OncoSimulR: forward genetic simulation in asexual populations with arbitrary epistatic interactions and a focus on modeling tumor progression.

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Contents

1 Introduction

OncoSimulR was originally developed to simulate tumor progression using several models of tumor progression with emphasis on allowing users to set restrictions in the accumulation of mutations as specified, for example, by Oncogenetic Trees (OT: Desper et al. 1999; A Szabo and Boucher 2008) or Conjunctive Bayesian Networks (CBN: Beerenwinkel, Eriksson, and Sturmfels 2007; Gerstung et al. 2009; Gerstung et al. 2011), with the possibility of adding passenger mutations to the simulations and allowing for several types of sampling.

Since then, OncoSimulR has been vastly extended to allow you to specify other types of restrictions in the accumulation of genes, as such as the XOR models of Korsunsky et al. (2014) or the "semimonotone" model of Farahani and Lagergren (Farahani and Lagergren 2013). Moreover, different fitness effects related to the order in which mutations appear can also be incorporated, involving arbitrary numbers of genes. This is different from "restrictions in the order of accumulation of mutations". With order effects, shown empirically in a recent cancer paper by Ortmann and collaborators (Ortmann et al. 2015), the effect of having both mutations "A" and "B" differs depending on whether "A" appeared before or after "B". More generally, now OncoSimulR also allows you to specify arbitrary epistatic interactions between arbitrary collections of genes and to model, for example, synthetic mortality or synthetic viability (again, involving an arbitrary number of genes, some of which might also depend on other genes, or show order effects with other genes). Moreover, it is possible to specify the above interactions in terms of modules, not genes. This idea is discussed in, for example, Raphael and Vandin (2015) and Gerstung et al. (2011): the restrictions encoded in, say, CBNs or OT can be considered to apply not to genes, but to modules, where each module is a set of genes (and the intersection between modules is the empty set) that performs a specific biological function. Modules, then, play the role of a "union operation" over the set of genes in a module. In addition, arbitrary numbers of genes without interactions (and with fitness effects coming from any distribution you might want) are also possible.

The models so far implemented are all continuous time models, which are simulated using the BNB algorithm of Mather, Hasty, and Tsimring (2012). The core of the code is implemented in C++, providing for fast execution. To help with simulation studies, code to simulate random graphs of the kind often seen in CBN, OTs, etc, is also available. Finally, OncoSimulR also allows for the generation of random fitness landscapes and the representation of fitness landscapes.

1.1 Key features of OncoSimulR

As mentioned above, OncoSimulR is now a very general package for forward genetic simulation, with applicability well beyond tumor progression. This is a summary of some of the key features:

- You can specify arbitrary interactions between genes, with arbitrary fitness effects, with explicit support for:

  - Restrictions in the accumulations of mutations, as specified by Oncogenetic Trees (OTs), Conjunctive Bayesian Networks (CBNs), semimonotone progression networks, and XOR relationships.
  - Epistatic interactions, including, but not limited to, synthetic viability and synthetic lethality.
  - Order effects.

- You can add passenger mutations.
- You can add mutator/antimutator effects.
- Fitness and mutation rates can be gene-specific.
- More generally, you can add arbitrary numbers of non-interacting genes with arbitrary fitness effects.
- You can allow for deviations from the OT, CBN, semimonotone, and XOR models, specifying a penalty for such deviations (the s_h parameter).
- You can conduct multiple simulations, and sample from them with different temporal schemes and using both whole tumor or single cell sampling.
- Right now, three different models are available, two that lead to exponential growth, one of them loosely based on Bozic et al. (2010), and another that leads to logistic-like growth, based on C. D. McFarland et al. (2013).
- You can use very large numbers of genes (e.g., see an example of 50000 in section 5.9).
- Simulations are generally very fast as I use C++ to implement the BNB algorithm.
- You can obtain the true sequence of events and the phylogenetic relationships between clones.
- You can generate random fitness landscapes (under the House of Cards, Rough Mount Fuji, or additive models, or combinations of the former) and use those landscapes as input to the simulation functions.
- You can plot fitness landscapes.

Further details about the motivation for wanting to simulate data this way in the context of tumor progression can be found in Diaz-Uriarte (2015), where additional comments about model parameters and caveats are discussed.

Are there similar programs? The Java program by Reiter et al. (2013) offers somewhat similar functionality to the previous version of OncoSimulR, but it is restricted to at most four drivers (whereas v.1 of OncoSimulR allowed for up to 64), you cannot use arbitrary CBNs or OTs (or XORs or semimonotone graphs) to specify restrictions, there is no allowance for passengers, and a single type of model (a discrete time Galton-Watson process) is implemented. The current functionality of OncoSimulR goes well beyond the the previous version (and, thus, also the TPT of (Reiter et al. 2013)). We now allow you to specify all types of fitness effects in other general forward genetic simulators such as FFPopSim (Zanini and Neher 2012), and some that, to our knowledge (e.g., order effects) are not available from any genetics simulator. In addition, the “Lego system” to flexibly combine different fitness specifications is also unique; by “Lego system” I mean that we can combine different pieces and blocks, similarly to what we do with Lego bricks. (I find this an intuitive and very graphical analogy which I have copied from Hothorn et al. (2006); Hothorn et al. (2008)).

1.2 Steps in using OncoSimulR

Using this package will often involve the following steps:

1. Specify the fitness effects: sections 2 and 4.

2. Simulate cancer progression: section 5. You can simulate for a single subject or for a set of subjects. You will need to:

    - Decide on a model. This basically amounts to choosing a model with exponential growth (“Exp” or “Bozic”) or a model with gompertz-like growth (“McFL”). If exponential growth, you can choose whether the the effects of mutations operate on the death rate (“Bozic”) or the birth rate (“Exp”)[1]

    - Specify other parameters of the simulation. In particular, decide when to stop the simulation, mutation rates, etc.

    Of course, at least for initial playing around, you can use the defaults.

3. Sample from the simulated data and do something with those simulated data (e.g., fit an OT model to them). What you do with the data, however, is outside the scope of this package.

Before anything else, let us load the package. We also explicitly load *graph* and *igraph* for the vignette to work (you do not need that for your usual interactive work). And I set the default color for vertices in igraph.

```
library(OncoSimulR)
library(graph)
library(igraph)
igraph_options(vertex.color = "SkyBlue2")
```

To be explicit, what version are we running?

[1] It is of course possible to do this with the gompertz-like models, but there probably is little reason to do it. C. D. McFarland et al. (2013) discuss this has little effect on their results, for example. In addition, decreasing the death rate will more easily lead to numerical problems as shown in section 5.13.

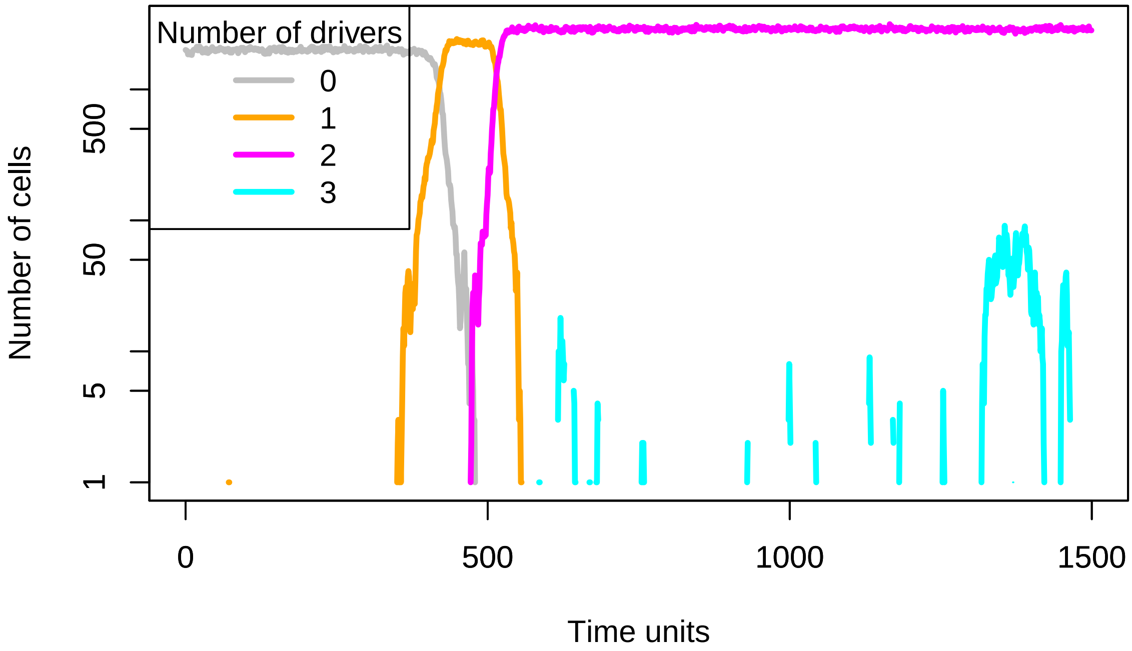
```
packageVersion("OncoSimulR")
## [1] '2.5.1'
```

1.3 Two quick examples

Following the above we will run two examples. First a model with a few genes and **epistasis**:

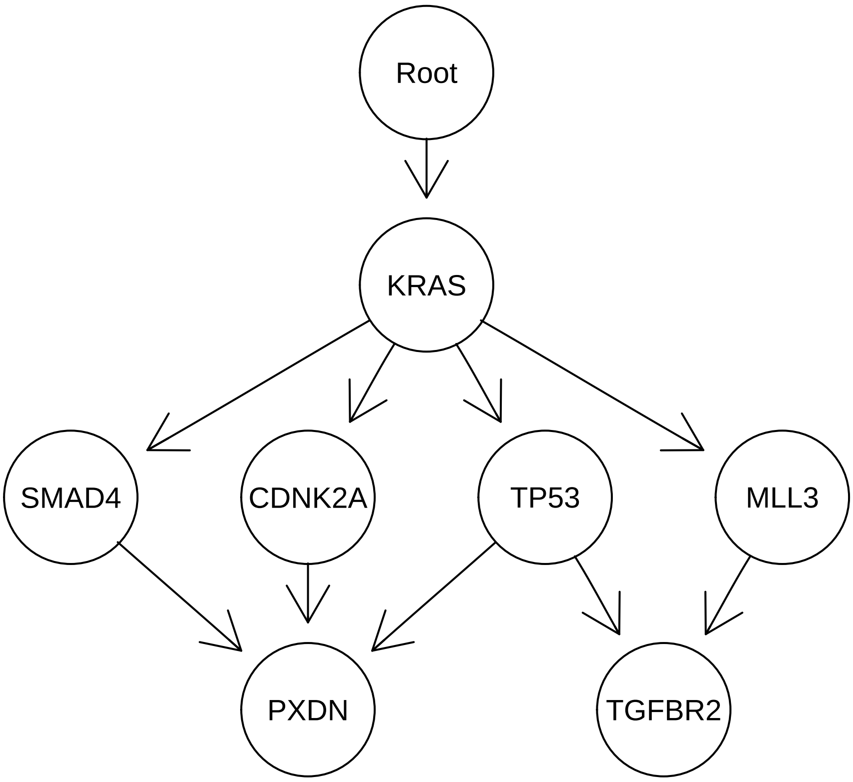
```
## 1. Fitness effects: here we specify an
##    epistatic model with modules.
sa <- 0.1
sb <- -0.2
sab <- 0.25
sac <- -0.1
sbc <- 0.25
sv2 <- allFitnessEffects(epistasis = c("-A : B" = sb,
                                       "A : -B" = sa,
                                       "A : C" = sac,
                                       "A:B" = sab,
                                       "-A:B:C" = sbc),
                         geneToModule = c(
                             "A" = "a1, a2",
                             "B" = "b",
                             "C" = "c"),
                         drvNames = c("a1", "a2", "b", "c"))
evalAllGenotypes(sv2, addwt = TRUE)
##        Genotype Fitness
## 1            WT   1.000
## 2            a1   1.100
## 3            a2   1.100
## 4             b   0.800
## 5             c   1.000
## 6        a1, a2   1.100
## 7         a1, b   1.250
## 8         a1, c   0.990
## 9         a2, b   1.250
## 10        a2, c   0.990
## 11         b, c   1.000
## 12    a1, a2, b   1.250
## 13    a1, a2, c   0.990
## 14     a1, b, c   1.125
## 15     a2, b, c   1.125
## 16 a1, a2, b, c   1.125

## 2. Simulate the data. Here we use the "McFL" model and set
##    explicitly parameters for mutation rate, initial size and size
##    of the population that will end the simulations, etc

RNGkind("Mersenne-Twister")
set.seed(983)
ep1 <- oncoSimulIndiv(sv2, model = "McFL",
                      mu = 5e-6,
                      sampleEvery = 0.02,
                      keepEvery = 0.5,
                      initSize = 2000,
                      finalTime = 3000,
                      onlyCancer = FALSE)
```

```
## 3. We will not analyze those data any further. We will only plot
## them.  For the sake of a small plot, we thin the data.
plot(ep1, show = "drivers", xlim = c(0, 1500),
     thinData = TRUE, thinData.keep = 0.5)
```



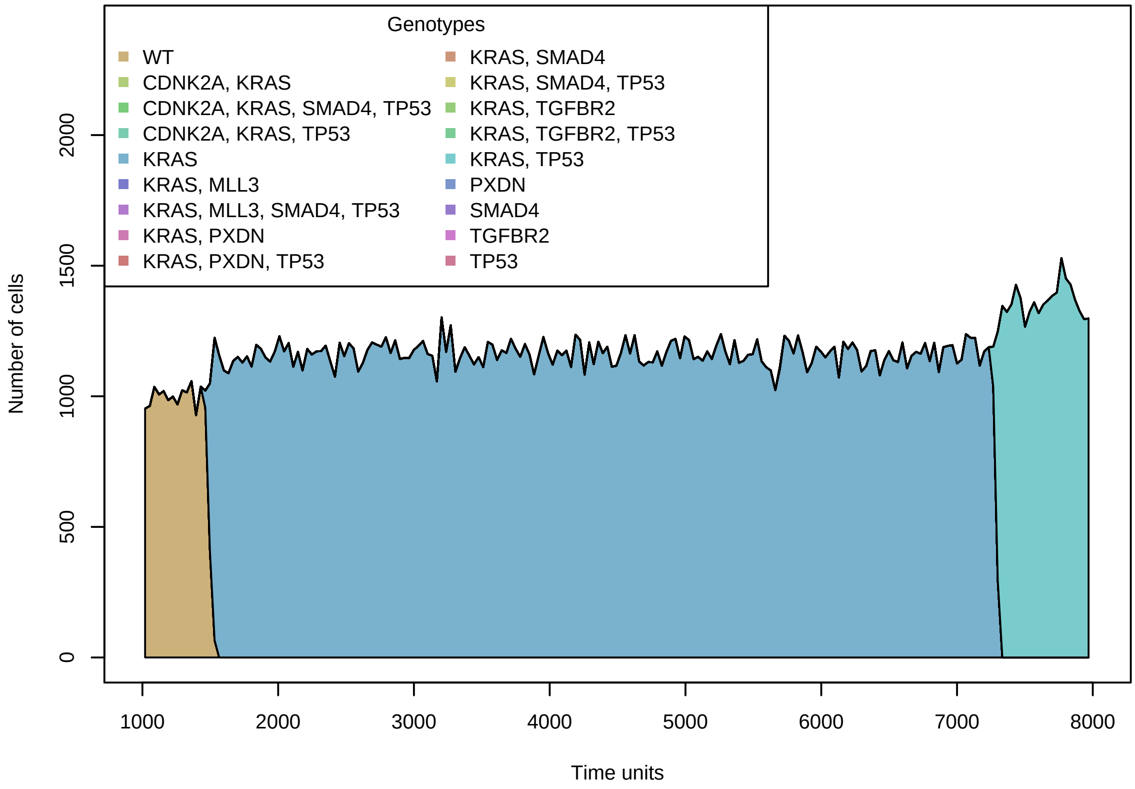
As a second example, we will use a model where we specify **restrictions in the order of accumulation of mutations** using the pancreatic cancer poset in Gerstung et al. (2011) (see more details in section 4.5):

```
## 1. Fitness effects:
pancr <- allFitnessEffects(
    data.frame(parent = c("Root", rep("KRAS", 4),
                          "SMAD4", "CDNK2A",
                          "TP53", "TP53", "MLL3"),
                   child = c("KRAS","SMAD4", "CDNK2A",
                          "TP53", "MLL3",
                    rep("PXDN", 3), rep("TGFBR2", 2)),
                s = 0.1,
                sh = -0.9,
                typeDep = "MN"),
    drvNames = c("KRAS", "SMAD4", "CDNK2A", "TP53",
                  "MLL3", "TGFBR2", "PXDN"))

## Plot the DAG of the fitnessEffects object
plot(pancr)
```



```
## 2. Simulate from it.

set.seed(4) ## Fix the seed, so we can repeat it
ep2 <- oncoSimulIndiv(pancr, model = "McFL",
                     mu = 1e-6,
                     sampleEvery = 0.02,
                     keepEvery = 1,
                     initSize = 1000,
                     finalTime = 10000,
                     onlyCancer = FALSE)
```

```
## 3. What genotypes and drivers we get? And play with limits
##    to show only parts of the data. We also aggressively thin
##    the data.
par(cex = 0.7)
plot(ep2, show = "genotypes", xlim = c(1000, 8000),
     ylim = c(0, 2400),
     thinData = TRUE, thinData.keep = 0.03)
```



1.4 How long does it take? How fast is OncoSimulR? How much space do the return objects take?

How long simulations take depend on several factors. This section gives a quick view. Longer examples and more detailed comments are provided in section ??.

- Your hardware, of course.
- The evolutionary model: using the "McFL" model is often much slower (this model includes density dependence and is more complex).
- The granularity of how often you keep data (`keepEvery` argument). Note that the default, which is to keep as often as you sample (so that we preserve all history) can lead to very slow execution times.
- The mutation rate, because higher mutation rates lead to more clones, and more clones mean we need to iterate over, well, more clones, and keep larger data structures.
- The fitness specification (more complex fitness specifications tend to be slightly slower).

- Whether or not you keep the complete clone history (this affects mainly size of return object, not speed).
- The stopping conditions.

To give you an idea, we will use the above examples, with the `detectionProb` stopping mechanism (see 5.4), two different growth models (exponential and McFarland) and will obtain 100 simulations. The results I show are for a laptop with an 8-core Intel Xeon E3-1505M CPU, running Debian GNU/Linux.

```
system.time(
    ep1_exp <- oncoSimulPop(100, sv2,
                            detectionProb = "default",
                            detectionSize = NA,
                            detectionDrivers = NA,
                            finalTime = NA,
                            keepEvery = 5,
                            model = "Exp",
                            sampleEvery = 0.02,
                            initSize = 500,
                            mc.cores = detectCores()))

system.time(
    ep1_mc <- oncoSimulPop(100, sv2,
                           detectionProb = "default",
                           detectionSize = NA,
                           detectionDrivers = NA,
                           finalTime = NA,
                           keepEvery = 5,
                           model = "McFL",
                           sampleEvery = 0.02,
                           initSize = 2000,
                           mc.cores = detectCores()))

system.time(
    ep2_exp <- oncoSimulPop(100, pancr,
                            detectionProb = "default",
                            detectionSize = NA,
                            detectionDrivers = NA,
                            finalTime = NA,
                            keepEvery = 5,
                            model = "Exp",
                            sampleEvery = 0.02,
                            initSize = 500,
                            mc.cores = detectCores()))

system.time(
    ep2_mc <- oncoSimulPop(100, pancr,
                           detectionProb = "default",
                           detectionSize = NA,
                           detectionDrivers = NA,
                           finalTime = NA,
                           keepEvery = 5,
                           model = "McFL",
                           sampleEvery = 0.02,
                           initSize = 2000,
                           mc.cores = detectCores()))
```

And we look at the sizes of objects using:

```
print(object.size(ep1_exp), units = "MB")
```

The above runs yield the following:

Simulation	Elapsed Time (s)	Object Size (MB)
ep1_exp	0.8	1.6
ep1_mc	6.6	16.6
ep2_exp	1.1	1.6
ep2_mc	5.8	20.6

There are large differences in speed between models and sizes of objects differ too: in these cases, the simulations using the "McFL" model run for much longer (2000 to 2000 time units, vs. 100 to 1500 of the "Exp" models) and also had a larger number of clones returned (5 to 16 vs. 1 to 3).

1.5 Citing OncoSimulR and other documentation

In R, you can do

```
citation("OncoSimulR")
##
## If you use OncoSimulR, please cite the OncoSimulR bioRxiv
## paper.  A former version of OncoSimulR has been used in a
## large comparative study of methods to infer restrictions,
## published in BMC Bioinformatics; you might want to cite that
## too, if appropriate, such as when referring to using
## evolutionary simulations to assess oncogenetic tree methods
## performance.
##
##   R Diaz-Uriarte. OncoSimulR: genetic simulation of cancer
##   progression with arbitrary epistasis and mutator genes.
##   2016. bioRxiv, http://dx.doi.org/10.1101/069500.
```

```
##
##   R Diaz-Uriarte. Identifying restrictions in the order of
##   accumulation of mutations during tumor progression:
##   effects of passengers, evolutionary models, and sampling
##   BMC Bioinformatics, 16(41), 2015.
```

which will tell you how to cite the package.

This is the URL for the bioRxiv paper: http://biorxiv.org/content/early/2016/08/14/069500. You can also take a look at this poster, http://dx.doi.org/10.7490/f1000research.1112860.1, presented at ECCB 2016.

1.5.1 HTML and PDF versions of the vignette

A PDF version of this vignette is available from https://rdiaz02.github.io/OncoSimul/pdfs/OncoSimulR.pdf. And an HTML version from https://rdiaz02.github.io/OncoSimul/OncoSimulR.html. These files correspond to the most recent, github version, of the package (i.e., they might include changes not yet available from the BioConudctor package).

1.6 Versions

In this vignette and the documentation I often refer to version 1 (v.1) and version 2 of OncoSimulR. Version 1 is the version available up to, and including, BioConductor v. 3.1. Version 2 of OncoSimulR is available starting from BioConductor 3.2 (and, of course, available too from development versions of BioC). So, if you are using the current stable or development version of BioConductor, or you grab the sources from github (https://github.com/rdiaz02/OncoSimul) you are using what we call version 2.

The functionality of version 1 will soon be removed.

2 Specifying fitness effects

2.1 Introduction to the specification of fitness effects

With OncoSimulR you can specify different types of effects on fitness:

- A special type of epistatic effect that is particularly amenable to be represented as a graph. In this graph, having, say, "B" be a child of "A" means that B can only accumulate if A is already present. This is what OT (Desper et al. 1999; A Szabo and Boucher 2008), CBN (Beerenwinkel, Eriksson, and Sturmfels 2007; Gerstung et al. 2009; Gerstung et al. 2011), progression networks (Farahani and Lagergren 2013), and other similar models (Korsunsky et al. 2014) generally mean. Details are provided in section 2.4. Note that this is not an order effect (discussed below): the fitness of a genotype from this DAGs is a function of whether or not the restrictions in the graph are satisfied, not the historical sequence of how they were satisfied.

- Effects where the order in which mutations are acquired matters, as illustrated in section 2.6. There is, in fact, empirical evidence of these effects (Ortmann et al. 2015). For instance, the fitness of genotype “A, B” would differ depending on whether A or B was acquired first.
- General epistatic effects (e.g., section 2.9), including synthetic viability (e.g., section 2.7) and synthetic lethality/mortality (e.g., section 2.8).
- Genes that have independent effects on fitness (section 2.3).

Modules (see section 2.5) allow you to specify any of the above effects (except those for genes without interactions, as it would not make sense there) in terms of modules (sets of genes), not individual genes. We will introduce them right after 2.4, and continue using them thereafter.

A guiding design principle of OncoSimulR is to try to make the specification of those effects as simple as possible but also as flexible as possible. Thus, there are two main ways of specifying fitness effects:

- Combining different types of effects in a single specification. For instance, you can combine epistasis with order effects with no interaction genes with modules. What you would do here is specify the effects that different mutations (or their combinations) have on fitness (the fitness effects) and then have OncoSimulR take care of combining them as if each of these were lego pieces. We will refer to this as the **lego system of fitness effects**.
- Explicitly passing to OncoSimulR a mapping of genotypes to fitness. Here you specify the fitness of each genotype. We will refer to this as the **explicit mapping of genotypes to fitness**.

Both approaches have advantages and disadvantages. Here I emphasize some relevant differences.

- With the lego system you can specify huge genomes with an enormous variety of interactions, since the possible genotypes are not constructed in advance. You would not be able to do this with the explicit mapping of genotypes to fitness if you wanted to, say, construct that mapping for a modest genotype of 500 genes (you’d have more genotypes than particles in the Universe).
- For many models/data you often intuitively start with the fitness of the genotypes, not the fitness consequences of the different mutations. In these cases, you’d need to do the math to specify the terms you want if you used the lego system.
- Sometimes you already have a moderate size genotype → fitness mapping and you certainly do not want to do the math by hand: here the lego system would be painful to use.

- But sometimes we do think in terms of “the effects on fitness of such and such mutations are” and that immediately calls for the lego system, where you focus on the effects, and let OncoSimulR take care of doing the math of combining.
- If you want to use order effects, you must use the lego system (at least for now).
- If you want to specify modules, you must use the lego system (the explicit mapping of genotypes is, by its very nature, ill-suited for this).
- The lego system might help you see what your model really means: in many cases, you can obtain fairly succinct specification of complex fitness models with just a few terms. Similarly, depending on what your emphasis is, you can often specify the same fitness landscape in several different ways.

Regardless of the route, you need to get that information into OncoSimulR’s functions. The main function we will use is *allFitnessEffects*: this is the function in charge of reading the fitness specifications. We also need to discuss how, what, and where you have to pass to *allFitnessEffects*.

2.1.1 Explicit mapping of genotypes to fitness

Conceptually, the simplest way to specify fitness is to specify the mapping of all genotypes to fitness explicitly. An example will make this clear. Let’s suppose you have a simple two-gene scenario, so a total of four genotypes, and you have a data frame with genotypes and fitness, where genoytpes are specified as character vectors, with mutated genes separated by commas:

```
m4 <- data.frame(G = c("WT", "A", "B", "A, B"), F = c(1, 2, 3, 4))
```

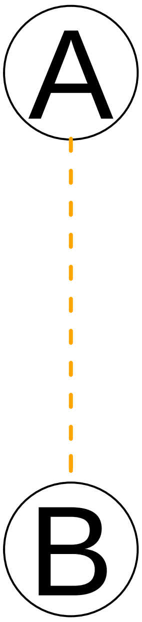
Now, let’s give that to the *allFitnessEffects* function:

```
fem4 <- allFitnessEffects(genotFitness = m4)
## Column names of object not Genotype and Fitness. Renaming them assuming that is
```

(The message is just telling you what the program guessed you wanted.)

That’s it. You can plot that fitnessEffects object

```
plot(fem4)
```



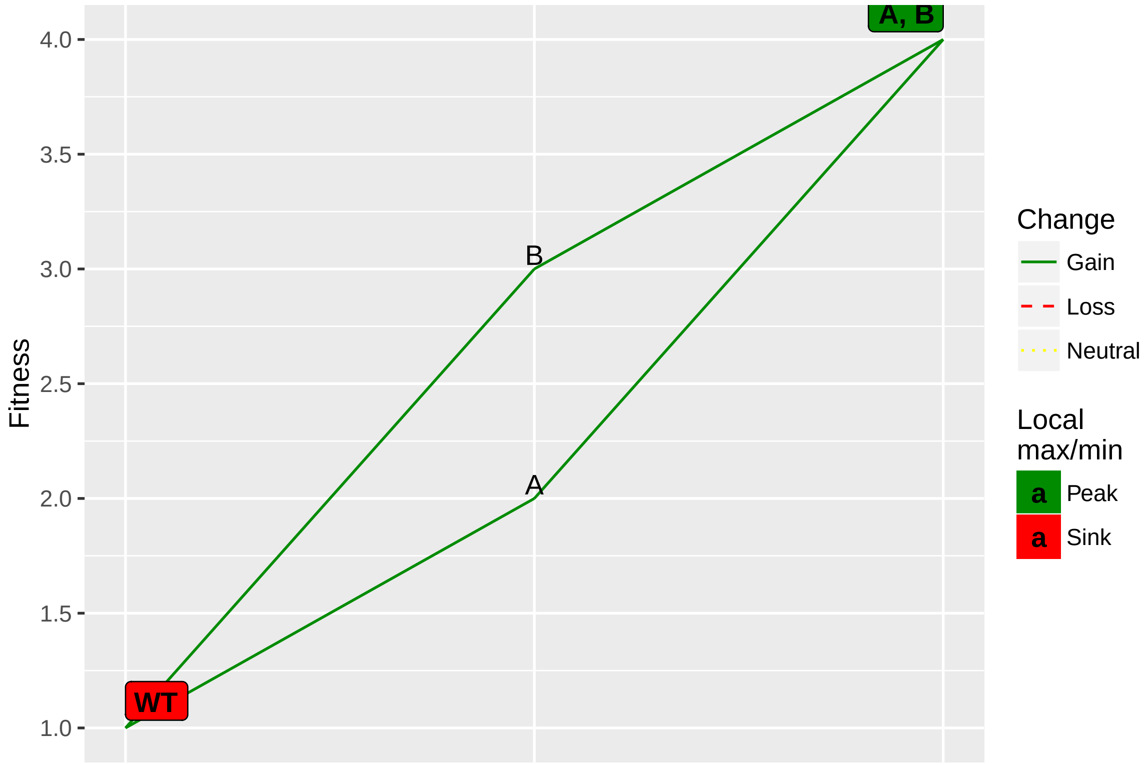
In this case, that plot is not very interesting (compare with the `plot(pancr)` we saw in 1.3 or the plots in 2.4).

You can also check what OncoSimulR thinks the fitnesses are, with the ***evalAllGenotypes*** function that we will use repeatedly below (of course, here we should see the same fitnesses we entered):

```
evalAllGenotypes(fem4, addwt = TRUE)
##   Genotype Fitness
## 1       WT       1
## 2        A       2
## 3        B       3
## 4     A, B       4
```

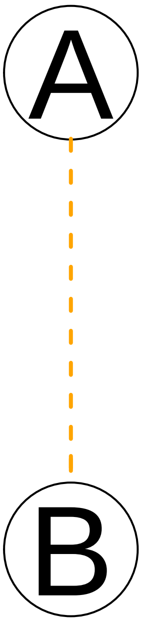
And you can plot the fitness landscape:

```
plotFitnessLandscape(evalAllGenotypes(fem4))
```

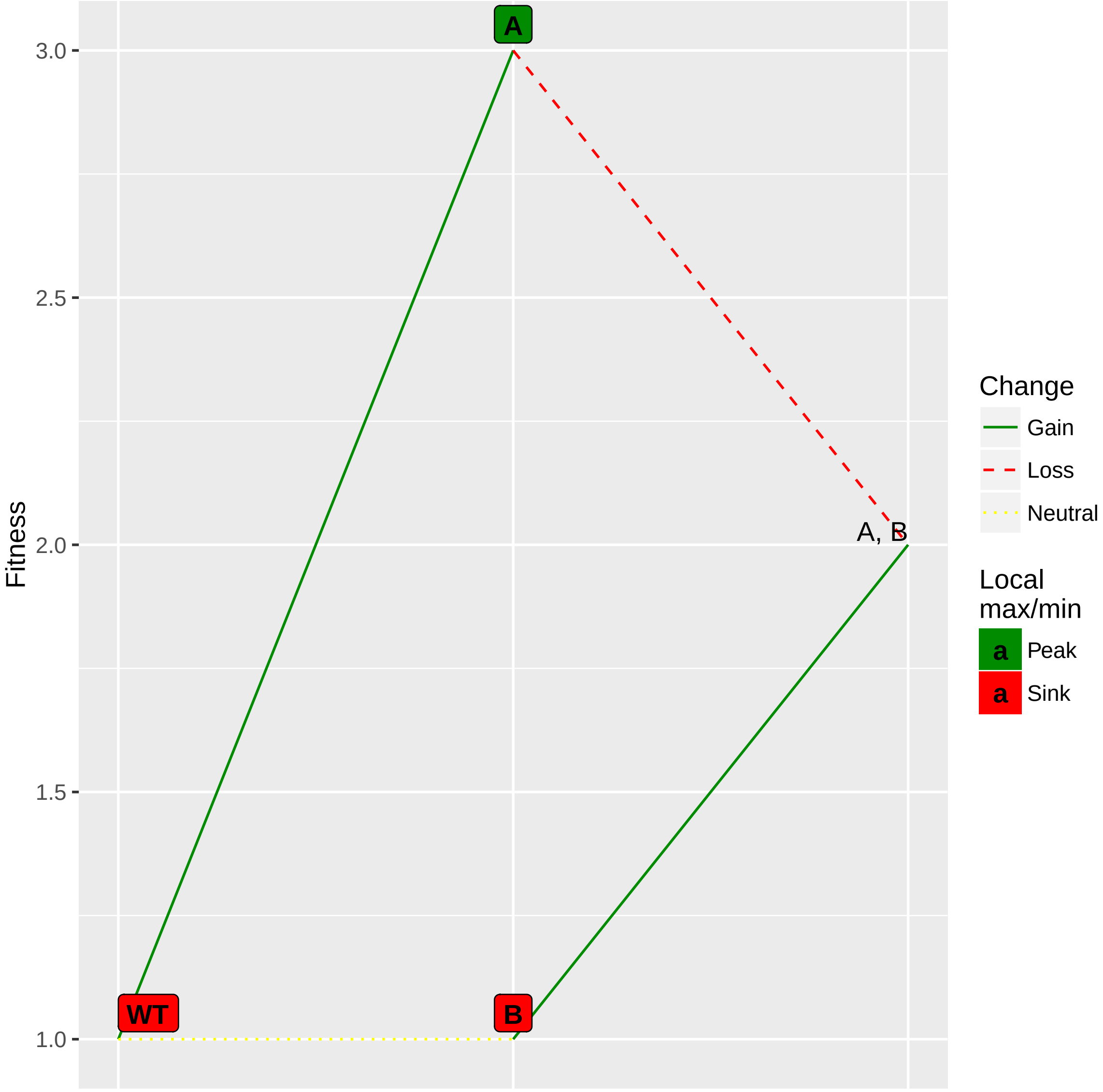


To specify the mapping you can also use a matrix (or data frame) with $g+1$ columns; each of the first g columns contains a 1 or a 0 indicating that the gene of that column is mutated or not. Column $g+1$ contains the fitness values. And you do not even need to specify all the genotypes (we will assume that the missing genotypes have fitness 1):

```
m6 <- cbind(c(1, 1), c(1, 0), c(2, 3))
fem6 <- allFitnessEffects(genotFitness = m6)
## Number of genotypes less than 2^L. Missing genotype will be set to 1
## Setting/resetting gene names because one or more are missing. If this is not wha
evalAllGenotypes(fem6, addwt = TRUE)
##   Genotype Fitness
## 1       WT       1
## 2        A       3
## 3        B       1
## 4     A, B       2
plot(fem6)
```



```
plotFitnessLandscape(evalAllGenotypes(fem6))
```



This way of giving a fitness specification to OncoSimulR might be ideal if you directly generate random mappings of genotypes to fitness (or random fitness landscapes), as we will do in section 9.

We will see an example of this way of passing fitness again in 4.1, where will compare it with the lego system.

2.1.2 How to specify fitness effects with the lego system

An alternative general approach followed in many genetic simulators is to specify how particular combinations of alleles modify the wildtype genotype or the genotype that contains the individual effects of the interacting genes (e.g., see equation 1 in the supplementary material for FFPopSim, Zanini and Neher (2012)). For example, if we specify that a mutation in "A" contributes 0.04, a mutation in "B" contributes 0.03, and the double mutation "A:B" contributes 0.1, that means that the fitness of the "A, B" genotype (the genotype with A and B mutated) is that of the wildtype (1, by default), plus (actually, times —see section 2.2) the effects of having A mutated, plus (times) the effects of having B mutated, plus (times) the effects of "A:B" both being mutated.

We will see below that with the "lego system" it is possible to do something very similar to the explicit mapping of section 2.1.1. But this will sometimes require a more cumbersome notation (and sometimes also will require your doing some math). We will see examples in sections 2.9.1, 2.9.2 and 2.9.3 or the example in 4.4.2. But then, if we can be explicit about (at least some of) the mappings $genotype \rightarrow fitness$, how are these procedures different? When you use the "lego system" you can combine both a partial explicit mapping of genotypes to fitness with arbitrary fitness effects of other genes/modules. In other words, with the "lego system" OncoSimulR makes it simple to be explicit about the mapping of specific genotypes, while also using the "how this specific effects modifies previous effects" logic, leading to a flexible specification. This also means that in many cases the same fitness effects can be specified in several different ways.

Most of the rest of this section is devoted to explaining how to combine those pieces. Before that, however, we need to discuss the fitness model we use.

2.2 Numeric values of fitness effects

We evaluate fitness using the usual (Zanini and Neher 2012; Gillespie 1993; Beerenwinkel, Eriksson, and Sturmfels 2007; Datta et al. 2013) multiplicative model: fitness is $\prod(1 + s_i)$ where s_i is the fitness effect of gene (or gene interaction) i. In all models except Bozic, this fitness refers to the growth rate (the death rate being fixed to 1[2]). The original model of C. D. McFarland et al. (2013) has a slightly different parameterization, but you can go easily from one to the other (see section 2.2.1).

For the Bozic model (Bozic et al. 2010), however, the birth rate is set to 1, and the death rate then becomes $\prod(1 - s_i)$.

2.2.1 McFarland parameterization

In the original model of C. D. McFarland et al. (2013), the effects of drivers contribute to the numerator of the birth rate, and those of the (deleterious) passengers to the denominator as: $\frac{(1+s)^D}{(1-s_p)^P}$, where D and P are, respectively, the total number of drivers and passengers in a genotype, and here the fitness effects of all drivers is the same (s) and that of all passengers the same too (s_p). However, we can map from this ratio to the usual product of terms by using a different value of s_p, that we will call $s_{pp} = -s_p/(1 + s_p)$ (see C. McFarland (2014), his eq. 2.1 in p.9). This reparameterization applies to v.2. In v.1 we use the same parameterization as in the original one in C. D. McFarland et al. (2013).

[2] You can change this if you really want to.

2.2.2 No viability of clones and types of models

For all models where fitness affects directly the birth rate (for now, all except Bozic), if you specify that some event (say, mutating gene A) has $s_A \leq -1$, if that event happens then birth rate becomes zero which is taken to indicate that the clone is not even viable and thus disappears immediately without any chance for mutation[3].

Models based on Bozic, however, have a birth rate of 1 and mutations affect the death rate. In this case, a death rate larger than birth rate, per se, does not signal immediate extinction and, moreover, even for death rates that are a few times larger than birth rates, the clone could mutate before becoming extinct[4].

In general, if you want to identify some mutations or some combinations of mutations as leading to immediate extinction (i.e., no viability), of the affected clone, set it to $-\infty$ as this would work even if how birth rates of 0 are handled changes. Most examples below evaluate fitness by its effects on the birth rate. You can see one where we do it both ways in Section 2.7.2.

2.3 Genes without interactions

This is a imple scenario. Each gene i has a fitness effect s_i if mutated. The s_i can come from any distribution you want. As an example let's use three genes. We know there are no order effects, but we will also see what happens if we examine genotypes as ordered.

```
ai1 <- evalAllGenotypes(allFitnessEffects(
    noIntGenes = c(0.05, -.2, .1)), order = FALSE)
```

We can easily verify the first results:

```
ai1
##   Genotype Fitness
## 1        1   1.050
## 2        2   0.800
## 3        3   1.100
```

[3] This is a shortcut that we take because we think that it is what you mean. Note, however, that technically a clone with birth rate of 0 might have a non-zero probability of mutating before becoming extinct because in the continuous time model we use mutation is not linked to reproduction. In the present code, we are not allowing for any mutation when birth rate is 0. There are other options, but none which I find really better. An alternative implementation makes a clone immediately extinct if and only if any of the $s_i = -\infty$. However, we still need to handle the case with $s_i < -1$ as a special case. We either make it identical to the case with any $s_i = -\infty$ or for any $s_i > -\infty$ we set $(1 + s_i) = \max(0, 1 + s_i)$ (i.e., if $s_i < -1$ then $(1 + s_i) = 0$), to avoid obtaining negative birth rates (that make no sense) and the problem of multiplying an even number of negative numbers. I think only the second would make sense as an alternative.

[4] We said "a few times". For a clone of population size 1 —which is the size at which all clones start from mutation—, if death rate is, say, 90 but birth rate is 1, the probability of mutating before becoming extinct is very, very close to zero for all reasonable values of mutation rate}. How do we signal immediate extinction or no viability in this case? You can set the value of $s = -\infty$.

```
## 4     1, 2   0.840
## 5     1, 3   1.155
## 6     2, 3   0.880
## 7  1, 2, 3   0.924
```

```
all(ai1[, "Fitness"]  == c( (1 + .05), (1 - .2), (1 + .1),
       (1 + .05) * (1 - .2),
       (1 + .05) * (1 + .1),
       (1 - .2) * (1 + .1),
       (1 + .05) * (1 - .2) * (1 + .1)))
## [1] TRUE
```

And we can see that considering the order of mutations (see section 2.6) makes no difference:

```
(ai2 <- evalAllGenotypes(allFitnessEffects(
    noIntGenes = c(0.05, -.2, .1)), order = TRUE,
    addwt = TRUE))
##     Genotype Fitness
## 1         WT   1.000
## 2          1   1.050
## 3          2   0.800
## 4          3   1.100
## 5      1 > 2   0.840
## 6      1 > 3   1.155
## 7      2 > 1   0.840
## 8      2 > 3   0.880
## 9      3 > 1   1.155
## 10     3 > 2   0.880
## 11 1 > 2 > 3   0.924
## 12 1 > 3 > 2   0.924
## 13 2 > 1 > 3   0.924
## 14 2 > 3 > 1   0.924
## 15 3 > 1 > 2   0.924
## 16 3 > 2 > 1   0.924
```

(The meaning of the notation in the output table is as follows: "WT" denotes the wild-type, or non-mutated clone. The notation $x > y$ means that a mutation in "x" happened before a mutation in "y". A genotype $x > y \_ z$ means that a mutation in "x" happened before a mutation in "y"; there is also a mutation in "z", but that is a gene for which order does not matter).

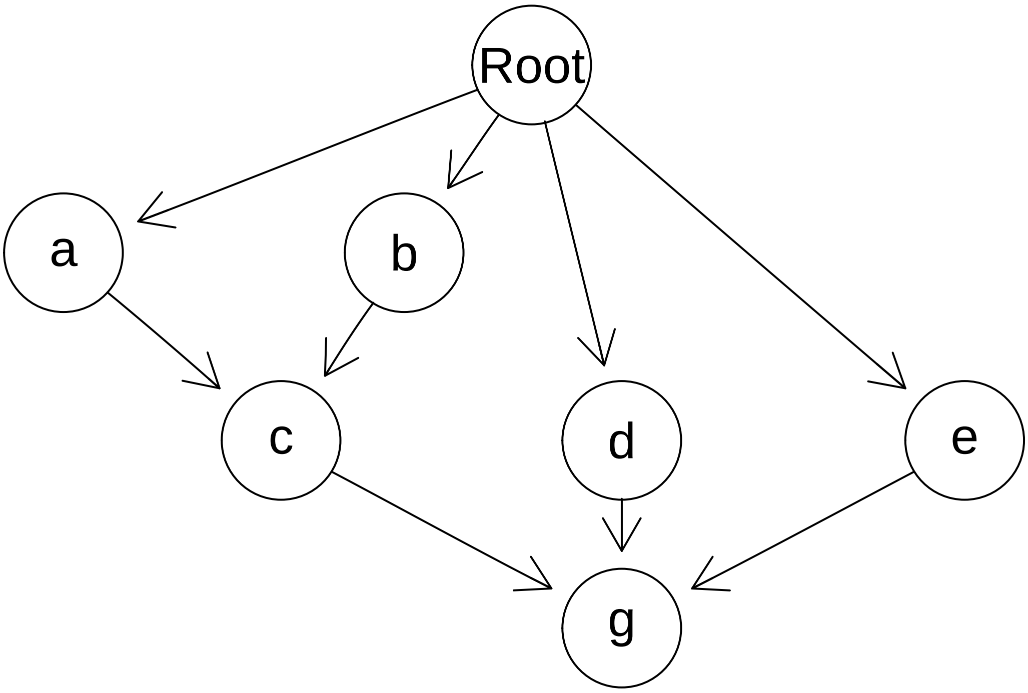
And what if I want genes without interactions but I want modules (see section 2.5)? Go to section 2.10.

2.4 Using DAGs: Restrictions in the order of mutations as extended posets

2.4.1 AND, OR, XOR relationships

The literature on oncogenetic trees, CBNs, etc, has used graphs as a way of showing the restrictions in the order in which mutations can accumulate. The meaning of "convergent arrows" in these graphs, however, differs. In Figure 1 of Korsunsky et al. (2014) we are shown a simple diagram that illustrates the three basic different meanings of convergent arrows using two parental nodes. We will illustrate it here with three. Suppose we focus on node "g" in the following figure (we will create it shortly)

```
data(examplesFitnessEffects)
plot(examplesFitnessEffects[["cbn1"]])
```



- In relationships of the type used in **Conjunctive Bayesian Networks (CBN)** (e.g., Gerstung et al. 2009), we are modeling an **AND** relationship, also called **CMPN** by Korsunsky et al. (2014) or **monotone** relationship by Farahani and Lagergren (2013). If the relationship in the graph is fully respected, then "g" will only appear if all of "c", "d", and "e" are already mutated.

- **Semimonotone** relationships *sensu* Farahani and Lagergren (2013) or **DMPN** *sensu* Korsunsky et al. (2014) are **OR** relationships: "g" will appear if one or more of "c", "d", or "e" are already mutated.

- **XMPN** relationships (Korsunsky et al. 2014) are **XOR** relationships: "g" will be present only if exactly one of "c", "d", or "e" is present.

Note that oncogenetic trees (Desper et al. 1999; A Szabo and Boucher 2008) need not deal with the above distinctions, since the DAGs are trees: no node has more than one incoming connection or more than one parent[5].

To have a flexible way of specifying all of these restrictions, we will want to be able to say what kind of dependency each child node has on its parents.

2.4.2 Fitness effects

Those DAGs specify dependencies and, as explained in Diaz-Uriarte (2015), it is simple to map them to a simple evolutionary model: any set of mutations that does not conform to the restrictions encoded in the graph will have a fitness of 0. However, we might not want to require absolute compliance with the DAG. This means we might want to allow deviations from the DAG with a corresponding penalization that is, however, not identical to setting fitness to 0 (again, see Diaz-Uriarte 2015). This we can do by being explicit about the fitness effects of the deviations from the restrictions encoded in the DAG. We will use below a column of `s` for the fitness effect when the restrictions are satisfied and a column of `sh` when they are not. (See also 2.2 for the details about the meaning of the fitness effects).

That way of specifying fitness effects makes it also trivial to use the model in Hjelm, Höglund, and Lagergren (2006) where all mutations might be allowed to occur, but the presence of some mutations increases the probability of occurrence of other mutations. For example, the values of `sh` could be all small positive ones (or for mildly deleterious effects, small negative numbers), while the values of `s` are much larger positive numbers.

2.4.3 Extended posets

In version 1 of this package we used posets in the sense of Beerenwinkel, Eriksson, and Sturmfels (2007) and Gerstung et al. (2009), as explained in section 11.1 and in the help for *poset*. Here, we continue using two columns, that specify parents and children, but we add columns for the specific values of fitness effects (both s and sh —i.e., fitness effects for what happens when restrictions are and are not satisfied) and for the type of dependency as explained in section 2.4.1.

We can now illustrate the specification of different fitness effects using DAGs.

2.4.4 DAGs: A first conjunction (AND) example

```
cs <-  data.frame(parent = c(rep("Root", 4), "a", "b", "d", "e", "c"),
                 child = c("a", "b", "d", "e", "c", "c", rep("g", 3)),
```

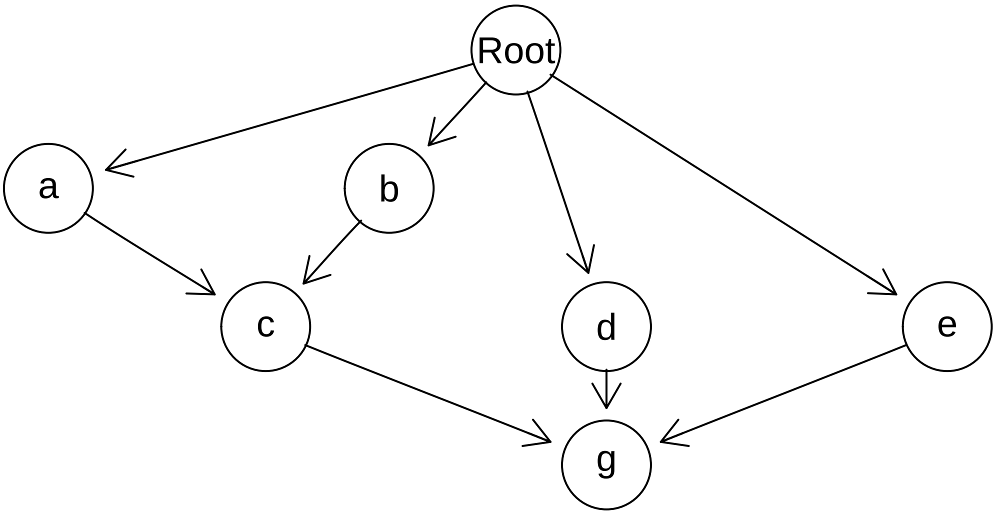
[5] OTs and CBNs have some other technical differences about the underlying model they assume, such as the exponential waiting time in CBNs. We will not discuss them here.

```
                        s = 0.1,
                        sh = -0.9,
                        typeDep = "MN")

cbn1 <- allFitnessEffects(cs)
```

(We skip one letter, just to show that names need not be consecutive or have any particular order.)

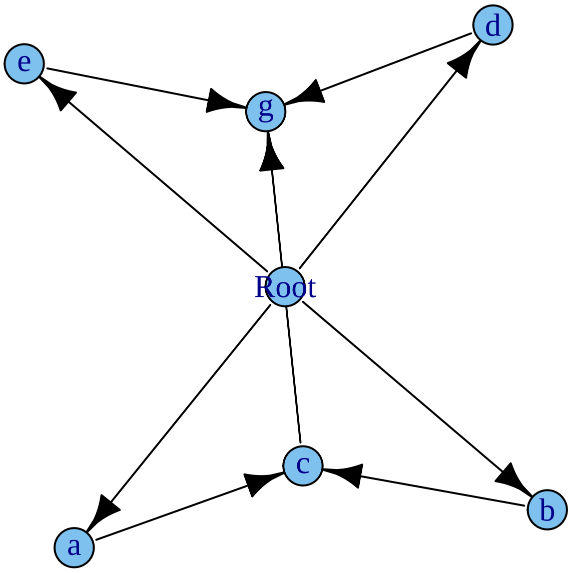
We can get a graphical representation using the default “graphNEL”

```
plot(cbn1)
```



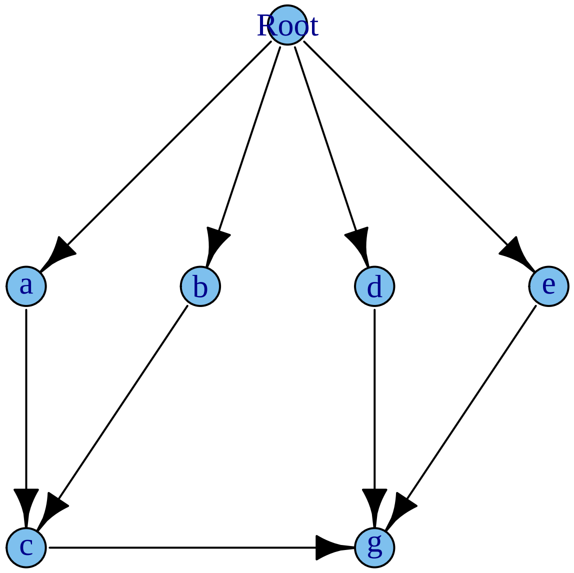
or one using “igraph”:

```
plot(cbn1, "igraph")
```



Since we have a parent and children, the reingold.tilford layout is probably the best here, so you might want to use that:

```
library(igraph) ## to make the reingold.tilford layout available
plot(cbn1, "igraph", layout = layout.reingold.tilford)
```



And what is the fitness of all genotypes?

```
gfs <- evalAllGenotypes(cbn1, order = FALSE, addwt = TRUE)

gfs[1:15, ]
##    Genotype Fitness
## 1        WT    1.00
## 2         a    1.10
## 3         b    1.10
## 4         c    0.10
## 5         d    1.10
## 6         e    1.10
## 7         g    0.10
## 8      a, b    1.21
## 9      a, c    0.11
## 10     a, d    1.21
## 11     a, e    1.21
## 12     a, g    0.11
## 13     b, c    0.11
## 14     b, d    1.21
## 15     b, e    1.21
```

You can verify that for each genotype, if a mutation is present without all of its dependencies present, you get a $(1-0.9)$ multiplier, and you get a $(1+0.1)$ multiplier for all the rest with its direct parents satisfied. For example, genotypes “a”, or “b”, or “d”, or “e” have fitness $(1 + 0.1)$, genotype “a, b, c” has fitness $(1 + 0.1)^3$, but genotype “a, c” has fitness $(1 + 0.1)(1 - 0.9) = 0.11$.

2.4.5 DAGs: A second conjunction example

Let's try a first attempt at a somewhat more complex example, where the fitness consequences of different genes differ.

```
c1 <- data.frame(parent = c(rep("Root", 4), "a", "b", "d", "e", "c"),
                 child = c("a", "b", "d", "e", "c", "c", rep("g", 3)),
                 s = c(0.01, 0.02, 0.03, 0.04, 0.1, 0.1, rep(0.2, 3)),
                 sh = c(rep(0, 4), c(-.1, -.2), c(-.05, -.06, -.07)),
                 typeDep = "MN")

try(fc1 <- allFitnessEffects(c1))
```

If you try this, you'll get an error. There is an error because the "sh" varies within a child, and we do not allow that for a poset-type specification, as it is ambiguous. If you need arbitrary fitness values for arbitrary combinations of genotypes, you can specify them using epistatic effects as in section 2.9 and order effects as in section 2.6.

Why do we need to specify as many "s" and "sh" as there are rows (or a single one, that gets expanded to those many) when the "s" and "sh" are properties of the child node, not of the edges? Because, for ease, we use a data.frame.

We fix the error in our specification. Notice that the "sh" is not set to -1 in these examples. If you want strict compliance with the poset restrictions, you should set $sh = -1$ or, better yet, $sh = -\infty$ (see section 2.2.2), but having an $sh > -1$ will lead to fitnesses that are > 0 and, thus, is a way of modeling small deviations from the poset (see discussion in Diaz-Uriarte 2015).

Note that for those nodes that depend only on "Root" the type of dependency is irrelevant.

```
c1 <- data.frame(parent = c(rep("Root", 4), "a", "b", "d", "e", "c"),
                 child = c("a", "b", "d", "e", "c", "c", rep("g", 3)),
                 s = c(0.01, 0.02, 0.03, 0.04, 0.1, 0.1, rep(0.2, 3)),
                 sh = c(rep(0, 4), c(-.9, -.9), rep(-.95, 3)),
                 typeDep = "MN")

cbn2 <- allFitnessEffects(c1)
```

We could get graphical representations but the figures would be the same as in the example in section 2.4.4, since the structure has not changed, only the numeric values.

What is the fitness of all possible genotypes? Here, order of events *per se* does not matter, beyond that considered in the poset. In other words, the fitness of genotype "a, b, c" is the same no matter how we got to "a, b, c". What matters is whether or not the genes on which each of "a", "b", and "c" depend are present or not (I only show the first 10 genotypes)

```
gcbn2 <- evalAllGenotypes(cbn2, order = FALSE)
gcbn2[1:10, ]
##    Genotype Fitness
## 1         a   1.010
## 2         b   1.020
## 3         c   0.100
## 4         d   1.030
## 5         e   1.040
## 6         g   0.050
## 7      a, b   1.030
## 8      a, c   0.101
## 9      a, d   1.040
## 10     a, e   1.050
```

Of course, if we were to look at genotypes but taking into account order of occurrence of mutations, we would see no differences

```
gcbn2o <- evalAllGenotypes(cbn2, order = TRUE, max = 1956)
gcbn2o[1:10, ]
##    Genotype Fitness
## 1         a   1.010
## 2         b   1.020
## 3         c   0.100
## 4         d   1.030
## 5         e   1.040
## 6         g   0.050
## 7     a > b   1.030
## 8     a > c   0.101
## 9     a > d   1.040
## 10    a > e   1.050
```

(The $max = 1956$ is there so that we show all the genotypes, even if they are more than 256, the default.)

You can check the output and verify things are as they should. For instance:

```
all.equal(
        gcbn2[c(1:21, 22, 28, 41, 44, 56, 63 ) , "Fitness"],
        c(1.01, 1.02, 0.1, 1.03, 1.04, 0.05,
          1.01 * c(1.02, 0.1, 1.03, 1.04, 0.05),
          1.02 * c(0.10, 1.03, 1.04, 0.05),
          0.1 * c(1.03, 1.04, 0.05),
          1.03 * c(1.04, 0.05),
          1.04 * 0.05,
          1.01 * 1.02 * 1.1,
          1.01 * 0.1 * 0.05,
          1.03 * 1.04 * 0.05,
          1.01 * 1.02 * 1.1 * 0.05,
          1.03 * 1.04 * 1.2 * 0.1, ## notice this
          1.01 * 1.02 * 1.03 * 1.04 * 1.1 * 1.2
          ))
## [1] TRUE
```

A particular one that is important to understand is genotype with mutated genes “c, d, e, g”:

```
gcbn2[56, ]
##      Genotype Fitness
## 56 c, d, e, g  0.1285
all.equal(gcbn2[56, "Fitness"], 1.03 * 1.04 * 1.2 * 0.10)
## [1] TRUE
```

where “g” is taken as if its dependencies are satisfied (as “c”, “d“, and”e" are present) even when the dependencies of “c” are not satisfied (and that is why the term for “c” is 0.9).

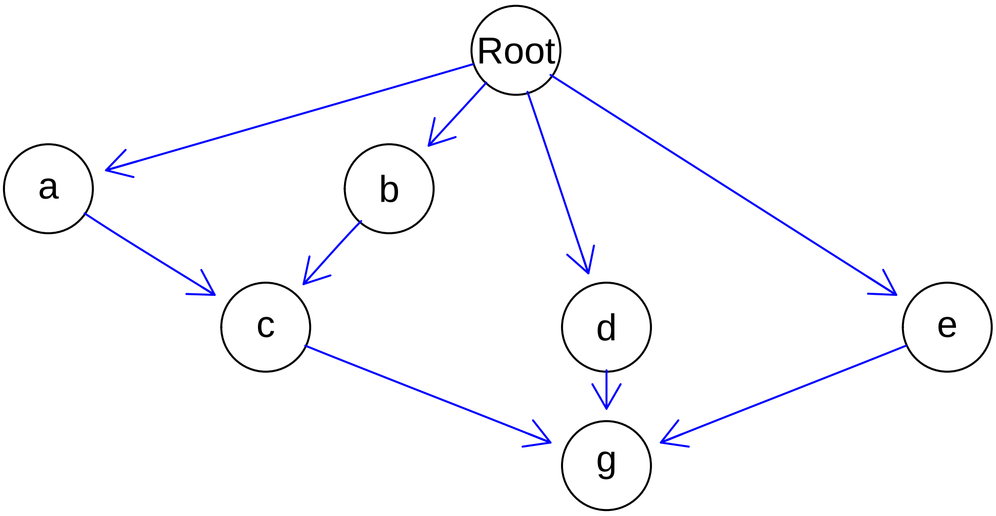
2.4.6 DAGs: A semimonotone or “OR” example

We will reuse the above example, changing the type of relationship:

```
s1 <- data.frame(parent = c(rep("Root", 4), "a", "b", "d", "e", "c"),
                 child = c("a", "b", "d", "e", "c", "c", rep("g", 3)),
                 s = c(0.01, 0.02, 0.03, 0.04, 0.1, 0.1, rep(0.2, 3)),
                 sh = c(rep(0, 4), c(-.9, -.9), rep(-.95, 3)),
                 typeDep = "SM")

smn1 <- allFitnessEffects(s1)
```

It looks like this (where edges are shown in blue to denote the semimonotone relationship):

```
plot(smn1)
```



```
gsmn1 <- evalAllGenotypes(smn1, order = FALSE)
```

Having just one parental dependency satisfied is now enough, in contrast to what happened before. For instance:

```
gcbn2[c(8, 12, 22), ]
##    Genotype Fitness
## 8      a, c   0.101
## 12     b, c   0.102
## 22  a, b, c   1.133
gsmn1[c(8, 12, 22), ]
##    Genotype Fitness
## 8      a, c   1.111
## 12     b, c   1.122
## 22  a, b, c   1.133

gcbn2[c(20:21, 28), ]
##    Genotype Fitness
## 20     d, g 0.05150
## 21     e, g 0.05200
## 28  a, c, g 0.00505
gsmn1[c(20:21, 28), ]
##    Genotype Fitness
## 20     d, g   1.236
## 21     e, g   1.248
## 28  a, c, g   1.333
```

2.4.7 An “XMPN” or “XOR” example

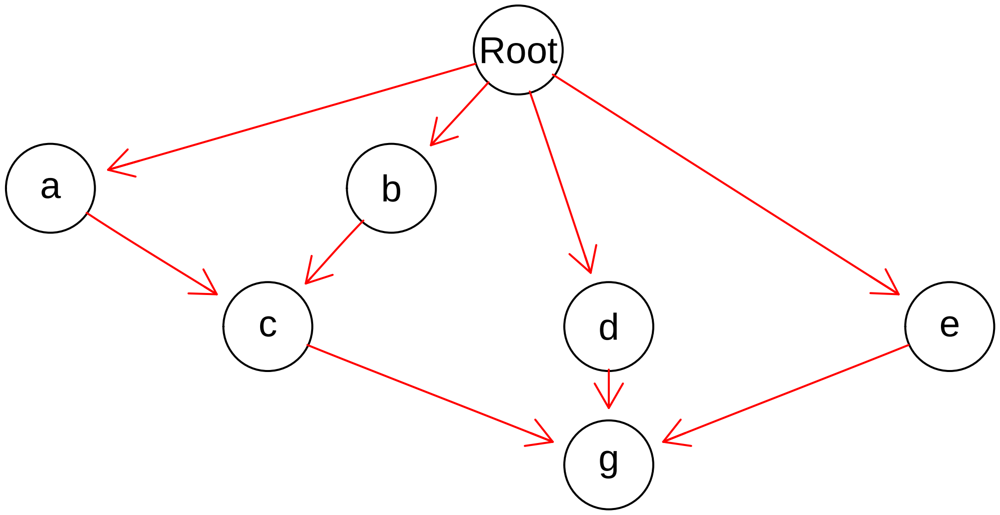
Again, we reuse the example above, changing the type of relationship:

```
x1 <- data.frame(parent = c(rep("Root", 4), "a", "b", "d", "e", "c"),
```

```r
                  child = c("a", "b", "d", "e", "c", "c", rep("g", 3)),
                  s = c(0.01, 0.02, 0.03, 0.04, 0.1, 0.1, rep(0.2, 3)),
                  sh = c(rep(0, 4), c(-.9, -.9), rep(-.95, 3)),
                  typeDep = "XMPN")

xor1 <- allFitnessEffects(x1)
```

It looks like this (edges in red to denote the “XOR” relationship):

```r
plot(xor1)
```



```r
gxor1 <- evalAllGenotypes(xor1, order = FALSE)
```

Whenever “c” is present with both “a” and “b”, the fitness component for “c” will be $(1 - 0.1)$. Similarly for “g” (if more than one of “d“,”e“, or”c" is present, it will show as $(1 - 0.05)$). For example:

```r
gxor1[c(22, 41), ]
##    Genotype Fitness
## 22  a, b, c 0.10302
## 41  d, e, g 0.05356
c(1.01 * 1.02 * 0.1, 1.03 * 1.04 * 0.05)
## [1] 0.10302 0.05356
```

However, having just both “a” and “b” is identical to the case with CBN and the monotone relationship (see sections 2.4.5 and 2.4.6). If you want the joint presence of “a” and “b” to result in different fitness than the product of the individual terms, without considering the presence of “c”, you can specify that using general epistatic effects (section 2.9).

We also see a very different pattern compared to CBN (section 2.4.5) here:

```r
gxor1[28, ]
##    Genotype Fitness
## 28  a, c, g   1.333
```

```
1.01 * 1.1 * 1.2
## [1] 1.333
```

as exactly one of the dependencies for both “c” and “g” are satisfied.

But

```
gxor1[44, ]
##       Genotype Fitness
## 44 a, b, c, g  0.1236
1.01 * 1.02 * 0.1 * 1.2
## [1] 0.1236
```

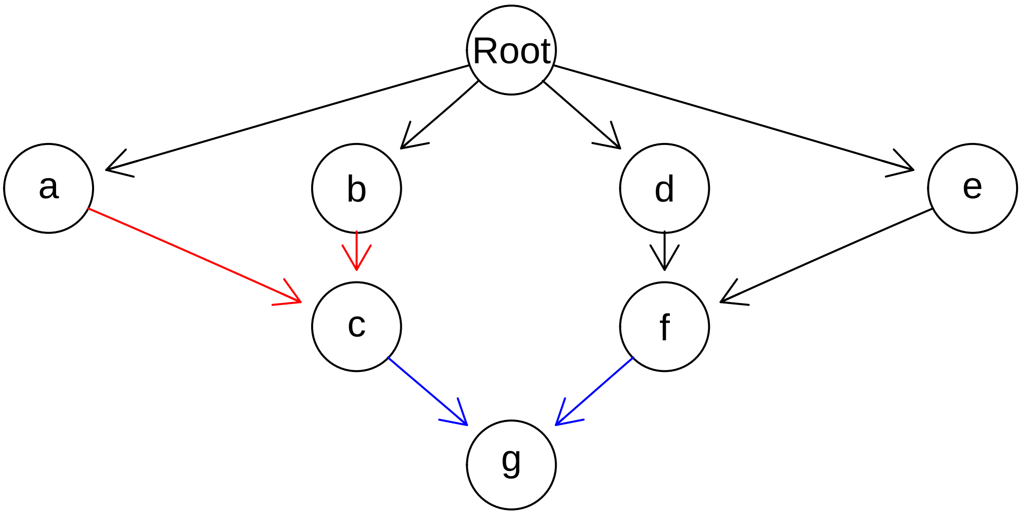
is the result of a 0.1 for “c” (and a 1.2 for “g” that has exactly one of its dependencies satisfied).

2.4.8 Posets: the three types of relationships

```

p3 <- data.frame(
    parent = c(rep("Root", 4), "a", "b", "d", "e", "c", "f"),
    child = c("a", "b", "d", "e", "c", "c", "f", "f", "g", "g"),
    s = c(0.01, 0.02, 0.03, 0.04, 0.1, 0.1, 0.2, 0.2, 0.3, 0.3),
    sh = c(rep(0, 4), c(-.9, -.9), c(-.95, -.95), c(-.99, -.99)),
    typeDep = c(rep("--", 4),
                "XMPN", "XMPN", "MN", "MN", "SM", "SM"))
fp3 <- allFitnessEffects(p3)
```

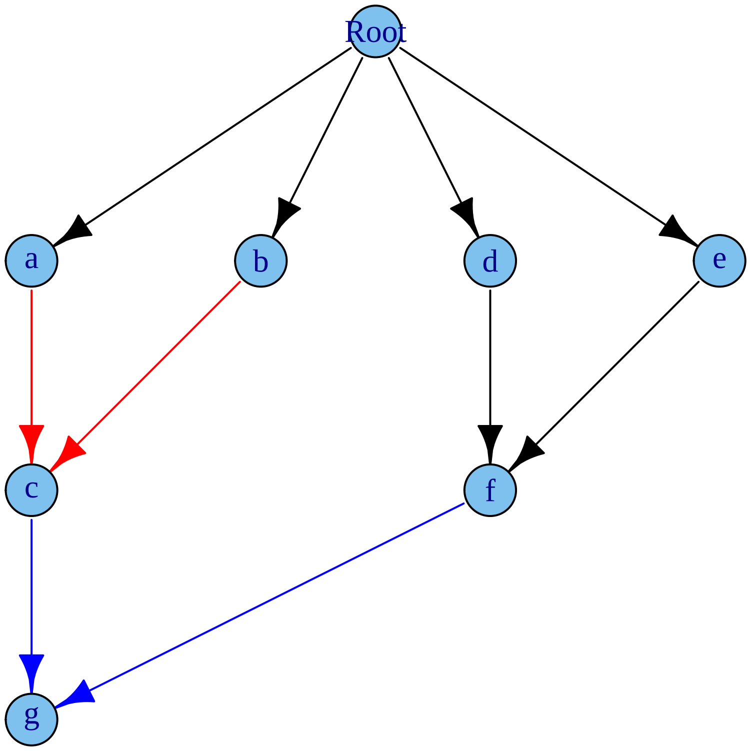
This is how it looks like:

```
plot(fp3)
```



We can also use “igraph”:

```
plot(fp3, "igraph", layout.reingold.tilford)
```



```
gfp3 <- evalAllGenotypes(fp3, order = FALSE)
```

Let's look at a few:

```
gfp3[c(9, 24, 29, 59, 60, 66, 119, 120, 126, 127), ]
##                 Genotype  Fitness
## 9                   a, c 1.111000
## 24                  d, f 0.051500
## 29               a, b, c 0.103020
## 59               c, f, g 0.006500
## 60               d, e, f 1.285440
## 66            a, b, c, f 0.005151
## 119        c, d, e, f, g 0.167107
## 120     a, b, c, d, e, f 0.132426
## 126     b, c, d, e, f, g 1.874943
## 127 a, b, c, d, e, f, g 0.172154

c(1.01 * 1.1, 1.03 * .05, 1.01 * 1.02 * 0.1, 0.1 * 0.05 * 1.3,
  1.03 * 1.04 * 1.2, 1.01 * 1.02 * 0.1 * 0.05,
  0.1 * 1.03 * 1.04 * 1.2 * 1.3,
  1.01 * 1.02 * 0.1 * 1.03 * 1.04 * 1.2,
  1.02 * 1.1 * 1.03 * 1.04 * 1.2 * 1.3,
  1.01 * 1.02 * 1.03 * 1.04 * 0.1 * 1.2 * 1.3)
##  [1] 1.111000 0.051500 0.103020 0.006500 1.285440 0.005151 0.167107
##  [8] 0.132426 1.874943 0.172154
```

As before, looking at the order of mutations makes no difference (look at the test directory to see a test that verifies this assertion).

2.5 Modules

As already mentioned, we can think in all the effects of fitness in terms not of individual genes but, rather, modules. This idea is discussed in, for example, Raphael and Vandin (2015), Gerstung et al. (2011): the restrictions encoded in, say, the DAGs can be considered to apply not to genes, but to modules, where each module is a set of genes (and the intersection between modules is the empty set). Modules, then, play the role of a "union operation" over sets of genes. Of course, if we can use modules for the restrictions in the DAGs we should also be able to use them for epistasis and order effects, as we will see later (e.g., 2.6.2).

2.5.1 What does a module provide

Modules can provide very compact ways of specifying relationships when you want to, well, model the existence of modules. For simplicity suppose there is a module, "A", made of genes "a1" and "a2", and a module "B", made of a single gene "b1". Module "B" can mutate if module "A" is mutated, but mutating both "a1" and "a2" provides no additional fitness advantage compared to mutating only a single one of them. We can specify this as:

```
s <- 0.2
sboth <- (1/(1 + s)) - 1
m0 <- allFitnessEffects(data.frame(
    parent = c("Root", "Root", "a1", "a2"),
    child = c("a1", "a2", "b", "b"),
    s = s,
    sh = -1,
    typeDep = "OR"),
                            epistasis = c("a1:a2" = sboth))
evalAllGenotypes(m0, order = FALSE, addwt = TRUE)
##    Genotype Fitness
## 1        WT    1.00
## 2        a1    1.20
## 3        a2    1.20
## 4         b    0.00
## 5    a1, a2    1.20
## 6     a1, b    1.44
## 7     a2, b    1.44
## 8 a1, a2, b    1.44
```

Note that we need to add an epistasis term, with value "sboth" to capture the idea of "mutating both"a1" and "a2" provides no additional fitness advantage compared to mutating only a single one of them"; see details in section 2.9.

Now, specify it using modules:

```
s <- 0.2
m1 <- allFitnessEffects(data.frame(
    parent = c("Root", "A"),
    child = c("A", "B"),
    s = s,
    sh = -1,
    typeDep = "OR"),
                        geneToModule = c("Root" = "Root",
                                         "A" = "a1, a2",
                                         "B" = "b1"))
evalAllGenotypes(m1, order = FALSE, addwt = TRUE)
##     Genotype Fitness
## 1         WT    1.00
## 2         a1    1.20
## 3         a2    1.20
## 4         b1    0.00
## 5     a1, a2    1.20
## 6     a1, b1    1.44
## 7     a2, b1    1.44
## 8 a1, a2, b1    1.44
```

This captures the ideas directly. The typing savings here are small, but they can be large with modules with many genes.

2.5.2 Specifying modules

How do you specify modules? The general procedure is simple: you pass a vector that makes explicit the mapping from modules to sets of genes. We just saw an example. There are several additional examples such as 2.5.3, 2.6.2, 2.9.4.

It is important to note that, once you specify modules, we expect all of the relationships (except those that involve the non interacting genes) to be specified as modules. Thus, all elements of the epistasis, posets (the DAGs) and order effects components should be specified in terms of modules. But you can, of course, specify a module as containing a single gene (and a single gene with the same name as the module).

What about the "Root" node? If you use a "restriction table", that restriction table (that DAG) must have a node named "Root" and in the mapping of genes to module there **must** be a first entry that has a module and gene named "Root", as we saw above with `geneToModule  = c("Root" = "Root", ...`. We force you to do this to be explicit about the "Root" node. This is not needed (thought it does not hurt) with other fitness specifications. For instance, if we have a model with two modules, one of them with two genes (see details in section 2.10) we do not need to pass a “Root” as in

```
fnme <- allFitnessEffects(epistasis = c("A" = 0.1,
                                        "B" = 0.2),
                          geneToModule = c("A" = "a1, a2",
                                           "B" = "b1"))
evalAllGenotypes(fnme, order = FALSE, addwt = TRUE)
##     Genotype Fitness
## 1         WT    1.00
## 2         a1    1.10
## 3         a2    1.10
## 4         b1    1.20
## 5     a1, a2    1.10
## 6     a1, b1    1.32
## 7     a2, b1    1.32
## 8 a1, a2, b1    1.32
```

but it is also OK to have a “Root” in the `geneToModule`:

```
fnme2 <- allFitnessEffects(epistasis = c("A" = 0.1,
                                         "B" = 0.2),
                           geneToModule = c(
                               "Root" = "Root",
                               "A" = "a1, a2",
                               "B" = "b1"))
evalAllGenotypes(fnme, order = FALSE, addwt = TRUE)
##     Genotype Fitness
## 1         WT    1.00
## 2         a1    1.10
## 3         a2    1.10
## 4         b1    1.20
## 5     a1, a2    1.10
## 6     a1, b1    1.32
## 7     a2, b1    1.32
## 8 a1, a2, b1    1.32
```

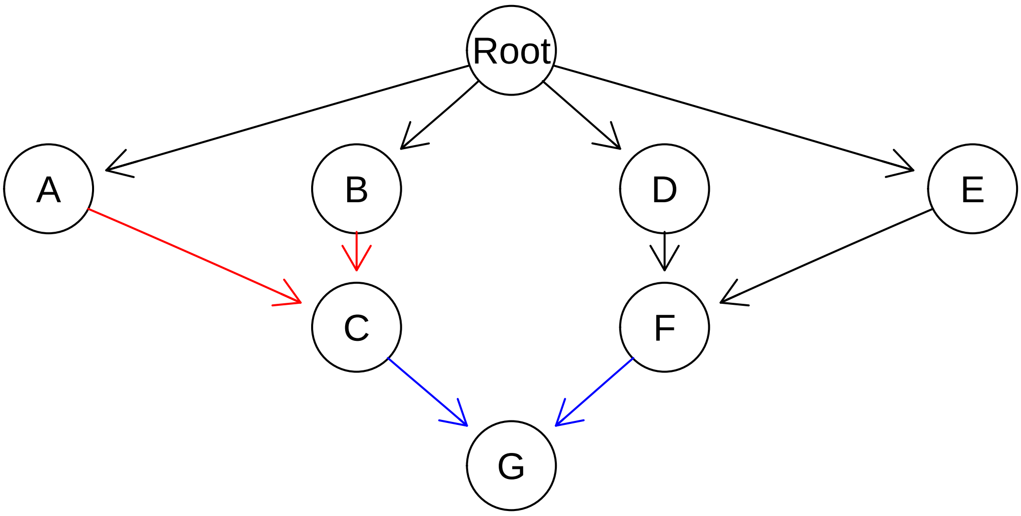
2.5.3 Modules and posets again: the three types of relationships and modules

We use the same specification of poset, but add modules. To keep it manageable, we only add a few genes for some modules, and have some modules with a single gene. Beware that the number of genotypes is starting to grow quite fast, though. We capitalize to differentiate modules (capital letters) from genes (lowercase with a number), but this is not needed.

```
p4 <- data.frame(
    parent = c(rep("Root", 4), "A", "B", "D", "E", "C", "F"),
    child = c("A", "B", "D", "E", "C", "C", "F", "F", "G", "G"),
    s = c(0.01, 0.02, 0.03, 0.04, 0.1, 0.1, 0.2, 0.2, 0.3, 0.3),
    sh = c(rep(0, 4), c(-.9, -.9), c(-.95, -.95), c(-.99, -.99)),
    typeDep = c(rep("--", 4),
                "XMPN", "XMPN", "MN", "MN", "SM", "SM"))

fp4m <- allFitnessEffects(
    p4,
    geneToModule = c("Root" = "Root", "A" = "a1",
                     "B" = "b1, b2", "C" = "c1",
                     "D" = "d1, d2", "E" = "e1",
                     "F" = "f1, f2", "G" = "g1"))
```

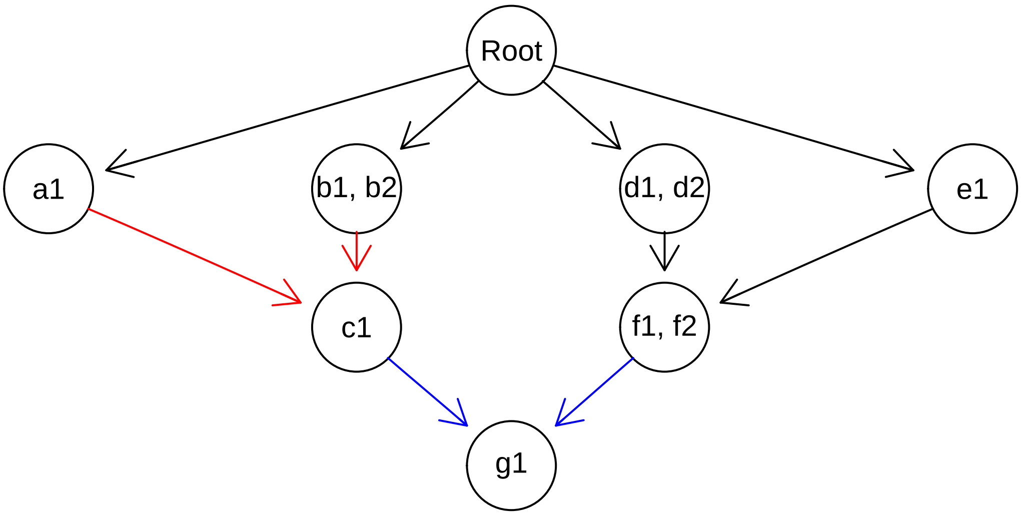
By default, plotting shows the modules:

```
plot(fp4m)
```



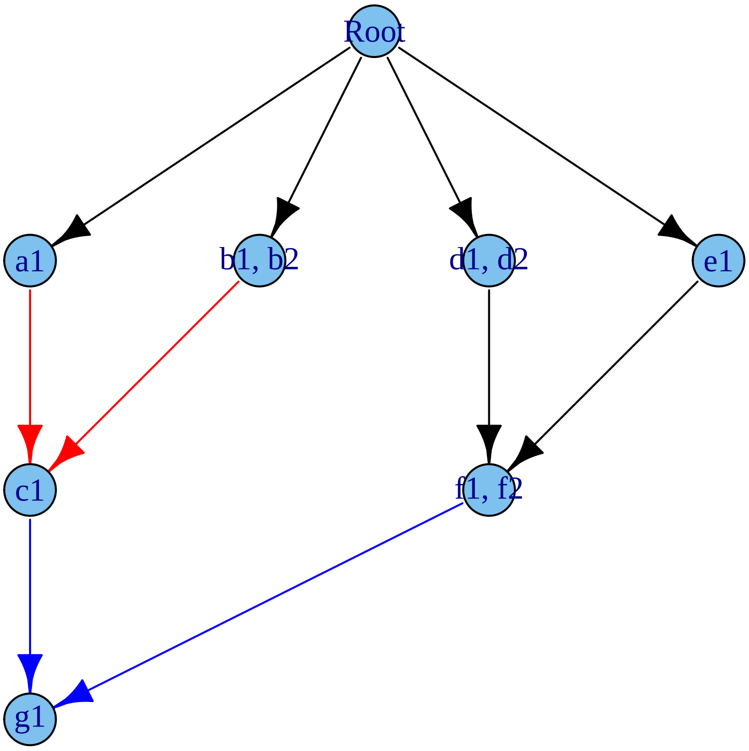
but we can show the gene names instead of the module names:

```
plot(fp4m, expandModules = TRUE)
```



or

```
plot(fp4m, "igraph", layout = layout.reingold.tilford,
     expandModules = TRUE)
```



We obtain the fitness of all genotypes in the usual way:

```
gfp4 <- evalAllGenotypes(fp4m, order = FALSE, max = 1024)
```

Let's look at a few of those:

```
gfp4[c(12, 20, 21, 40, 41, 46, 50, 55, 64, 92,
       155, 157, 163, 372, 632, 828), ]
##                    Genotype Fitness
## 12                  a1, b2  1.0302
## 20                  b1, b2  1.0200
## 21                  b1, c1  1.1220
## 40                  c1, g1  0.1300
## 41                  d1, d2  1.0300
## 46                  d2, e1  1.0712
## 50                  e1, f1  0.0520
## 55                  f2, g1  0.0650
## 64              a1, b2, c1  0.1030
## 92              b1, b2, c1  1.1220
## 155             c1, f2, g1  0.0065
## 157             d1, d2, f1  0.0515
## 163             d1, f1, f2  0.0515
## 372         d1, d2, e1, f2  1.2854
## 632     d1, d2, e1, f1, f2  1.2854
## 828 b2, c1, d1, e1, f2, g1  1.8749

c(1.01 * 1.02, 1.02, 1.02 * 1.1, 0.1 * 1.3, 1.03,
  1.03 * 1.04, 1.04 * 0.05, 0.05 * 1.3,
  1.01 * 1.02 * 0.1, 1.02 * 1.1, 0.1 * 0.05 * 1.3,
  1.03 * 0.05, 1.03 * 0.05, 1.03 * 1.04 * 1.2, 1.03 * 1.04 * 1.2,
  1.02 * 1.1 * 1.03 * 1.04 * 1.2 * 1.3)
##  [1] 1.0302 1.0200 1.1220 0.1300 1.0300 1.0712 0.0520 0.0650 0.1030
## [10] 1.1220 0.0065 0.0515 0.0515 1.2854 1.2854 1.8749
```

2.6 Order effects

As explained in the introduction (1), by order effects we mean a phenomenon such as the one shown empirically by Ortmann et al. (2015): the fitness of a double mutant "A", "B" is different depending on whether "A" was acquired before "B" or "B" before "A". This, of course, can be generalized to more than two genes.

Note that these order effects are different from the order restrictions discussed in section 2.4. In there we might say that acquiring "B" depends or is facilitated by having "A" mutated (and, unless we allowed for multiple mutations, having "A" mutated means having "A" mutated before "B"). However, once you have the genotype "A, B", its fitness does not depend on the order in which "A" and "B" appeared.

2.6.1 Order effects: three-gene orders

Consider this case, where three specific three-gene orders and two two-gene orders (one of them a subset of one of the three) lead to different fitness compared to the wild-type. We add also modules, to show its usage (but just limit ourselves to using one gene per module here).

Order effects are specified using a $x > y$, that means that that order effect is satisfied when module x is mutated before module y.

```
o3 <- allFitnessEffects(orderEffects = c(
                            "F > D > M" = -0.3,
                            "D > F > M" = 0.4,
                            "D > M > F" = 0.2,
                            "D > M"     = 0.1,
                            "M > D"     = 0.5),
                        geneToModule =
                            c("M" = "m",
                              "F" = "f",
                              "D" = "d") )


(ag <- evalAllGenotypes(o3, addwt = TRUE, order = TRUE))
##      Genotype Fitness
## 1          WT    1.00
## 2           d    1.00
## 3           f    1.00
## 4           m    1.00
## 5       d > f    1.00
## 6       d > m    1.10
## 7       f > d    1.00
## 8       f > m    1.00
## 9       m > d    1.50
## 10      m > f    1.00
## 11 d > f > m    1.54
## 12 d > m > f    1.32
## 13 f > d > m    0.77
## 14 f > m > d    1.50
## 15 m > d > f    1.50
## 16 m > f > d    1.50
```

(The meaning of the notation in the output table is as follows: "WT" denotes the wild-type, or non-mutated clone. The notation $x > y$ means that a mutation in "x" happened before a mutation in "y". A genotype $x > y$ _ z means that a mutation in "x" happened before a mutation in "y"; there is also a mutation in "z", but that is a gene for which order does not matter).

The values for the first nine genotypes come directly from the fitness specifications. The 10th genotype matches $D > F > M$ $(= (1 + 0.4))$ but also $D > M$ $((1 + 0.1))$. The 11th matches $D > M > F$ and $D > M$. The 12th matches $F > D > M$ but also $D > M$. Etc.

2.6.2 Order effects and modules with multiple genes

Consider the following case:

```
ofe1 <- allFitnessEffects(
    orderEffects = c("F > D" = -0.3, "D > F" = 0.4),
    geneToModule =
        c("F" = "f1, f2",
          "D" = "d1, d2") )

ag <- evalAllGenotypes(ofe1, order = TRUE)
```

There are four genes, $d1, d2, f1, f2$, where each d belongs to module D and each f belongs to module F.

What to expect for cases such as $d1 > f1$ or $f1 > d1$ is clear, as shown in

```
ag[5:16,]
##    Genotype Fitness
## 5   d1 > d2     1.0
## 6   d1 > f1     1.4
## 7   d1 > f2     1.4
## 8   d2 > d1     1.0
## 9   d2 > f1     1.4
## 10  d2 > f2     1.4
## 11  f1 > d1     0.7
## 12  f1 > d2     0.7
## 13  f1 > f2     1.0
## 14  f2 > d1     0.7
## 15  f2 > d2     0.7
## 16  f2 > f1     1.0
```

Likewise, cases such as $d1 > d2 > f1$ or $f2 > f1 > d1$ are clear, because in terms of modules they map to \$ D > F\$ or $F > D$: the observed order of mutation $d1 > d2 > f1$ means that module D was mutated first and module F was mutated second. Similar for $d1 > f1 > f2$ or $f1 > d1 > d2$: those map to $D > F$ and $F > D$. We can see the fitness of those four case in:

```
ag[c(17, 39, 19, 29), ]
##         Genotype Fitness
## 17 d1 > d2 > f1     1.4
## 39 f2 > f1 > d1     0.7
## 19 d1 > f1 > d2     1.4
## 29 f1 > d1 > d2     0.7
```

and they correspond to the values of those order effects, where $F > D = (1 - 0.3)$ and $D > F = (1 + 0.4)$:

```
ag[c(17, 39, 19, 29), "Fitness"] == c(1.4, 0.7, 1.4, 0.7)
## [1] TRUE TRUE TRUE TRUE
```

What if we match several patterns? For example, $d1 > f1 > d2 > f2$ and $d1 > f1 > f2 > d2$? The first maps to $D > F > D > F$ and the second to $D > F > D$. But since we are concerned with which one happened first and which happened second we should expect those two to correspond to the same fitness, that of pattern $D > F$, as is the case:

```
ag[c(43, 44),]
##               Genotype Fitness
## 43 d1 > f1 > d2 > f2     1.4
## 44 d1 > f1 > f2 > d2     1.4
ag[c(43, 44), "Fitness"] == c(1.4, 1.4)
## [1] TRUE TRUE
```

More generally, that applies to all the patterns that start with one of the “d” genes:

```
all(ag[41:52, "Fitness"] == 1.4)
## [1] TRUE
```

Similar arguments apply to the opposite pattern, $F > D$, which apply to all the possible gene mutation orders that start with one of the “f” genes. For example:

```
all(ag[53:64, "Fitness"] == 0.7)
## [1] TRUE
```

2.6.3 Order and modules with 325 genotypes

We can of course have more than two genes per module. This just repeats the above, with five genes (there are 325 genotypes, and that is why we pass the “max” argument to ***evalAllGenotypes***, to allow for more than the default 256).

```
ofe2 <- allFitnessEffects(
    orderEffects = c("F > D" = -0.3, "D > F" = 0.4),
    geneToModule =
        c("F" = "f1, f2, f3",
          "D" = "d1, d2") )
ag2 <- evalAllGenotypes(ofe2, max = 325, order = TRUE)
```

We can verify that any combination that starts with a “d” gene and then contains at least one “f” gene will have a fitness of $1 + 0.4$. And any combination that starts with an “f” gene and contains at least one “d” genes will have a fitness of $1 - 0.3$. All other genotypes have a fitness of 1:

```
all(ag2[grep("^d.*f.*", ag2[, 1]), "Fitness"] == 1.4)
## [1] TRUE
all(ag2[grep("^f.*d.*", ag2[, 1]), "Fitness"] == 0.7)
```

```
## [1] TRUE
oe <- c(grep("^f.*d.*", ag2[, 1]), grep("^d.*f.*", ag2[, 1]))
all(ag2[-oe, "Fitness"] == 1)
## [1] TRUE
```

2.6.4 Order effects and genes without interactions

We will now look at both order effects and interactions. To make things more interesting, we name genes so that the ordered names do split nicely between those with and those without order effects (this, thus, also serves as a test of messy orders of names).

```
foi1 <- allFitnessEffects(
    orderEffects = c("D>B" = -0.2, "B > D" = 0.3),
    noIntGenes = c("A" = 0.05, "C" = -.2, "E" = .1))
```

You can get a verbose view of what the gene names and modules are (and their automatically created numeric codes) by:

```
foi1[c("geneModule", "long.geneNoInt")]
## $geneModule
##   Gene Module GeneNumID ModuleNumID
## 1 Root   Root         0           0
## 2    B      B         1           1
## 3    D      D         2           2
##
## $long.geneNoInt
##   Gene GeneNumID     s
## A    A         3  0.05
## C    C         4 -0.20
## E    E         5  0.10
```

We can get the fitness of all genotypes (we set $max = 325$ because that is the number of possible genotypes):

```
agoi1 <- evalAllGenotypes(foi1,  max = 325, order = TRUE)
head(agoi1)
##   Genotype Fitness
## 1        B    1.00
## 2        D    1.00
## 3        A    1.05
## 4        C    0.80
## 5        E    1.10
## 6    B > D    1.30
```

Now:

```
rn <- 1:nrow(agoi1)
names(rn) <- agoi1[, 1]

agoi1[rn[LETTERS[1:5]], "Fitness"] == c(1.05, 1, 0.8, 1, 1.1)
## [1] TRUE TRUE TRUE TRUE TRUE
```

According to the fitness effects we have specified, we also know that any genotype with only two mutations, one of which is either “A”, “C” “E” and the other is “B” or “D” will have the fitness corresponding to “A”, “C” or “E”, respectively:

```
agoi1[grep("^A > [BD]$", names(rn)), "Fitness"] == 1.05
## [1] TRUE TRUE
agoi1[grep("^C > [BD]$", names(rn)), "Fitness"] == 0.8
## [1] TRUE TRUE
agoi1[grep("^E > [BD]$", names(rn)), "Fitness"] == 1.1
## [1] TRUE TRUE
agoi1[grep("^[BD] > A$", names(rn)), "Fitness"] == 1.05
## [1] TRUE TRUE
agoi1[grep("^[BD] > C$", names(rn)), "Fitness"] == 0.8
## [1] TRUE TRUE
agoi1[grep("^[BD] > E$", names(rn)), "Fitness"] == 1.1
## [1] TRUE TRUE
```

We will not be playing many additional games with regular expressions, but let us check those that start with “D” and have all the other mutations, which occupy rows 230 to 253; fitness should be equal (within numerical error, because of floating point arithmetic) to the order effect of “D” before “B” times the other effects $(1 - 0.3) * 1.05 * 0.8 * 1.1 = 0.7392$

```
all.equal(agoi1[230:253, "Fitness"] ,
          rep((1 - 0.2) * 1.05 * 0.8 * 1.1, 24))
## [1] TRUE
```

and that will also be the value of any genotype with the five mutations where “D” comes before “B” such as those in rows 260 to 265, 277, or 322 and 323, but it will be equal to $(1 + 0.3) * 1.05 * 0.8 * 1.1 = 1.2012$ in those where “B” comes before “D”. Analogous arguments apply to four, three, and two mutation genotypes.

2.7 Synthetic viability

Synthetic viability and synthetic lethality (e.g., Ashworth, Lord, and Reis-Filho 2011; Hartman, Garvik, and Hartwell 2001) are just special cases of epistasis (section 2.9) but we deal with them here separately.

2.7.1 A simple synthetic viability example

A simple and extreme example of synthetic viability is shown in the following table, where the joint mutant has fitness larger than the wild type, but each single mutant is lethal.

A	B	Fitness
wt	wt	1
wt	M	0
M	wt	0
M	M	(1 + s)

where "wt" denotes wild type and "M" denotes mutant.

We can specify this (setting $s = 0.2$) as (I play around with spaces, to show there is a certain flexibility with them):

```
s <- 0.2
sv <- allFitnessEffects(epistasis = c("-A : B" = -1,
                                      "A : -B" = -1,
                                      "A:B" = s))
```

Now, let's look at all the genotypes (we use "addwt" to also get the wt, which by decree has fitness of 1), and disregard order:

```
(asv <- evalAllGenotypes(sv, order = FALSE, addwt = TRUE))
##   Genotype Fitness
## 1       WT     1.0
## 2        A     0.0
## 3        B     0.0
## 4     A, B     1.2
```

Asking the program to consider the order of mutations of course makes no difference:

```
evalAllGenotypes(sv, order = TRUE, addwt = TRUE)
##   Genotype Fitness
## 1       WT     1.0
## 2        A     0.0
## 3        B     0.0
## 4    A > B     1.2
## 5    B > A     1.2
```

Another example of synthetic viability is shown in section 4.2.2.

Of course, if multiple simultaneous mutations are not possible in the simulations, it is not possible to go from the wildtype to the double mutant in this model where the single mutants are not viable.

2.7.2 Synthetic viability using Bozic model

If we were to use the above specification with Bozic's models, we might not get what we think we should get:

```
evalAllGenotypes(sv, order = FALSE, addwt = TRUE, model = "Bozic")
##   Genotype Death_rate
## 1       WT        1.0
## 2        A        2.0
## 3        B        2.0
## 4     A, B        0.8
```

What gives here? The simulation code would alert you of this (see section 2.7.2) in this particular case because there are "-1", which might indicate that this is not what you want. The problem is that you probably want the Death rate to be infinity (the birth rate was 0, so no clone viability, when we used birth rates —section 2.2.2).

Let us say so explicitly:

```
s <- 0.2
svB <- allFitnessEffects(epistasis = c("-A : B" = -Inf,
                                       "A : -B" = -Inf,
                                       "A:B" = s))
evalAllGenotypes(svB, order = FALSE, addwt = TRUE, model = "Bozic")
##   Genotype Death_rate
## 1       WT        1.0
## 2        A        Inf
## 3        B        Inf
## 4     A, B        0.8
```

Likewise, values of s larger than one have no effect beyond setting $s = 1$ (a single term of $(1 - 1)$ will drive the product to 0, and as we cannot allow negative death rates negative values are set to 0):

```
s <- 1
svB1 <- allFitnessEffects(epistasis = c("-A : B" = -Inf,
                                        "A : -B" = -Inf,
                                        "A:B" = s))

evalAllGenotypes(svB1, order = FALSE, addwt = TRUE, model = "Bozic")
##   Genotype Death_rate
## 1       WT          1
## 2        A        Inf
## 3        B        Inf
## 4     A, B          0


s <- 3
```

```
svB3 <- allFitnessEffects(epistasis = c("-A : B" = -Inf,
                                       "A : -B" = -Inf,
                                       "A:B" = s))

evalAllGenotypes(svB3, order = FALSE, addwt = TRUE, model = "Bozic")
##   Genotype Death_rate
## 1       WT          1
## 2        A        Inf
## 3        B        Inf
## 4     A, B          0
```

Of course, death rates of 0.0 are likely to lead to trouble down the road, when we actually conduct simulations (see section 5.13).

2.7.3 Synthetic viability, non-zero fitness, and modules

This is a slightly more elaborate case, where there is one module and the single mutants have different fitness between themselves, which is non-zero. Without the modules, this is the same as in Misra et al. Misra, Szczurek, and Vingron (2014), Figure 1b, which we go over in section 4.2.

A	B	Fitness
wt	wt	1
wt	M	$1 + s_b$
M	wt	$1 + s_a$
M	M	$1 + s_{ab}$

where $s_a, s_b < 0$ but $s_{ab} > 0$.

```
sa <- -0.1
sb <- -0.2
sab <- 0.25
sv2 <- allFitnessEffects(epistasis = c("-A : B" = sb,
                                       "A : -B" = sa,
                                       "A:B" = sab),
                                   geneToModule = c(
                                       "A" = "a1, a2",
                                       "B" = "b"))
evalAllGenotypes(sv2, order = FALSE, addwt = TRUE)
##    Genotype Fitness
## 1        WT    1.00
## 2        a1    0.90
## 3        a2    0.90
## 4         b    0.80
## 5    a1, a2    0.90
## 6     a1, b    1.25
## 7     a2, b    1.25
## 8 a1, a2, b    1.25
```

And if we look at order, of course it makes no difference:

```
evalAllGenotypes(sv2, order = TRUE, addwt = TRUE)
##        Genotype Fitness
## 1            WT    1.00
## 2            a1    0.90
## 3            a2    0.90
## 4             b    0.80
## 5       a1 > a2    0.90
## 6        a1 > b    1.25
## 7       a2 > a1    0.90
## 8        a2 > b    1.25
## 9        b > a1    1.25
## 10       b > a2    1.25
## 11 a1 > a2 > b    1.25
## 12 a1 > b > a2    1.25
## 13 a2 > a1 > b    1.25
## 14 a2 > b > a1    1.25
## 15 b > a1 > a2    1.25
## 16 b > a2 > a1    1.25
```

2.8 Synthetic mortality or synthetic lethality

In contrast to section 2.7, here the joint mutant has decreased viability:

A	B	Fitness
wt	wt	1
wt	M	$1 + s_b$
M	wt	$1 + s_a$
M	M	$1 + s_{ab}$

where $s_a, s_b > 0$ but $s_{ab} < 0$.

```
sa <- 0.1
sb <- 0.2
sab <- -0.8
sm1 <- allFitnessEffects(epistasis = c("-A : B" = sb,
                                        "A : -B" = sa,
                                        "A:B" = sab))
evalAllGenotypes(sm1, order = FALSE, addwt = TRUE)
##   Genotype Fitness
## 1       WT     1.0
## 2        A     1.1
## 3        B     1.2
## 4     A, B     0.2
```

And if we look at order, of course it makes no difference:

```
evalAllGenotypes(sm1, order = TRUE, addwt = TRUE)
##   Genotype Fitness
## 1       WT     1.0
## 2        A     1.1
## 3        B     1.2
## 4    A > B     0.2
## 5    B > A     0.2
```

2.9 Epistasis

2.9.1 Epistasis: two alternative specifications

We want the following mapping of genotypes to fitness:

A	B	Fitness
wt	wt	1
wt	M	$1 + s_b$
M	wt	$1 + s_a$
M	M	$1 + s_{ab}$

Suppose that the actual numerical values are $s_a = 0.2, s_b = 0.3, s_{ab} = 0.7$.

We specify the above as follows:

```
sa <- 0.2
sb <- 0.3
sab <- 0.7

e2 <- allFitnessEffects(epistasis =
                            c("A: -B" = sa,
                              "-A:B" = sb,
                              "A : B" = sab))
evalAllGenotypes(e2, order = FALSE, addwt = TRUE)
##   Genotype Fitness
## 1       WT     1.0
## 2        A     1.2
## 3        B     1.3
## 4     A, B     1.7
```

That uses the “-” specification, so we explicitly exclude some patterns: with “A:-B” we say “A when there is no B”.

But we can also use a specification where we do not use the “-”. That requires a different numerical value of the interaction, because now, as we are rewriting the interaction term as genotype “A is mutant, B is mutant” the double mutant will incorporate the effects of “A mutant”, “B mutant" and “both A and B mutants”. We can define a new s_2 that satisfies $(1 + s_{ab}) = (1 + s_a)(1 + s_b)(1 + s_2)$ so $(1 + s_2) = (1 + s_{ab})/((1 + s_a)(1 + s_b))$ and therefore specify as:

```
s2 <- ((1 + sab)/((1 + sa) * (1 + sb))) - 1

e3 <- allFitnessEffects(epistasis =
                            c("A" = sa,
                              "B" = sb,
                              "A : B" = s2))
evalAllGenotypes(e3, order = FALSE, addwt = TRUE)
##   Genotype Fitness
## 1       WT     1.0
## 2        A     1.2
## 3        B     1.3
## 4     A, B     1.7
```

Note that this is the way you would specify effects with FFPopsim Zanini and Neher (2012). Whether this specification or the previous one with “-” is simpler will depend on the model. For synthetic mortality and viability, I think the one using “-” is simpler to map genotype tables to fitness effects. See also section 2.9.2 and 2.9.3 and the example in section 4.4.2.

Finally, note that we can also specify some of these effects by combining the graph and the epistasis, as shown in section 4.2.1 or 4.4.2.

2.9.2 Epistasis with three genes and two alternative specifications

Suppose we have

A	B	C	Fitness
M	wt	wt	$1 + s_a$
wt	M	wt	$1 + s_b$
wt	wt	M	$1 + s_c$
M	M	wt	$1 + s_{ab}$
wt	M	M	$1 + s_{bc}$
M	wt	M	$(1 + s_a)(1 + s_c)$
M	M	M	$1 + s_{abc}$

where missing rows have a fitness of 1 (they have been deleted for conciseness). Note that the mutant for exactly A and C has a fitness that is the product of the individual terms (so there is no epistasis in that case).

```
sa <- 0.1
sb <- 0.15
sc <- 0.2
sab <- 0.3
sbc <- -0.25
sabc <- 0.4

sac <- (1 + sa) * (1 + sc) - 1

E3A <- allFitnessEffects(epistasis =
                             c("A:-B:-C" = sa,
                               "-A:B:-C" = sb,
                               "-A:-B:C" = sc,
                               "A:B:-C" = sab,
                               "-A:B:C" = sbc,
                               "A:-B:C" = sac,
                               "A : B : C" = sabc)
                                             )

evalAllGenotypes(E3A, order = FALSE, addwt = FALSE)
##   Genotype Fitness
## 1        A    1.10
## 2        B    1.15
## 3        C    1.20
## 4     A, B    1.30
## 5     A, C    1.32
## 6     B, C    0.75
## 7  A, B, C    1.40
```

We needed to pass the s_{ac} coefficient explicitly, even if it that term was just the product. We can try to avoid using the “-”, however (but we will need to do other calculations). For simplicity, I use capital “S" in what follows where the letters differ from the previous specification:

```
sa <- 0.1
sb <- 0.15
sc <- 0.2
sab <- 0.3
Sab <- ( (1 + sab)/((1 + sa) * (1 + sb))) - 1
Sbc <- ( (1 + sbc)/((1 + sb) * (1 + sc))) - 1
Sabc <- ( (1 + sabc)/
          ( (1 + sa) * (1 + sb) * (1 + sc) *
            (1 + Sab) * (1 + Sbc) ) ) - 1
```

```
E3B <- allFitnessEffects(epistasis =
                             c("A" = sa,
                               "B" = sb,
                               "C" = sc,
                               "A:B" = Sab,
                               "B:C" = Sbc,
                               ## "A:C" = sac, ## not needed now
                               "A : B : C" = Sabc)
                                                )
evalAllGenotypes(E3B, order = FALSE, addwt = FALSE)
##   Genotype Fitness
## 1        A    1.10
## 2        B    1.15
## 3        C    1.20
## 4     A, B    1.30
## 5     A, C    1.32
## 6     B, C    0.75
## 7  A, B, C    1.40
```

The above two are, of course, identical:

```
all(evalAllGenotypes(E3A, order = FALSE, addwt = FALSE) ==
    evalAllGenotypes(E3B, order = FALSE, addwt = FALSE))
## [1] TRUE
```

We avoid specifying the “A:C”, as it just follows from the individual “A” and “C” terms, but given a specified genotype table, we need to do a little bit of addition and multiplication to get the coefficients.

2.9.3 Why can we specify some effects with a “-”?

Let’s suppose we want to specify the synthetic viability example seen before:

A	B	Fitness
wt	wt	1
wt	M	0
M	wt	0
M	M	(1 + s)

where “wt” denotes wild type and “M” denotes mutant.

If you want to directly map the above table to the fitness table for the program, to specify the genotype “A is wt, B is a mutant” you can specify it as “-A,B”, not just as “B”. Why? Because just the presence of a “B” is also compatible with genotype “A is mutant and B is mutant”. If you use “-” you are explicitly saying what should not be there so that “-A,B” is NOT compatible with “A, B”. Otherwise, you need to carefully add coefficients. Depending on what you are trying to model, different specifications might be simpler. See the examples in section 2.9.1 and 2.9.2. You have both options.

2.9.4 Epistasis: modules

There is nothing conceptually new, but we will show an example here:

```
sa <- 0.2
sb <- 0.3
sab <- 0.7

em <- allFitnessEffects(epistasis =
                            c("A: -B" = sa,
                              "-A:B" = sb,
                              "A : B" = sab),
                        geneToModule = c("A" = "a1, a2",
                                         "B" = "b1, b2"))
evalAllGenotypes(em, order = FALSE, addwt = TRUE)
##          Genotype Fitness
## 1              WT     1.0
## 2              a1     1.2
## 3              a2     1.2
## 4              b1     1.3
## 5              b2     1.3
## 6          a1, a2     1.2
## 7          a1, b1     1.7
## 8          a1, b2     1.7
## 9          a2, b1     1.7
## 10         a2, b2     1.7
## 11         b1, b2     1.3
## 12     a1, a2, b1     1.7
## 13     a1, a2, b2     1.7
## 14     a1, b1, b2     1.7
## 15     a2, b1, b2     1.7
## 16 a1, a2, b1, b2     1.7
```

Of course, we can do the same thing without using the “-”, as in section 2.9.1:

```
s2 <- ((1 + sab)/((1 + sa) * (1 + sb))) - 1

em2 <- allFitnessEffects(epistasis =
                             c("A" = sa,
                               "B" = sb,
                               "A : B" = s2),
                         geneToModule = c("A" = "a1, a2",
                                         "B" = "b1, b2")
                         )
evalAllGenotypes(em2, order = FALSE, addwt = TRUE)
##          Genotype Fitness
## 1              WT     1.0
## 2              a1     1.2
## 3              a2     1.2
## 4              b1     1.3
## 5              b2     1.3
## 6          a1, a2     1.2
## 7          a1, b1     1.7
## 8          a1, b2     1.7
## 9          a2, b1     1.7
## 10         a2, b2     1.7
## 11         b1, b2     1.3
## 12     a1, a2, b1     1.7
## 13     a1, a2, b2     1.7
## 14     a1, b1, b2     1.7
## 15     a2, b1, b2     1.7
## 16 a1, a2, b1, b2     1.7
```

2.10 I do not want epistasis, but I want modules!

Sometimes you might want something like having several modules, say "A" and "B", each with a number of genes, but with "A" and "B" showing no interaction.

It is a terminological issue whether we should allow `noIntGenes` (no interaction genes), as explained in section 2.3 to actually be modules. The reasoning for not allowing them is that the situation depicted above (several genes in module A, for example) actually is one of interaction: the members of "A" are combined using an "OR" operator (i.e., the fitness consequences of having one or more genes of A mutated are the same), not just simply multiplying their fitness; similarly for "B“. This is why no interaction genes also mean no modules allowed.

So how do you get what you want in this case? Enter the names of the modules in the `epistasis` component but have no term for ":" (the colon). Let's see an example:

```
fnme <- allFitnessEffects(epistasis = c("A" = 0.1,
                                        "B" = 0.2),
                          geneToModule = c("A" = "a1, a2",
                                           "B" = "b1, b2, b3"))

evalAllGenotypes(fnme, order = FALSE, addwt = TRUE)
##              Genotype Fitness
## 1                  WT    1.00
## 2                  a1    1.10
## 3                  a2    1.10
## 4                  b1    1.20
## 5                  b2    1.20
## 6                  b3    1.20
## 7              a1, a2    1.10
## 8              a1, b1    1.32
## 9              a1, b2    1.32
## 10             a1, b3    1.32
## 11             a2, b1    1.32
## 12             a2, b2    1.32
## 13             a2, b3    1.32
## 14             b1, b2    1.20
## 15             b1, b3    1.20
## 16             b2, b3    1.20
## 17         a1, a2, b1    1.32
## 18         a1, a2, b2    1.32
## 19         a1, a2, b3    1.32
## 20         a1, b1, b2    1.32
## 21         a1, b1, b3    1.32
## 22         a1, b2, b3    1.32
## 23         a2, b1, b2    1.32
## 24         a2, b1, b3    1.32
## 25         a2, b2, b3    1.32
## 26         b1, b2, b3    1.20
## 27     a1, a2, b1, b2    1.32
## 28     a1, a2, b1, b3    1.32
## 29     a1, a2, b2, b3    1.32
## 30     a1, b1, b2, b3    1.32
## 31     a2, b1, b2, b3    1.32
## 32 a1, a2, b1, b2, b3    1.32
```

In previous versions these was possible using the longer, still accepted way of specifying a `:` with a value of 0, but this is no longer needed:

```
fnme <- allFitnessEffects(epistasis = c("A" = 0.1,
                                        "B" = 0.2,
                                        "A : B" = 0.0),
                          geneToModule = c("A" = "a1, a2",
                                           "B" = "b1, b2, b3"))

evalAllGenotypes(fnme, order = FALSE, addwt = TRUE)
##              Genotype Fitness
## 1                  WT    1.00
## 2                  a1    1.10
## 3                  a2    1.10
## 4                    b1    1.20
## 5                    b2    1.20
## 6                    b3    1.20
## 7                a1, a2    1.10
## 8                a1, b1    1.32
## 9                a1, b2    1.32
## 10               a1, b3    1.32
## 11               a2, b1    1.32
## 12               a2, b2    1.32
## 13               a2, b3    1.32
## 14               b1, b2    1.20
## 15               b1, b3    1.20
## 16               b2, b3    1.20
## 17           a1, a2, b1    1.32
## 18           a1, a2, b2    1.32
## 19           a1, a2, b3    1.32
## 20           a1, b1, b2    1.32
## 21           a1, b1, b3    1.32
## 22           a1, b2, b3    1.32
## 23           a2, b1, b2    1.32
## 24           a2, b1, b3    1.32
## 25           a2, b2, b3    1.32
## 26           b1, b2, b3    1.20
## 27       a1, a2, b1, b2    1.32
## 28       a1, a2, b1, b3    1.32
## 29       a1, a2, b2, b3    1.32
## 30       a1, b1, b2, b3    1.32
## 31       a2, b1, b2, b3    1.32
## 32 a1, a2, b1, b2, b3    1.32
```

This can, of course, be extended to more modules.

2.11 A longer example: Poset, epistasis, synthetic mortality and viability, order effects and genes without interactions, with some modules

We will now put together a complex example. We will use the poset from section 2.5.3 but will also add:

- Order effects that involve genes in the poset. In this case, if C happens before F, fitness decreases by $1 - 0.1$. If it happens the other way around, there is no effect on fitness beyond their individual contributions.
- Order effects that involve two new modules, “H” and “I” (with genes “h1, h2” and “i1”, respectively), so that if H happens before I fitness increases by $1 + 0.12$.

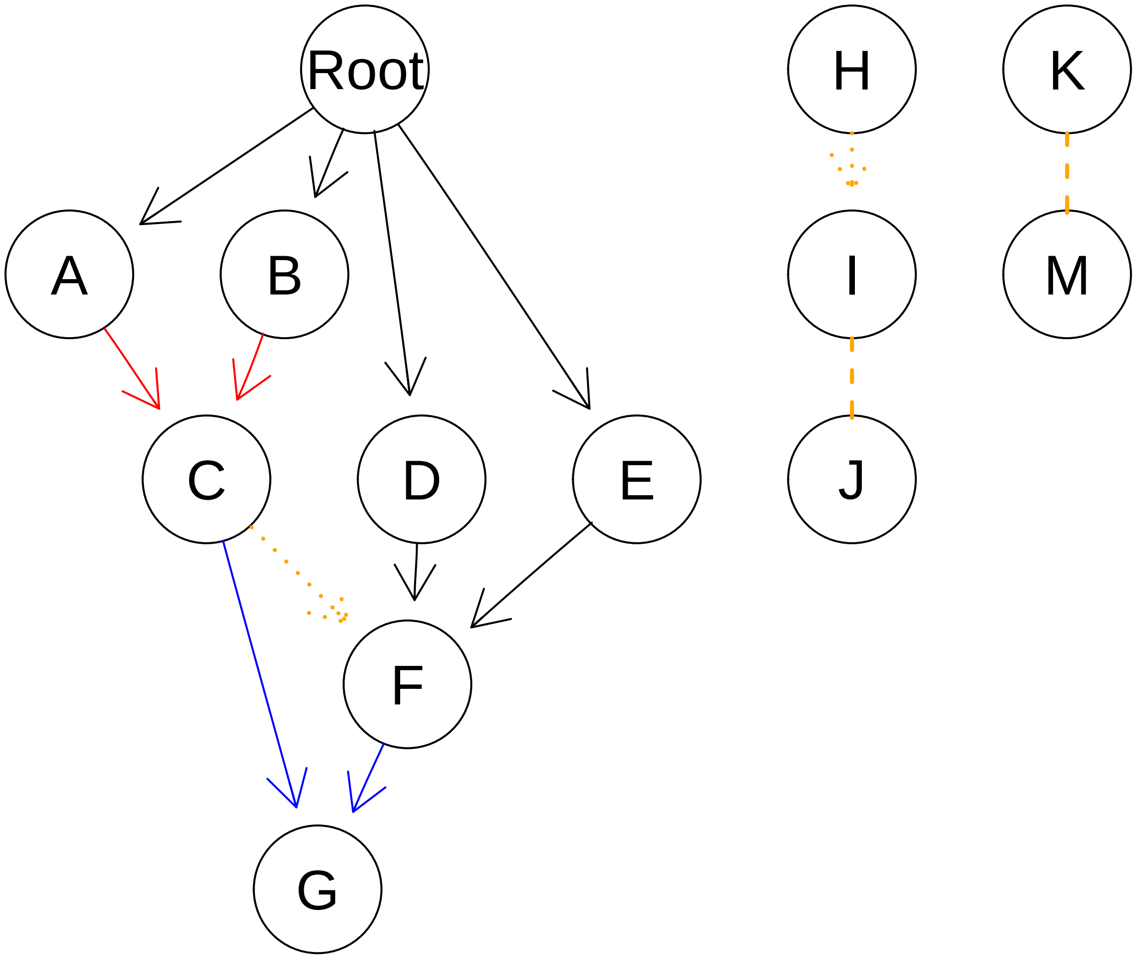
- Synthetic mortality between modules “I” (already present in the epistatic interaction) and “J” (with genes “j1” and “j2”): the joint presence of these modules leads to cell death (fitness of 0).
- Synthetic viability between modules “K” and “M” (with genes “k1”, “k2” and “m1”, respectively), so that their joint presence is viable but adds nothing to fitness (i.e., mutation of both has fitness 1), whereas each single mutant has a fitness of $1 - 0.5$.
- A set of 5 driver genes ($n1, \ldots, n5$) with fitness that comes from an exponential distribution with rate of 10.

As we are specifying many different things, we will start by writing each set of effects separately:

```
p4 <- data.frame(
    parent = c(rep("Root", 4), "A", "B", "D", "E", "C", "F"),
    child = c("A", "B", "D", "E", "C", "C", "F", "F", "G", "G"),
    s = c(0.01, 0.02, 0.03, 0.04, 0.1, 0.1, 0.2, 0.2, 0.3, 0.3),
    sh = c(rep(0, 4), c(-.9, -.9), c(-.95, -.95), c(-.99, -.99)),
    typeDep = c(rep("--", 4),
                "XMPN", "XMPN", "MN", "MN", "SM", "SM"))

oe <- c("C > F" = -0.1, "H > I" = 0.12)
sm <- c("I:J"  = -1)
sv <- c("-K:M" = -.5, "K:-M" = -.5)
epist <- c(sm, sv)

modules <- c("Root" = "Root", "A" = "a1",
             "B" = "b1, b2", "C" = "c1",
             "D" = "d1, d2", "E" = "e1",
             "F" = "f1, f2", "G" = "g1",
             "H" = "h1, h2", "I" = "i1",
             "J" = "j1, j2", "K" = "k1, k2", "M" = "m1")

set.seed(1) ## for repeatability
noint <- rexp(5, 10)
names(noint) <- paste0("n", 1:5)

fea <- allFitnessEffects(rT = p4, epistasis = epist,
                         orderEffects = oe,
                         noIntGenes = noint,
                         geneToModule = modules)
```

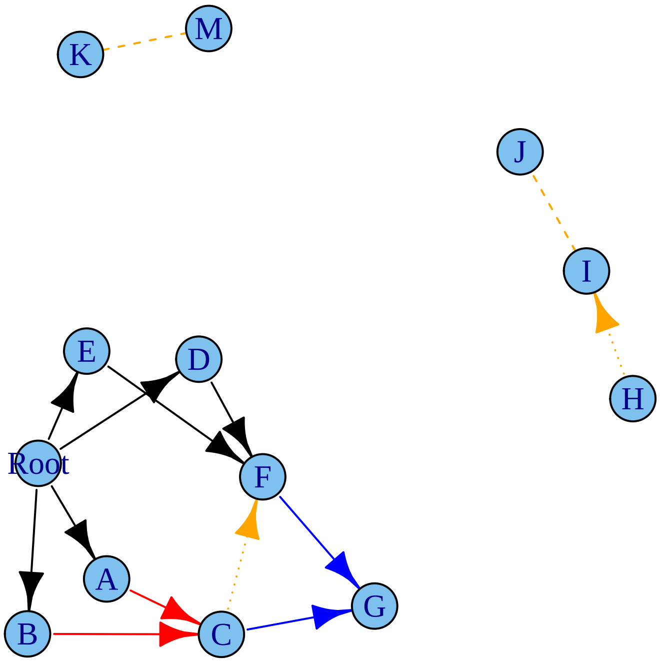
How does it look?

```
plot(fea)
```



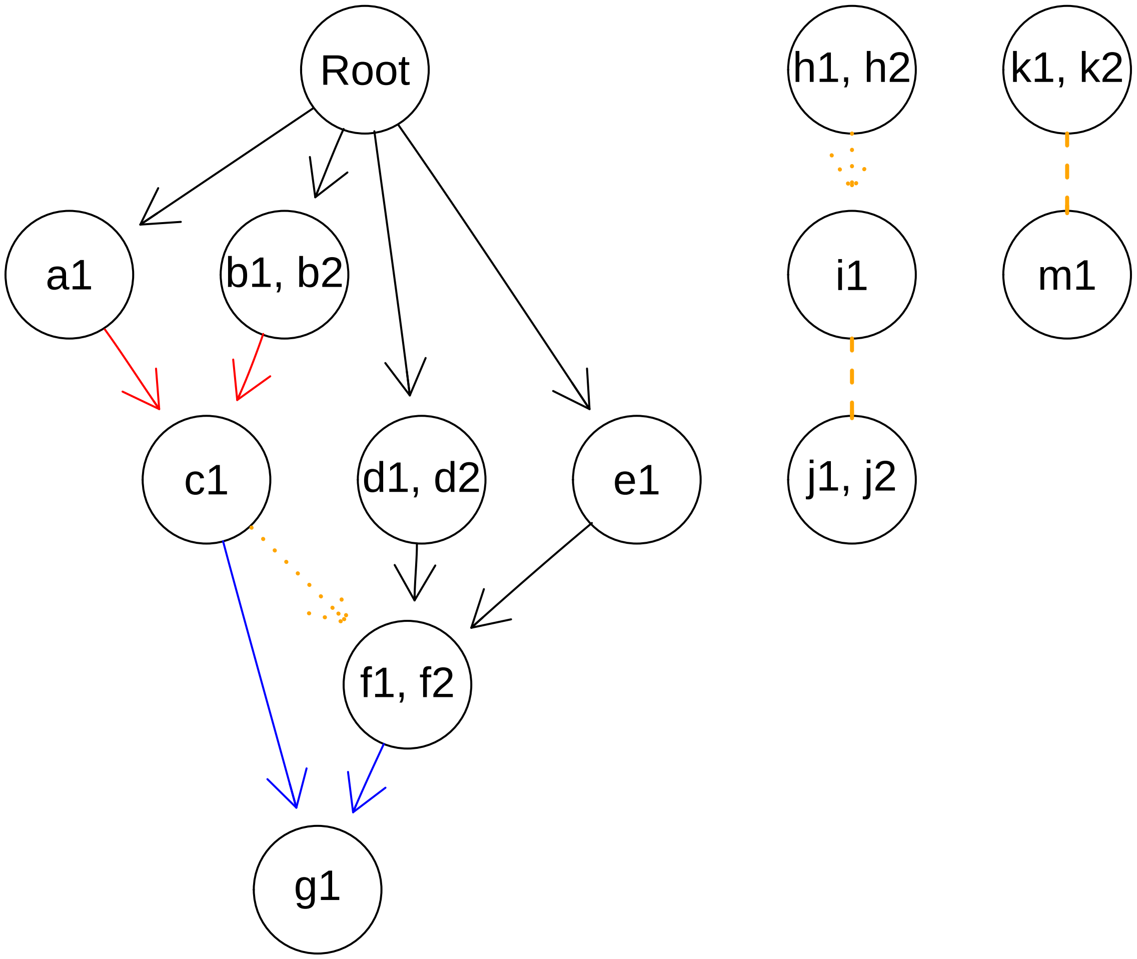
or

```r
plot(fea, "igraph")
```



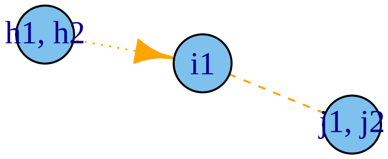
We can, if we want, expand the modules using a "graphNEL" graph

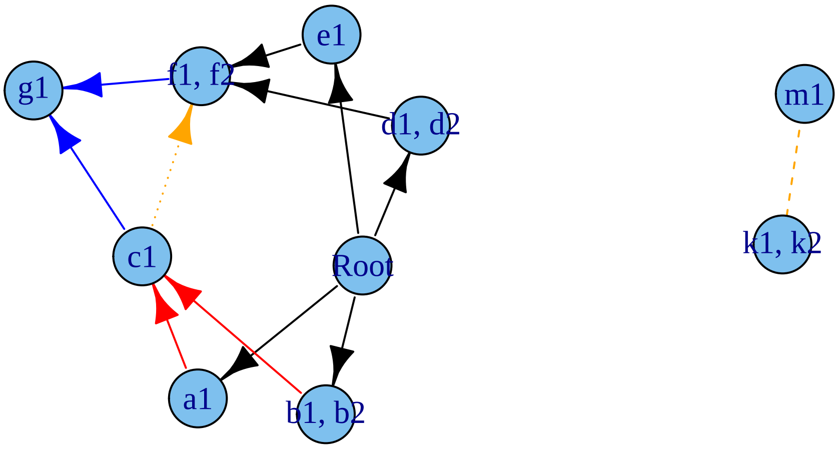
```
plot(fea, expandModules = TRUE)
```



or an "igraph" one

```
plot(fea, "igraph", expandModules = TRUE)
```





We will not evaluate the fitness of all genotypes, since the number of all ordered genotypes is $> 7 * 10^{22}$. We will look at some specific genotypes:

```
evalGenotype("k1 > i1 > h2", fea) ## 0.5
## [1] 0.5
evalGenotype("k1 > h1 > i1", fea) ## 0.5 * 1.12
## [1] 0.56

evalGenotype("k2 > m1 > h1 > i1", fea) ## 1.12
## [1] 1.12

evalGenotype("k2 > m1 > h1 > i1 > c1 > n3 > f2", fea)
## [1] 0.005113
## 1.12 * 0.1 * (1 + noint[3]) * 0.05 * 0.9
```

Finally, let's generate some ordered genotypes randomly:

```
randomGenotype <- function(fe, ns = NULL) {
    gn <- setdiff(c(fe$geneModule$Gene,
                    fe$long.geneNoInt$Gene), "Root")
    if(is.null(ns)) ns <- sample(length(gn), 1)
    return(paste(sample(gn, ns), collapse = " > "))
}
```

```
set.seed(2) ## for reproducibility

evalGenotype(randomGenotype(fea), fea, echo = TRUE, verbose = TRUE)
## Genotype:  k2 > i1 > c1 > n1 > m1
##  Individual s terms are : 0.0755182 -0.9
##  Fitness:  0.1076
## [1] 0.1076
## Genotype:  k2 > i1 > c1 > n1 > m1
##  Individual s terms are : 0.0755182 -0.9
##  Fitness:  0.107552
evalGenotype(randomGenotype(fea), fea, echo = TRUE, verbose = TRUE)
## Genotype:  n2 > h1 > h2
##  Individual s terms are : 0.118164
##  Fitness:  1.118
## [1] 1.118
## Genotype:  n2 > h1 > h2
##  Individual s terms are : 0.118164
##  Fitness:  1.11816
evalGenotype(randomGenotype(fea), fea, echo = TRUE, verbose = TRUE)
## Genotype:  d2 > k2 > c1 > f2 > n4 > m1 > n3 > f1 > b1 > g1 > n5 > h1 > j2
##  Individual s terms are : 0.0145707 0.0139795 0.0436069 0.02 0.1 0.03 -0.95 0.3
##  Fitness:  0.07258
## [1] 0.07258
## Genotype:  d2 > k2 > c1 > f2 > n4 > m1 > n3 > f1 > b1 > g1 > n5 > h1 > j2
##  Individual s terms are : 0.0145707 0.0139795 0.0436069 0.02 0.1 0.03 -0.95 0.3
##  Fitness:  0.0725829
evalGenotype(randomGenotype(fea), fea, echo = TRUE, verbose = TRUE)
## Genotype:  h2 > c1 > f1 > n2 > b2 > a1 > n1 > i1
##  Individual s terms are : 0.0755182 0.118164 0.01 0.02 -0.9 -0.95 -0.1 0.12
##  Fitness:  0.006244
## [1] 0.006244
## Genotype:  h2 > c1 > f1 > n2 > b2 > a1 > n1 > i1
##  Individual s terms are : 0.0755182 0.118164 0.01 0.02 -0.9 -0.95 -0.1 0.12
##  Fitness:  0.00624418
evalGenotype(randomGenotype(fea), fea, echo = TRUE, verbose = TRUE)
## Genotype:  h2 > j1 > m1 > d2 > i1 > b2 > k2 > d1 > b1 > n3 > n1 > g1 > h1 > c1 >
##  Individual s terms are : 0.0755182 0.0145707 0.0436069 0.01 0.02 -0.9 0.03 0.04
##  Fitness:  0
## [1] 0
## Genotype:  h2 > j1 > m1 > d2 > i1 > b2 > k2 > d1 > b1 > n3 > n1 > g1 > h1 > c1 >
##  Individual s terms are : 0.0755182 0.0145707 0.0436069 0.01 0.02 -0.9 0.03 0.04
##  Fitness:  0
evalGenotype(randomGenotype(fea), fea, echo = TRUE, verbose = TRUE)
## Genotype:  n1 > m1 > n3 > i1 > j1 > n5 > k1
##  Individual s terms are : 0.0755182 0.0145707 0.0436069 -1
##  Fitness:  0
## [1] 0
## Genotype:  n1 > m1 > n3 > i1 > j1 > n5 > k1
##  Individual s terms are : 0.0755182 0.0145707 0.0436069 -1
##  Fitness:  0
evalGenotype(randomGenotype(fea), fea, echo = TRUE, verbose = TRUE)
## Genotype:  d2 > n1 > g1 > f1 > f2 > c1 > b1 > d1 > k1 > a1 > b2 > i1 > n4 > h2 >
##  Individual s terms are : 0.0755182 0.118164 0.0139795 0.01 0.02 -0.9 0.03 -0.95
##  Fitness:  0.004205
## [1] 0.004205
## Genotype:  d2 > n1 > g1 > f1 > f2 > c1 > b1 > d1 > k1 > a1 > b2 > i1 > n4 > h2 >
##  Individual s terms are : 0.0755182 0.118164 0.0139795 0.01 0.02 -0.9 0.03 -0.95
##  Fitness:  0.00420528
evalGenotype(randomGenotype(fea), fea, echo = TRUE, verbose = TRUE)
## Genotype:  j1 > f1 > j2 > a1 > n4 > c1 > n3 > k1 > d1 > h1
##  Individual s terms are : 0.0145707 0.0139795 0.01 0.1 0.03 -0.95 -0.5
##  Fitness:  0.02943
## [1] 0.02943
## Genotype:  j1 > f1 > j2 > a1 > n4 > c1 > n3 > k1 > d1 > h1
##  Individual s terms are : 0.0145707 0.0139795 0.01 0.1 0.03 -0.95 -0.5
##  Fitness:  0.0294308
evalGenotype(randomGenotype(fea), fea, echo = TRUE, verbose = TRUE)
## Genotype:  n5 > f2 > f1 > h2 > n4 > c1 > n3 > b1
##  Individual s terms are : 0.0145707 0.0139795 0.0436069 0.02 0.1 -0.95
##  Fitness:  0.06023
## [1] 0.06023
## Genotype:  n5 > f2 > f1 > h2 > n4 > c1 > n3 > b1
##  Individual s terms are : 0.0145707 0.0139795 0.0436069 0.02 0.1 -0.95
##  Fitness:  0.0602298
evalGenotype(randomGenotype(fea), fea, echo = TRUE, verbose = TRUE)
## Genotype:  h1 > d1 > f2
##  Individual s terms are : 0.03 -0.95
##  Fitness:  0.0515
## [1] 0.0515
## Genotype:  h1 > d1 > f2
##  Individual s terms are : 0.03 -0.95
##  Fitness:  0.0515
```

2.12 Homozygosity, heterozygosity, oncogenes, tumor suppressors

We are using what is conceptually a single linear chromosome. However, you can use it to model scenarios where the numbers of copies affected matter, by properly duplicating the genes.

Suppose we have a tumor suppressor gene, G, with two copies, one from Mom and one from Dad. We can have a table like:

O_M	O_D	Fitness
wt	wt	1
wt	M	1
M	wt	1
M	M	$(1 + s)$

where $s > 0$, meaning that you need two hits, one in each copy, to trigger the clonal expansion.

What about oncogenes? A simple model is that one single hit leads to clonal expansion and additional hits lead to no additional changes, as in this table for gene O, where again the M or D subscript denotes the copy from Mom or from Dad:

O_M	O_D	Fitness
wt	wt	1
wt	M	$(1 + s)$
M	wt	$(1 + s)$
M	M	$(1 + s)$

If you have multiple copies you can proceed similarly. As you can see, these are nothing but special cases of synthetic mortality (2.8), synthetic viability (2.7) and epistasis (2.9).

2.13 Gene-specific mutation rates

You can specify gene-specific mutation rates. Instead of passing a scalar value for `mu`, you pass a named vector. (This does not work with the old v. 1 format, though; yet another reason to stop using that format). This is a simple example (many more are available in the tests, see file `./tests/testthat/test.per-gene-mutation-rates.R`).

```
muvar2 <- c("U" = 1e-6, "z" = 5e-5, "e" = 5e-4, "m" = 5e-3,
            "D" = 1e-4)
ni1 <- rep(0, 5)
names(ni1) <- names(muvar2) ## We use the same names, of course
fe1 <- allFitnessEffects(noIntGenes = ni1)
bb <- oncoSimulIndiv(fe1,
                     mu = muvar2, onlyCancer = FALSE,
                     initSize = 1e5,
                     finalTime = 25,
                     seed =NULL)
```

2.14 Mutator genes

You can specify mutator/antimutator genes (e.g. Gerrish et al. 2007; Tomlinson, Novelli, and Bodmer 1996). These are genes that, when mutated, lead to an increase/decrease in the mutation rate all over the genome (similar to what happens with, say, mutations in mismatch-repair genes or microsatellite instability in cancer).

The specification is very similar to that for fitness effects, except we do not (at least for now) the use of DAGs for poset-like structures nor allow for order effects (we have seen no reference in the literature to suggest any of these would be relevant). You can, however, specify epistasis and use modules. Note that the mutator genes must be a subset of the genes in the fitness effects; if you want to have mutator genes that have no direct fitness effects, give them a fitness effect of 0.

This first is a very simple example with simple fitness effects and modules for mutators. We will specify the fitness and mutator effects and evaluate the fitness and mutator effects:

```
fe2 <- allFitnessEffects(noIntGenes =
                         c(a1 = 0.1, a2 = 0.2,
                           b1 = 0.01, b2 = 0.3, b3 = 0.2,
                           c1 = 0.3, c2 = -0.2))

fm2 <- allMutatorEffects(epistasis = c("A" = 5,
                                       "B" = 10,
                                       "C" = 3),
                         geneToModule = c("A" = "a1, a2",
                                          "B" = "b1, b2, b3",
                                          "C" = "c1, c2"))

## Show the fitness effect of a specific genotype
evalGenotype("a1, c2", fe2, verbose = TRUE)
##
##  Individual s terms are : 0.1 -0.2
## [1] 0.88

## Show the mutator effect of a specific genotype
evalGenotypeMut("a1, c2", fm2, verbose = TRUE)
##
##  Individual mutator product terms are : 5 3
## [1] 15

## Fitness and mutator of a specific genotype
evalGenotypeFitAndMut("a1, c2", fe2, fm2, verbose = TRUE)
##
##  Individual s terms are : 0.1 -0.2
##
##  Individual mutator product terms are : 5 3
## [1]  0.88 15.00
```

You can also use the `evalAll` functions. We do not show the output here to avoid cluttering the vignette:

```
## Show only all the fitness effects
evalAllGenotypes(fe2, order = FALSE)

## Show only all mutator effects
evalAllGenotypesMut(fm2)

## Show all fitness and mutator
evalAllGenotypesFitAndMut(fe2, fm2, order = FALSE)
```

Building upon the above, the next is an example where we have a bunch of no interaction genes that affect fitness, and a small set of genes that affect the mutation rate (but have no fitness effects).

```
set.seed(1) ## for reproducibility
## 17 genes, 7 with no direct fitness effects
ni <- c(rep(0, 7), runif(10, min = -0.01, max = 0.1))
names(ni) <- c("a1", "a2", "b1", "b2", "b3", "c1", "c2",
               paste0("g", 1:10))

fe3 <- allFitnessEffects(noIntGenes = ni)

fm3 <- allMutatorEffects(epistasis = c("A" = 5,
                                       "B" = 10,
                                       "C" = 3,
                                       "A:C" = 70),
                         geneToModule = c("A" = "a1, a2",
                                          "B" = "b1, b2, b3",
                                          "C" = "c1, c2"))
```

Let us check what the effects are of a few genotypes:

```
## These only affect mutation, not fitness
evalGenotypeFitAndMut("a1, a2", fe3, fm3, verbose = TRUE)
##
##  Individual s terms are : 0 0
##
##  Individual mutator product terms are : 5
## [1] 1 5
evalGenotypeFitAndMut("a1, b3", fe3, fm3, verbose = TRUE)
##
##  Individual s terms are : 0 0
##
##  Individual mutator product terms are : 5 10
## [1]  1 50

## These only affect fitness: the mutator multiplier is 1
evalGenotypeFitAndMut("g1", fe3, fm3, verbose = TRUE)
##
##  Individual s terms are : 0.019206
## [1] 1.019 1.000
evalGenotypeFitAndMut("g3, g9", fe3, fm3, verbose = TRUE)
##
##  Individual s terms are : 0.0530139 0.0592025
## [1] 1.115 1.000

## These affect both
evalGenotypeFitAndMut("g3, g9, a2, b3", fe3, fm3, verbose = TRUE)
##
##  Individual s terms are : 0 0 0.0530139 0.0592025
##
##  Individual mutator product terms are : 5 10
## [1]  1.115 50.000
```

Finally, we will do a simulation with those data

```
set.seed(1) ## so that it is easy to reproduce
mue1 <- oncoSimulIndiv(fe3, muEF = fm3,
                       mu = 1e-6,
                       initSize = 1e5,
                       model = "McFL",
                       detectionSize = 5e6,
                       finalTime = 500,
                       onlyCancer = FALSE)
```

```
## We do not show this in the vignette to avoid cluttering it
## with output
mue1
```

Of course, it is up to you to keep things reasonable: mutator effects are multiplicative, so if you specify, say, 20 genes (without modules), or 20 modules, each with a mutator effect of 50, the overall mutation rate can be increased by a factor of 50^{20} and that is unlikely to be what you really want.

You can play with the following case (an extension of the example above), where a clone with a mutator phenotype and some fitness enhancing mutations starts giving rise to many other clones, some with additional mutator effects, and thus leading to the number of clones blowing up (as some also accumulate additional fitness-enhancing mutations). Things start getting out of hand shortly after time 250. The code below takes a few minutes and is commented, but you can run it to get an idea of the increase in the number of clones and their relationships (the usage of *`plotClonePhylog`* is explained in section 8).

```r
set.seed(1) ## for reproducibility
## 17 genes, 7 with no direct fitness effects
ni <- c(rep(0, 7), runif(10, min = -0.01, max = 0.1))
names(ni) <- c("a1", "a2", "b1", "b2", "b3", "c1", "c2",
               paste0("g", 1:10))

## Next is for nicer figure labeling.
## consider drivers those with s >0
gp <- which(ni > 0)

fe3 <- allFitnessEffects(noIntGenes = ni,
                         drvNames = names(ni)[gp])


set.seed(12)
mue1 <- oncoSimulIndiv(fe3, muEF = fm3,
                       mu = 1e-6,
                       initSize = 1e5,
                       model = "McFL",
                       detectionSize = 5e6,
                       finalTime = 270,
                       keepPhylog = TRUE,
                       onlyCancer = FALSE)
mue1
## If you decrease N even further it gets even more cluttered
op <- par(ask = TRUE)
plotClonePhylog(mue1, N = 10, timeEvents = TRUE)
plot(mue1, plotDrivers = TRUE, addtot = TRUE,
     plotDiversity = TRUE)
## The stacked plot is slow; be patient
## Most clones have tiny population sizes, and their lines
## are piled on top of each other
plot(mue1, addtot = TRUE,
     plotDiversity = TRUE, type = "stacked")
par(op)
```

3 Plotting fitness landscapes

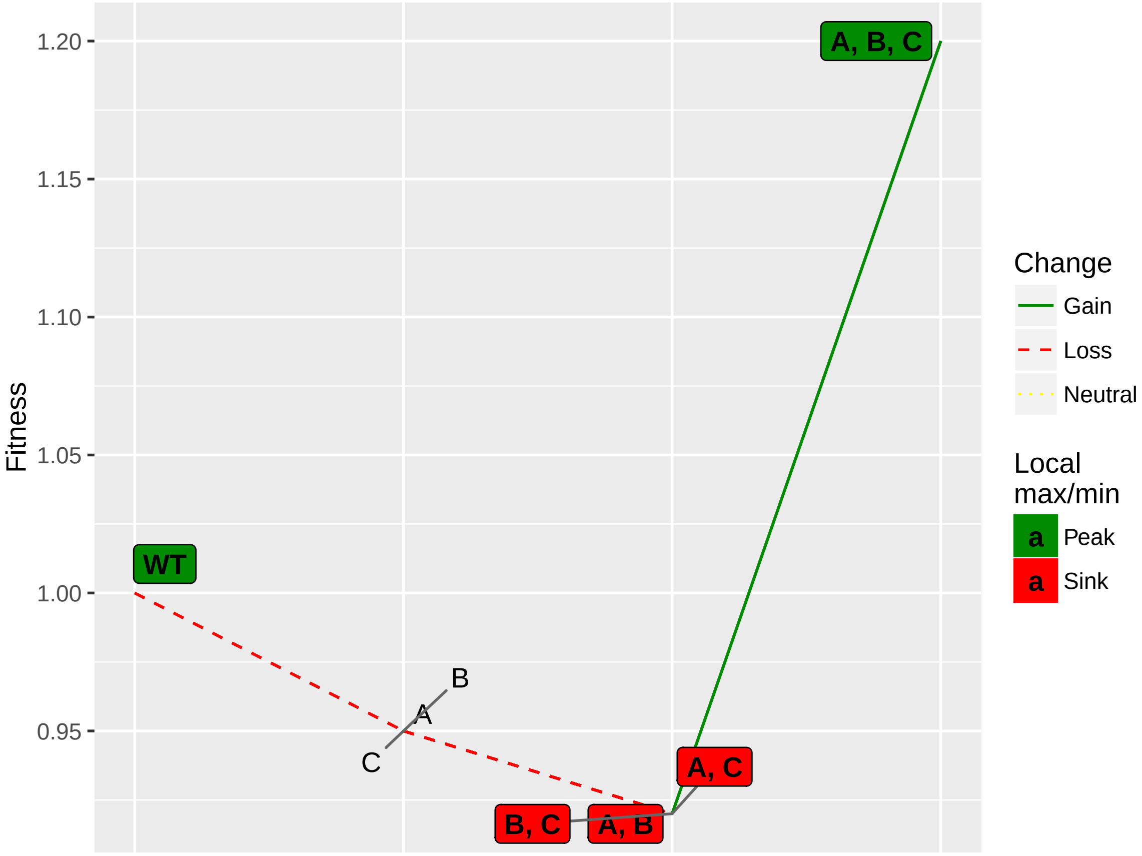
The *evalAllGenotypes* and related functions allow you to obtain tables of the genotype to fitness mappings. It might be more convenient to actually plot that, allowing us to quickly identify local minima and maxima and get an idea of how the fitness landscape looks.

In *plotFitnessLandscape* I have blatantly and shamelessly copied most of the looks of the plots of MAGELLAN (Brouillet et al. 2015) (see also http://wwwabi.snv.jussieu.fr/public/Magellan/), a very nice web-based tool for fitness landscape plotting and analysis (MAGELLAN provides some other extra functionality and epistasis statistics not provided here).

As an example, let us show the previous example of Weissman et al. we saw in 4.4:

```
d1 <- -0.05 ## single mutant fitness 0.95
d2 <- -0.08 ## double mutant fitness 0.92
d3 <- 0.2   ## triple mutant fitness 1.2
s2 <- ((1 + d2)/(1 + d1)^2) - 1
s3 <- ( (1 + d3)/((1 + d1)^3 * (1 + s2)^3) ) - 1

wb <- allFitnessEffects(
    epistasis = c(
        "A" = d1,
        "B" = d1,
        "C" = d1,
        "A:B" = s2,
        "A:C" = s2,
        "B:C" = s2,
        "A:B:C" = s3))
```

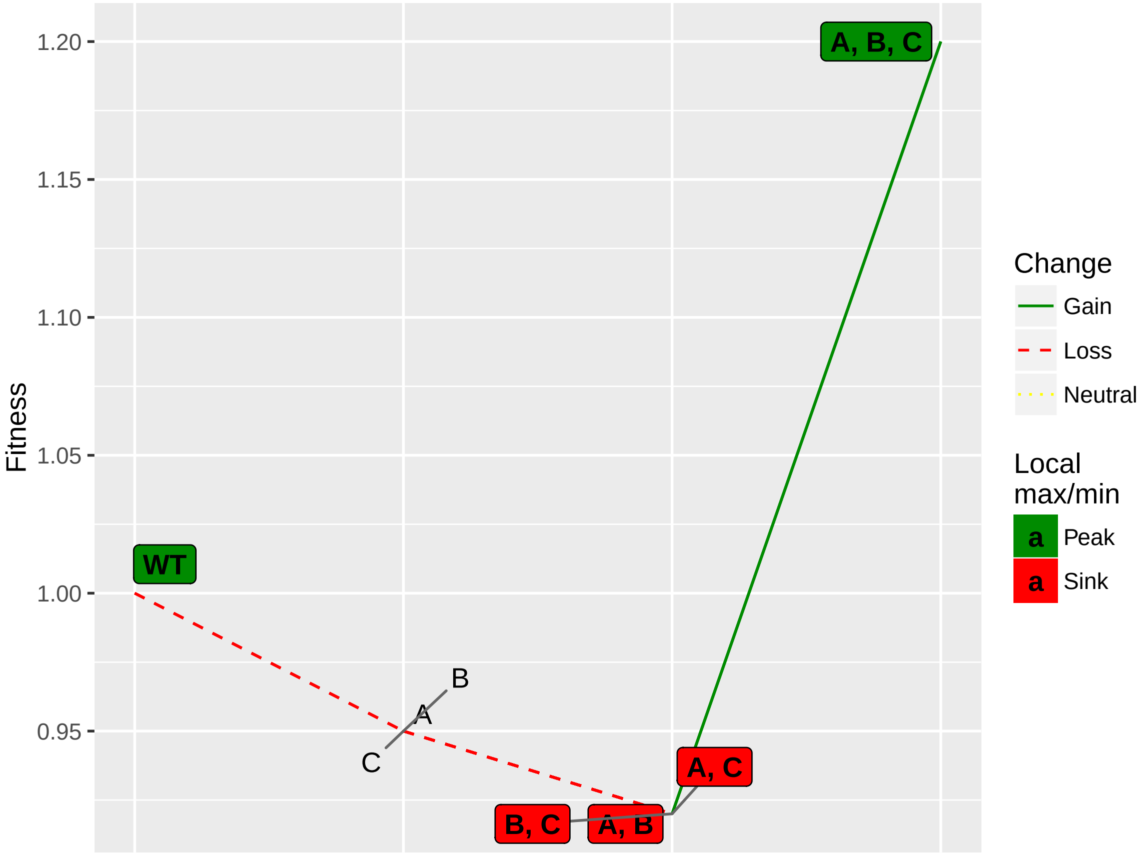
```
plotFitnessLandscape(wb, use_ggrepel = TRUE)
```



We have set `use_ggrepel = TRUE` to avoid overlap of labels.

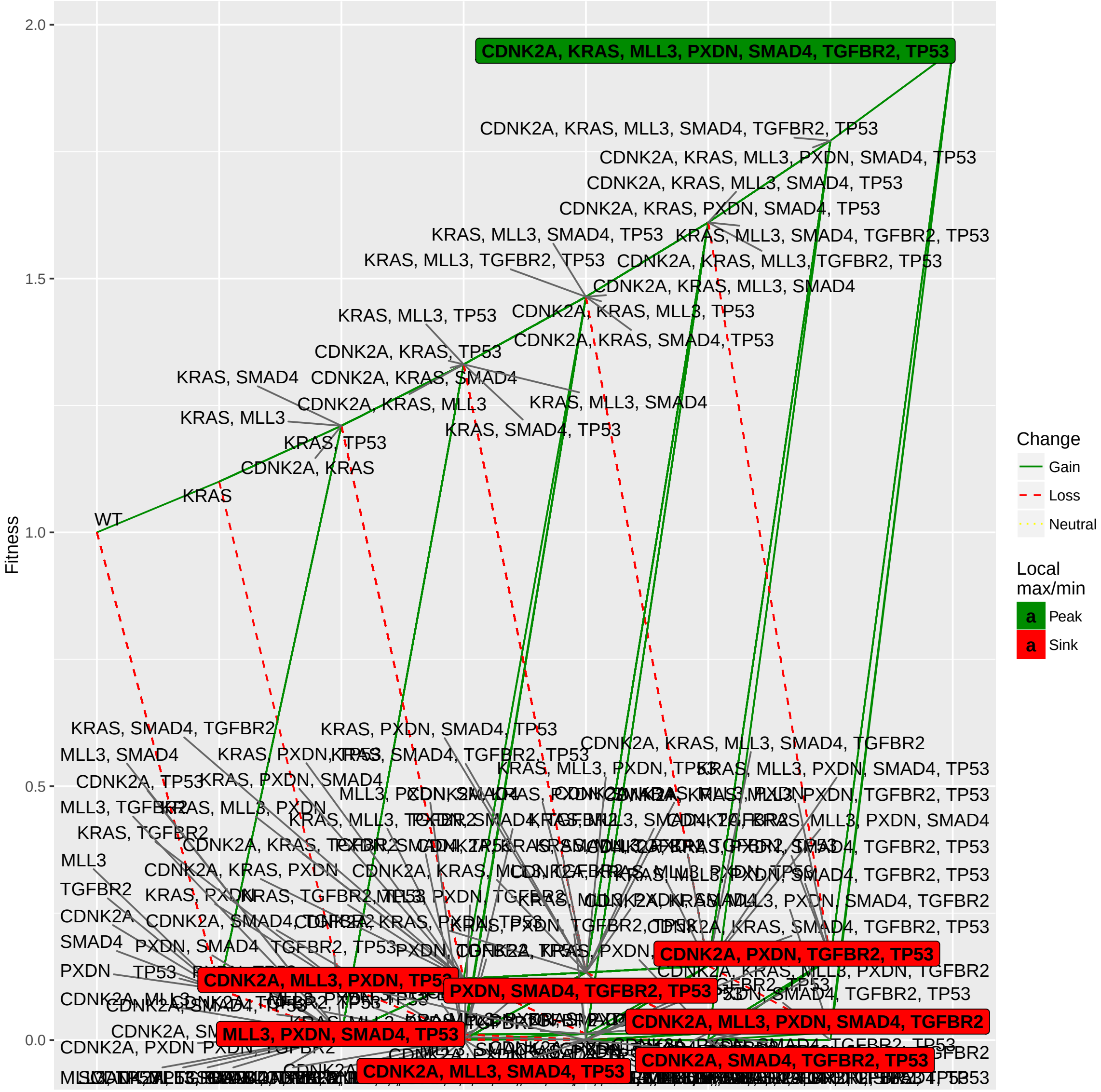
For some types of objects, directly invoking *`plot`* will give you the fitness plot

```
(ewb <- evalAllGenotypes(wb, order = FALSE))
##   Genotype Fitness
## 1        A    0.95
## 2        B    0.95
## 3        C    0.95
## 4     A, B    0.92
## 5     A, C    0.92
## 6     B, C    0.92
## 7  A, B, C    1.20
plot(ewb, use_ggrepel = TRUE)
```



This is another example we saw before (4.5), that will give a very busy plot:

```
par(cex = 0.7)
pancr <- allFitnessEffects(
    data.frame(parent = c("Root", rep("KRAS", 4),
                   "SMAD4", "CDNK2A",
                   "TP53", "TP53", "MLL3"),
               child = c("KRAS","SMAD4", "CDNK2A",
                   "TP53", "MLL3",
                   rep("PXDN", 3), rep("TGFBR2", 2)),
               s = 0.1,
               sh = -0.9,
               typeDep = "MN"))
plot(evalAllGenotypes(pancr, order = FALSE), use_ggrepel = TRUE)
```



4 Specifying fitness effects: some examples from the literature

4.1 Bauer et al., 2014

In the model of Bauer and collaborators (Bauer, Siebert, and Traulsen 2014, 54) we have “For cells without the primary driver mutation, each secondary driver mutation leads to a change in the cell’s fitness by s_P. For cells with the primary driver mutation, the fitness advantage obtained with each secondary driver mutation is s_{DP}."

The proliferation probability is given as:

- $\frac{1}{2}(1+s_p)^k$ when there are k secondary drivers mutated and no primary diver;
- $\frac{1}{2}\frac{1+S_D^+}{1+S_D^-}(1+S_{DP})^k$ when the primary driver is mutated;

apoptosis is one minus the proliferation rate.

4.1.1 Using a DAG

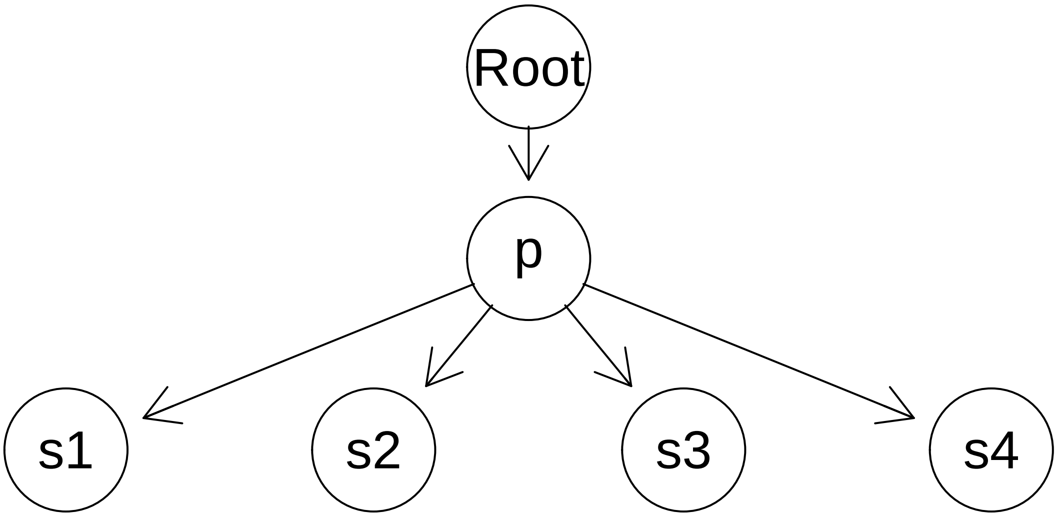
We cannot find a simple mapping from their expressions to our fitness parameterization, but we can get fairly close by using a DAG; in this one, note the unusual feature of having one of the "s" terms (that for the driver dependency on root) be negative. Using the parameters given in the legend of their Figure 3 for $s_p, S_D^+, S_D^-, S_{DP}$ and obtaining that negative value for the dependency of the driver on root we can do:

```
K <- 4
sp <- 1e-5
sdp <- 0.015
sdplus <- 0.05
sdminus <- 0.1
cnt <- (1 + sdplus)/(1 + sdminus)
prod_cnt <- cnt - 1
bauer <- data.frame(parent = c("Root", rep("p", K)),
                    child = c("p", paste0("s", 1:K)),
                    s = c(prod_cnt, rep(sdp, K)),
                    sh = c(0, rep(sp, K)),
                    typeDep = "MN")
fbauer <- allFitnessEffects(bauer)
(b1 <- evalAllGenotypes(fbauer, order = FALSE, addwt = TRUE))
##             Genotype Fitness
## 1                 WT  1.0000
## 2                  p  0.9545
## 3                 s1  1.0000
## 4                 s2  1.0000
## 5                 s3  1.0000
## 6                 s4  1.0000
## 7              p, s1  0.9689
## 8              p, s2  0.9689
## 9              p, s3  0.9689
## 10             p, s4  0.9689
## 11            s1, s2  1.0000
## 12            s1, s3  1.0000
## 13            s1, s4  1.0000
## 14            s2, s3  1.0000
## 15              s2, s4  1.0000
## 16              s3, s4  1.0000
## 17           p, s1, s2  0.9834
## 18           p, s1, s3  0.9834
## 19           p, s1, s4  0.9834
## 20           p, s2, s3  0.9834
## 21           p, s2, s4  0.9834
## 22           p, s3, s4  0.9834
## 23          s1, s2, s3  1.0000
## 24          s1, s2, s4  1.0000
## 25          s1, s3, s4  1.0000
## 26          s2, s3, s4  1.0000
## 27       p, s1, s2, s3  0.9981
## 28       p, s1, s2, s4  0.9981
## 29       p, s1, s3, s4  0.9981
## 30       p, s2, s3, s4  0.9981
## 31      s1, s2, s3, s4  1.0000
## 32 p, s1, s2, s3, s4  1.0131
```

Note that what we specify as "typeDep" is irrelevant (MN, SMN, or XMPN make no difference).

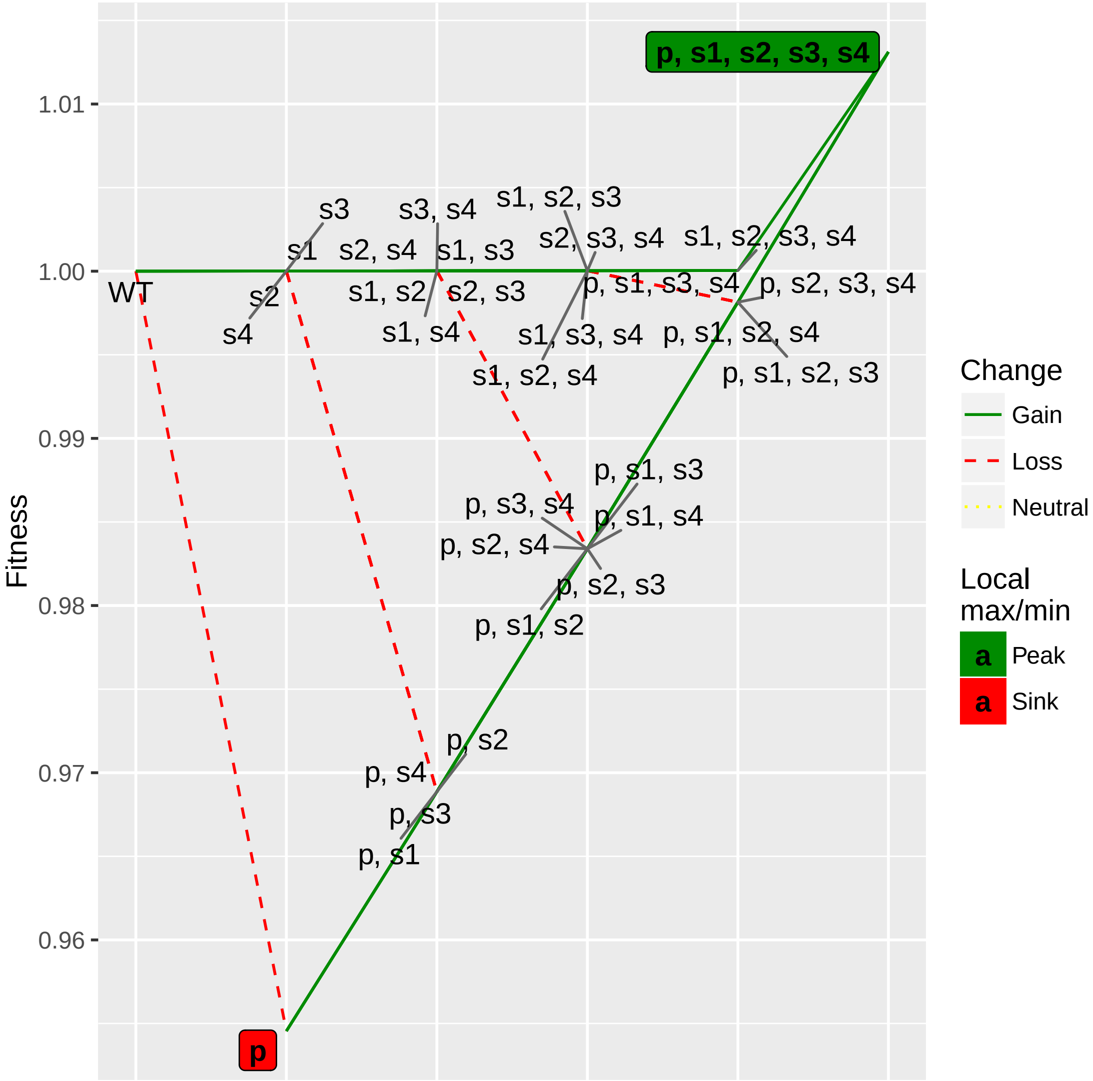
This is the DAG:

```
plot(fbauer)
```



And if you compare the tabular output of `evalAllGenotypes` you can see that the values of fitness reproduces the fitness landscape that they show in their Figure 1. We can also use our plot for fitness landscapes:

```
plot(b1, use_ggrepel = TRUE)
```



4.1.2 Specifying fitness of genotypes directly

An alternative approach to specify the fitness, if the number of genotypes is reasonably small, is to directly evaluate fitness as given by their expressions. Then, use the `genotFitness` argument to *`allFitnessEffects`*.

We will create all possible genotypes; then we will write a function that gives the fitness of each genotype according to their expression; finally, we will call this function on the data frame of genotypes, and pass this data frame to *`allFitnessEffects`*.

```
m1 <- expand.grid(p = c(1, 0), s1 = c(1, 0), s2 = c(1, 0),
                  s3 = c(1, 0), s4 = c(1, 0))

fitness_bauer <- function(p, s1, s2, s3, s4,
                          sp = 1e-5, sdp = 0.015, sdplus = 0.05,
                              sdminus = 0.1) {
    if(!p) {
        b <- 0.5 * ( (1 + sp)^(sum(c(s1, s2, s3, s4))))
    } else {
        b <- 0.5 *
            (((1 + sdplus)/(1 + sdminus)  *
              (1 + sdp)^(sum(c(s1, s2, s3, s4)))))
    }
    fitness <- b - (1 - b)
    our_fitness <- 1 + fitness ## prevent negative fitness and
    ## make wt fitness = 1
    return(our_fitness)
}

m1$Fitness <-
    apply(m1, 1, function(x) do.call(fitness_bauer, as.list(x)))

bauer2 <- allFitnessEffects(genotFitness = m1)
```

Now, show the fitness of all genotypes:

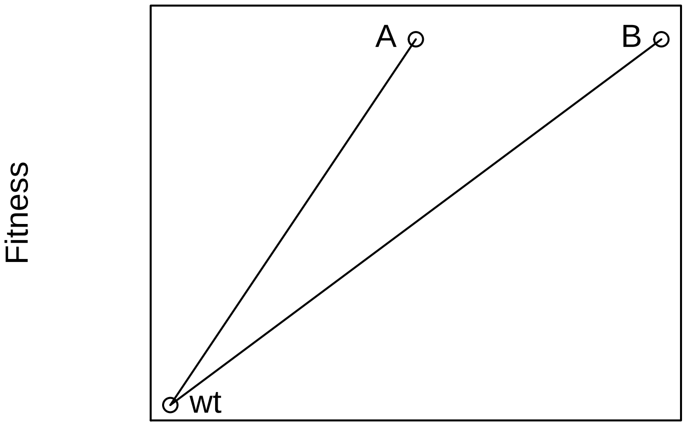
```
evalAllGenotypes(bauer2, order = FALSE, addwt = TRUE)
##              Genotype Fitness
## 1                  WT  1.0000
## 2                   p  0.9545
## 3                  s1  1.0000
## 4                  s2  1.0000
## 5                  s3  1.0000
## 6                  s4  1.0000
## 7               p, s1  0.9689
## 8               p, s2  0.9689
## 9               p, s3  0.9689
## 10              p, s4  0.9689
## 11             s1, s2  1.0000
## 12             s1, s3  1.0000
## 13             s1, s4  1.0000
## 14             s2, s3  1.0000
## 15             s2, s4  1.0000
## 16             s3, s4  1.0000
## 17          p, s1, s2  0.9834
## 18          p, s1, s3  0.9834
## 19          p, s1, s4  0.9834
## 20          p, s2, s3  0.9834
## 21          p, s2, s4  0.9834
## 22          p, s3, s4  0.9834
## 23         s1, s2, s3  1.0000
## 24        s1, s2, s4  1.0000
## 25        s1, s3, s4  1.0000
## 26        s2, s3, s4  1.0000
## 27     p, s1, s2, s3  0.9981
## 28     p, s1, s2, s4  0.9981
## 29     p, s1, s3, s4  0.9981
## 30     p, s2, s3, s4  0.9981
## 31    s1, s2, s3, s4  1.0000
## 32 p, s1, s2, s3, s4  1.0131
```

Can we use modules in this example, if we use the "lego system"? Sure, as in any other case.

4.2 Misra et al., 2014

Figure 1 of Misra, Szczurek, and Vingron (2014) presents three scenarios which are different types of epistasis.

4.2.1 Example 1.a



In that figure it is evident that the fitness effect of "A" and "B" are the same. There are two different models depending on whether "AB" is just the product of both, or there is epistasis. In the first case probably the simplest is:

```
s <- 0.1 ## or whatever number
m1a1 <- allFitnessEffects(data.frame(parent = c("Root", "Root"),
                                     child = c("A", "B"),
                                     s = s,
                                     sh = 0,
                                     typeDep = "MN"))
evalAllGenotypes(m1a1, order = FALSE, addwt = TRUE)
##   Genotype Fitness
## 1       WT    1.00
## 2         A    1.10
## 3         B    1.10
## 4      A, B    1.21
```

If the double mutant shows epistasis, as we saw before (section 2.9.1) we have a range of options. For example:

```
s <- 0.1
sab <- 0.3
m1a2 <- allFitnessEffects(epistasis = c("A:-B" = s,
                                        "-A:B" = s,
                                        "A:B" = sab))
evalAllGenotypes(m1a2, order = FALSE, addwt = TRUE)
##   Genotype Fitness
## 1       WT     1.0
## 2        A     1.1
## 3        B     1.1
## 4     A, B     1.3
```

But we could also modify the graph dependency structure, and we have to change the value of the coefficient, since that is what multiplies each of the terms for "A" and "B": $(1 + s_{AB}) = (1 + s)^2(1 + s_{AB3}) $

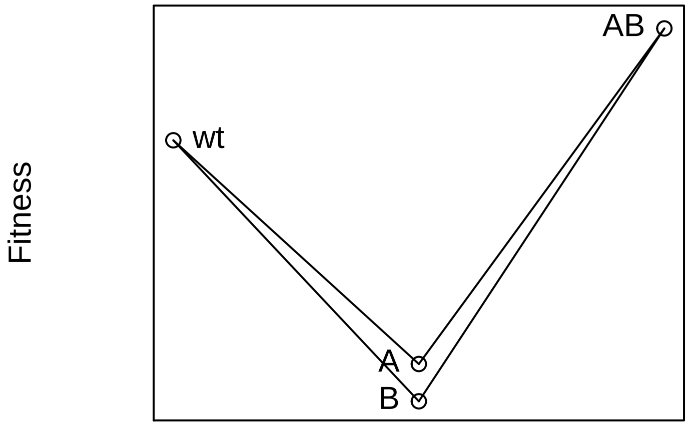
```
sab3 <- ((1 + sab)/((1 + s)^2)) - 1
m1a3 <- allFitnessEffects(data.frame(parent = c("Root", "Root"),
                                     child = c("A", "B"),
                                     s = s,
                                     sh = 0,
                                     typeDep = "MN"),
                          epistasis = c("A:B" = sab3))
evalAllGenotypes(m1a3, order = FALSE, addwt = TRUE)
##   Genotype Fitness
## 1       WT     1.0
## 2        A     1.1
## 3        B     1.1
## 4     A, B     1.3
```

And, obviously

```
all.equal(evalAllGenotypes(m1a2, order = FALSE, addwt = TRUE),
          evalAllGenotypes(m1a3, order = FALSE, addwt = TRUE))
## [1] TRUE
```

4.2.2 Example 1.b

This is a specific case of synthetic viability (see also section 2.7):



Here, $S_A, S_B < 0$, $S_B < 0$, $S_{AB} > 0$ and $(1 + S_{AB})(1 + S_A)(1 + S_B) > 1$.

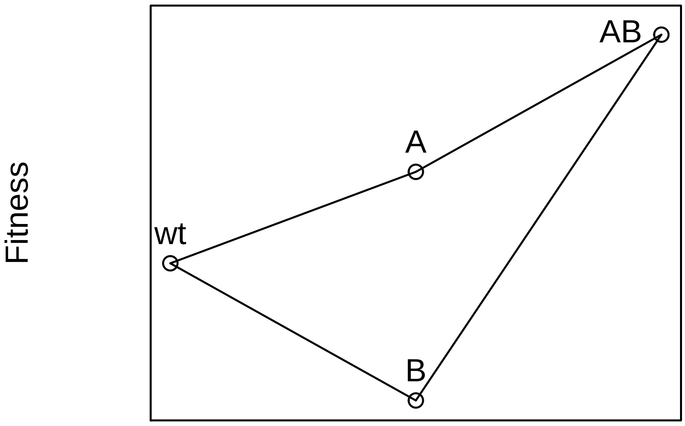
As before, we can specify this in several different ways. The simplest is to specify all genotypes:

```
sa <- -0.6
sb <- -0.7
sab <- 0.3
m1b1 <- allFitnessEffects(epistasis = c("A:-B" = sa,
                                         "-A:B" = sb,
                                         "A:B" = sab))
evalAllGenotypes(m1b1, order = FALSE, addwt = TRUE)
##   Genotype Fitness
## 1       WT     1.0
## 2        A     0.4
## 3        B     0.3
## 4     A, B     1.3
```

We could also use a tree and modify the "sab" for the epistasis, as before (4.2.1).

4.2.3 Example 1.c

The final case, in figure 1.c of Misra et al., is just epistasis, where a mutation in one of the genes is deleterious (possibly only mildly), in the other is beneficial, and the double mutation has fitness larger than any of the other two.



Here we have that $s_A > 0$, $s_B < 0$, $(1 + s_{AB})(1 + s_A)(1 + s_B) > (1 + s_{AB})$ so $s_{AB} > \frac{-s_B}{1+s_B}$

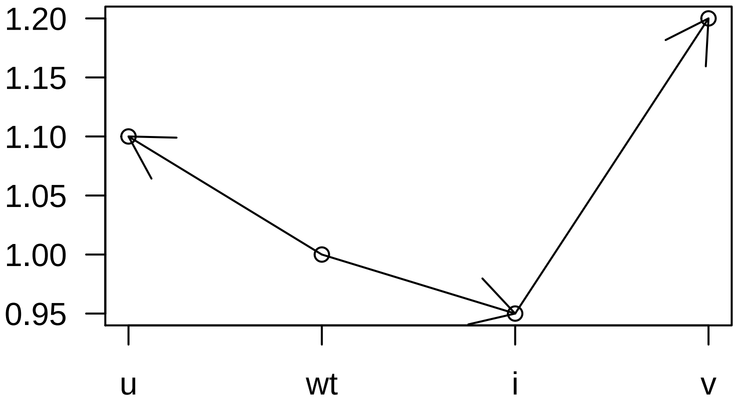
As before, we can specify this in several different ways. The simplest is to specify all genotypes:

```
sa <- 0.2
sb <- -0.3
sab <- 0.5
m1c1 <- allFitnessEffects(epistasis = c("A:-B" = sa,
                                        "-A:B" = sb,
                                        "A:B" = sab))
evalAllGenotypes(m1c1, order = FALSE, addwt = TRUE)
##   Genotype Fitness
## 1       WT     1.0
## 2        A     1.2
## 3        B     0.7
## 4     A, B     1.5
```

We could also use a tree and modify the “sab” for the epistasis, as before (4.2.1).

4.3 Ochs and Desai, 2015

In Ochs and Desai (2015) the authors present a model shown graphically as (the actual numerical values are arbitrarily set by me):



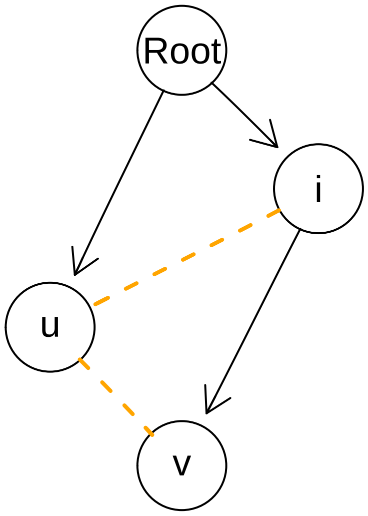
In their model, $s_u > 0$, $s_v > s_u$, $s_i < 0$, we can only arrive at v from i, and the mutants “ui” and “uv” can never appear as their fitness is 0, or $-\infty$, so $s_{ui} = s_{uv} = -1$ (or $-\infty$).

We can specify this combining a graph and epistasis specifications:

```
su <- 0.1
si <- -0.05
fvi <- 1.2 ## the fitnes of the vi mutant
sv <- (fvi/(1 + si)) - 1
sui <- suv <- -1
od <- allFitnessEffects(
    data.frame(parent = c("Root", "Root", "i"),
               child = c("u", "i", "v"),
               s = c(su, si, sv),
               sh = -1,
               typeDep = "MN"),
    epistasis = c(
        "u:i" = sui,
        "u:v" = suv))
```

A figure showing that model is

```
plot(od)
```



And the fitness of all genotype is

```
evalAllGenotypes(od, order = FALSE, addwt = TRUE)
##   Genotype Fitness
## 1       WT    1.00
## 2        i    0.95
## 3        u    1.10
## 4        v    0.00
## 5     i, u    0.00
## 6     i, v    1.20
## 7     u, v    0.00
## 8  i, u, v    0.00
```

We could alternatively have specified fitness either directly specifying the fitness of each genotype or specifying epistatic effects. Let us use the second approach:

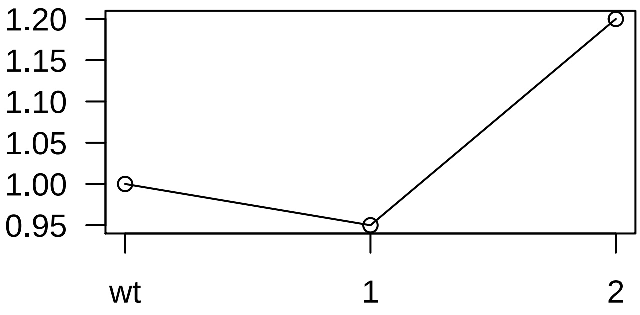
```
u <- 0.2; i <- -0.02; vi <- 0.6; ui <- uv <- -Inf
od2 <- allFitnessEffects(
    epistasis = c("u" = u,  "u:i" = ui,
                  "u:v" = uv, "i" = i,
                  "v:-i" = -Inf, "v:i" = vi))
evalAllGenotypes(od, addwt = TRUE)
##   Genotype Fitness
## 1       WT    1.00
## 2        i    0.95
## 3        u    1.10
## 4        v    0.00
## 5     i, u    0.00
## 6     i, v    1.20
## 7     u, v    0.00
## 8  i, u, v    0.00
```

We will return to this model when we explain the usage of `fixation` for stopping the simulations (see @{fixation}).

4.4 Weissman et al., 2009

In their figure 1a, Weissman et al. (2009) present this model (actual numeric values are set arbitrarily)

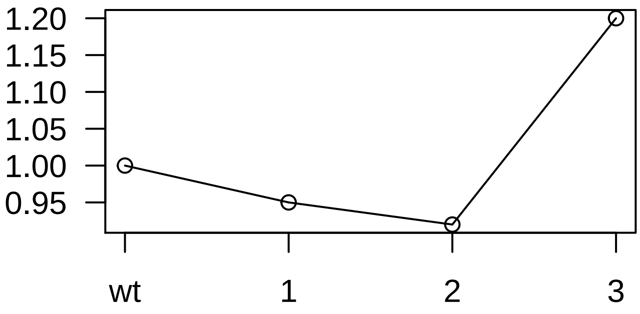
4.4.1 Figure 1.a}



where the 1" and2" refer to the total number of mutations in two different loci. This is, therefore, very similar to the example in section 4.2.2. Here we have, in their notation, $\delta_1 < 0$, fitness of single "A" or single "B" $= 1 + \delta_1$, $S_{AB} > 0$, $(1 + S_{AB})(1 + \delta_1)^2 > 1$.

4.4.2 Figure 1.b

In their figure 1b they show



Where, as before, 1, 2, 3, denote the total number of mutations over three different loci and $\delta_1 < 0$, $\delta_2 < 0$, fitness of single mutant is $(1 + \delta_1)$, of double mutant is $(1 + \delta_2)$ so that $(1 + \delta_2) = (1 + \delta_1)^2(1 + s_2)$ and of triple mutant is $(1 + \delta_3)$, so that $(1 + \delta_3) = (1 + \delta_1)^3(1 + s_2)^3(1 + s_3)$.

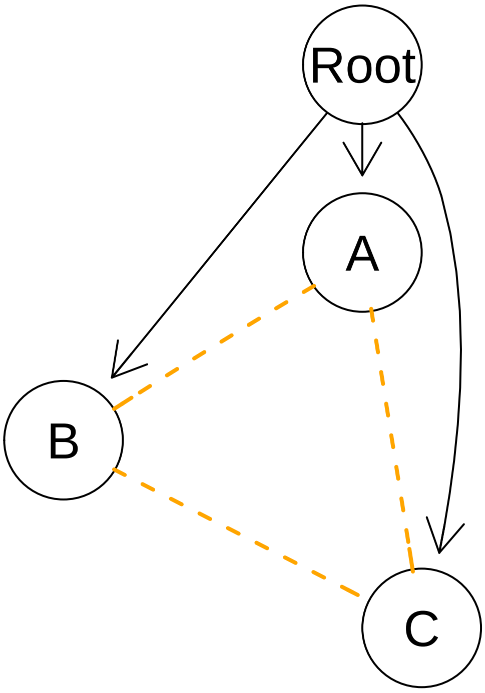
We can specify this combining a graph with epistasis:

```
d1 <- -0.05 ## single mutant fitness 0.95
d2 <- -0.08 ## double mutant fitness 0.92
d3 <- 0.2   ## triple mutant fitness 1.2

s2 <- ((1 + d2)/(1 + d1)^2) - 1
s3 <- ( (1 + d3)/((1 + d1)^3 * (1 + s2)^3) ) - 1
```

```
w <- allFitnessEffects(
    data.frame(parent = c("Root", "Root", "Root"),
               child = c("A", "B", "C"),
               s = d1,
               sh = -1,
               typeDep = "MN"),
    epistasis = c(
        "A:B" = s2,
        "A:C" = s2,
        "B:C" = s2,
        "A:B:C" = s3))
```

The model can be shown graphically as:

```
plot(w)
```



And fitness of all genotypes is:

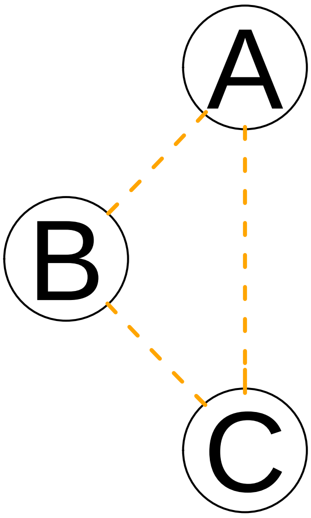
```
evalAllGenotypes(w, order = FALSE, addwt = TRUE)
##   Genotype Fitness
## 1       WT    1.00
## 2        A    0.95
## 3        B    0.95
## 4        C    0.95
## 5     A, B    0.92
## 6     A, C    0.92
## 7     B, C    0.92
## 8  A, B, C    1.20
```

Alternatively, we can directly specify what each genotype adds to the fitness, given the included genotype. This is basically replacing the graph by giving each of "A", "B", and "C" directly:

```
wb <- allFitnessEffects(
    epistasis = c(
        "A" = d1,
        "B" = d1,
        "C" = d1,
        "A:B" = s2,
        "A:C" = s2,
        "B:C" = s2,
        "A:B:C" = s3))

evalAllGenotypes(wb, order = FALSE, addwt = TRUE)
##   Genotype Fitness
## 1       WT    1.00
## 2        A    0.95
## 3        B    0.95
## 4        C    0.95
## 5     A, B    0.92
## 6     A, C    0.92
## 7     B, C    0.92
## 8  A, B, C    1.20
```

The plot, of course, is not very revealing and we cannot show that there is a three-way interaction (only all three two-way interactions):

```
plot(wb)
```



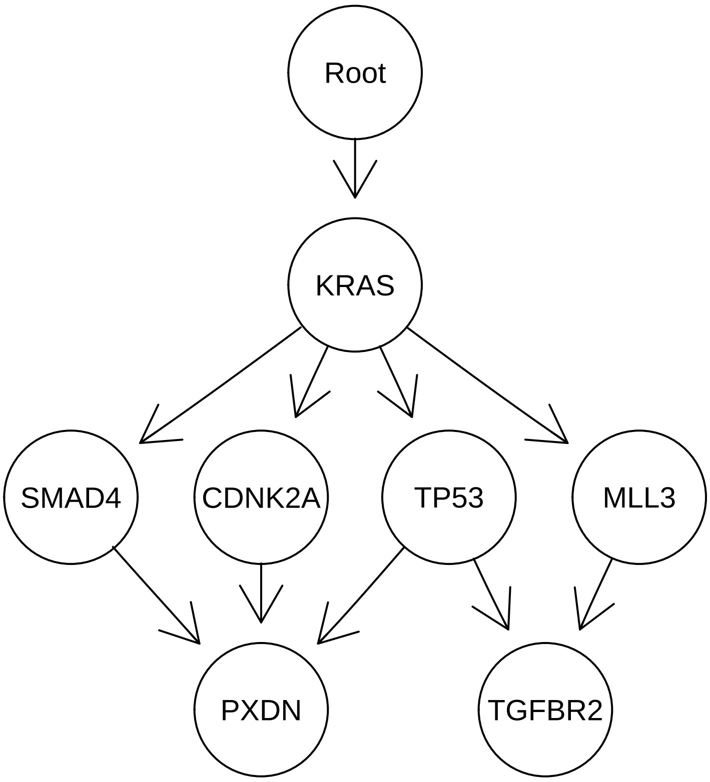
As we have seen several times already (sections 2.9.1, 2.9.2, 2.9.3) we can also give the genotypes directly and, consequently, the fitness of each genotype (not the added contribution):

```
wc <- allFitnessEffects(
    epistasis = c(
        "A:-B:-C" = d1,
        "B:-C:-A" = d1,
        "C:-A:-B" = d1,
        "A:B:-C" = d2,
        "A:C:-B" = d2,
        "B:C:-A" = d2,
        "A:B:C" = d3))
evalAllGenotypes(wc, order = FALSE, addwt = TRUE)
##   Genotype Fitness
## 1       WT    1.00
## 2        A    0.95
## 3        B    0.95
## 4        C    0.95
## 5     A, B    0.92
## 6     A, C    0.92
## 7     B, C    0.92
## 8  A, B, C    1.20
```

4.5 Gerstung et al., 2011, pancreatic cancer poset

Similar to what we did in v.1 (see section 11.1) we can specify the pancreatic cancer poset in Gerstung et al. (2011) (their figure 2B, left). We use directly the names of the genes, since that is immediately supported by the new version.

```
pancr <- allFitnessEffects(
    data.frame(parent = c("Root", rep("KRAS", 4),
                   "SMAD4", "CDNK2A",
                   "TP53", "TP53", "MLL3"),
               child = c("KRAS","SMAD4", "CDNK2A",
                   "TP53", "MLL3",
                   rep("PXDN", 3), rep("TGFBR2", 2)),
               s = 0.1,
               sh = -0.9,
               typeDep = "MN"))

plot(pancr)
```

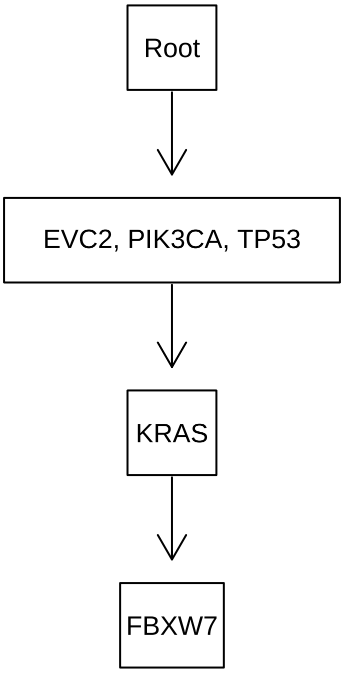


Of course the “s” and “sh” are set arbitrarily here.

4.6 Raphael and Vandin’s 2014 modules

In Raphael and Vandin (2015), the authors show several progression models in terms of modules. We can code the extended poset for the colorectal cancer model in their Figure 4.a is (s and sh are arbitrary):

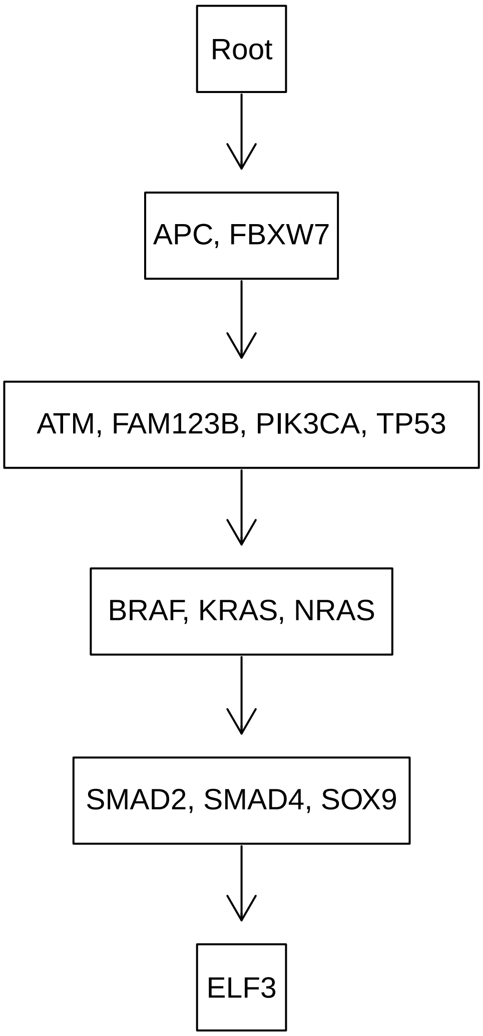
```r
rv1 <- allFitnessEffects(data.frame(parent = c("Root", "A", "KRAS"),
                                    child = c("A", "KRAS", "FBXW7"),
                                    s = 0.1,
                                    sh = -0.01,
                                    typeDep = "MN"),
                         geneToModule = c("Root" = "Root",
                             "A" = "EVC2, PIK3CA, TP53",
                             "KRAS" = "KRAS",
                             "FBXW7" = "FBXW7"))

plot(rv1, expandModules = TRUE, autofit = TRUE)
```



We have used the (experimental) `autofit` option to fit the labels to the edges. Note how we can use the same name for genes and modules, but we need to specify all the modules.

Their Figure 5b is

```
rv2 <- allFitnessEffects(
    data.frame(parent = c("Root", "1", "2", "3", "4"),
               child = c("1", "2", "3", "4", "ELF3"),
               s = 0.1,
               sh = -0.01,
               typeDep = "MN"),
    geneToModule = c("Root" = "Root",
                     "1" = "APC, FBXW7",
                     "2" = "ATM, FAM123B, PIK3CA, TP53",
                     "3" = "BRAF, KRAS, NRAS",
                     "4" = "SMAD2, SMAD4, SOX9",
                     "ELF3" = "ELF3"))

plot(rv2, expandModules = TRUE,   autofit = TRUE)
```



5 Running and plotting the simulations: starting, ending, and examples

5.1 Starting and ending

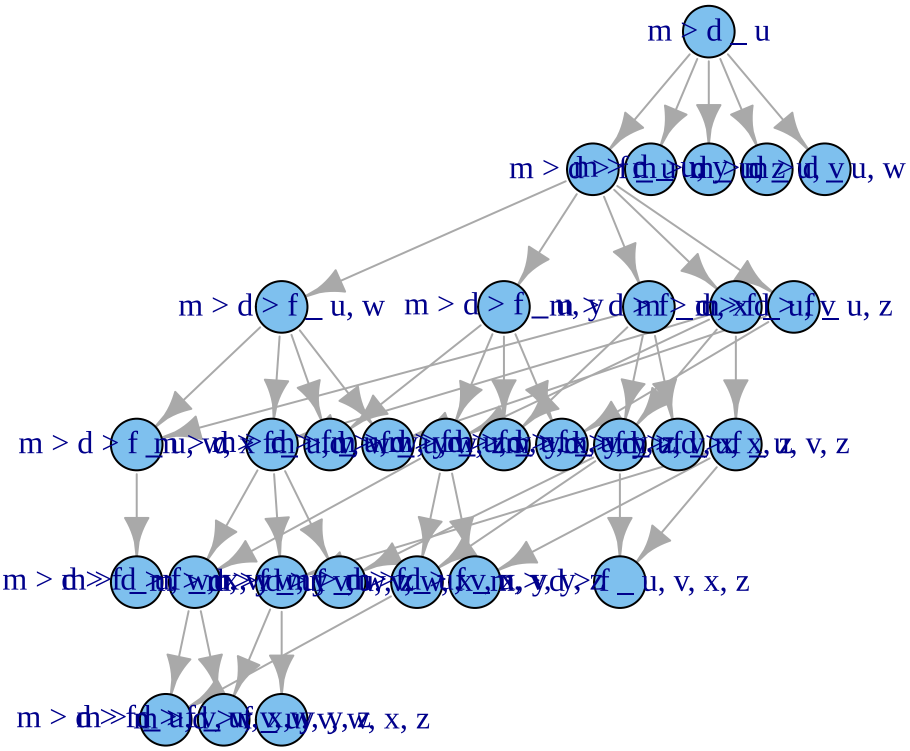
After you have decided the specifics of the fitness effects and the model, you need to decide:

- Where will you start your simulation from. This involves deciding the initial population size (argument `initSize`) and, possibly, the genotype of the initial population; the later is covered in section 5.2.
- When will you stop it: how long to run it, and whether or not to require simulations to reach cancer (under some definition of what it means to reach cancer). This is covered in 5.3.

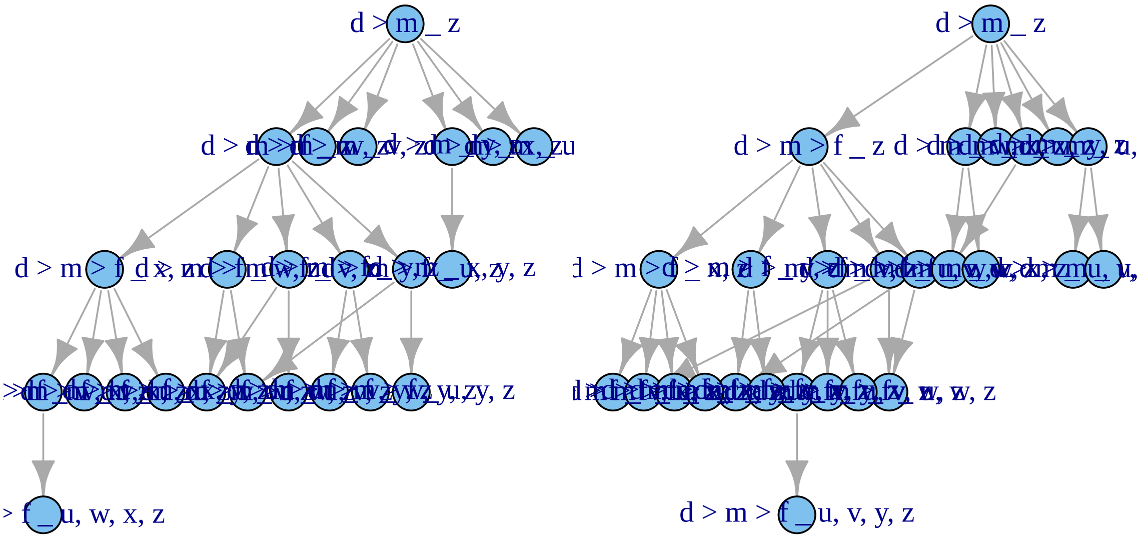
5.2 Can I start the simulation from a specific mutant?

You bet. In v.1 you can only give the initial mutant as one with a single mutated gene. In version 2, however, you can specify the genotype for the initial mutant with the same flexibility as in *evalGenotype*. Here we show a couple of examples (we use the representation of the parent-child relationships —discussed in section 8— of the clones so that you can see which clones appear, and from which, and check that we are not making mistakes).

```
o3init <- allFitnessEffects(orderEffects = c(
                              "M > D > F" = 0.99,
                              "D > M > F" = 0.2,
                              "D > M"     = 0.1,
                              "M > D"     = 0.9),
                          noIntGenes = c("u" = 0.01,
                                         "v" = 0.01,
                                         "w" = 0.001,
                                         "x" = 0.0001,
                                         "y" = -0.0001,
                                         "z" = -0.001),
                          geneToModule =
                              c("M" = "m",
                                "F" = "f",
                                "D" = "d") )

oneI <- oncoSimulIndiv(o3init, model = "McFL",
                         mu = 5e-5, finalTime = 500,
                         detectionDrivers = 3,
                         onlyCancer = FALSE,
                         initSize = 1000,
                         keepPhylog = TRUE,
                         initMutant = c("m > u > d")
                         )
plotClonePhylog(oneI, N = 0)
```



```
## Note we also disable the stopping stochastically as a function of size
## to allow the population to grow large and generate may different
## clones.
ospI <- oncoSimulPop(2,
                     o3init, model = "Exp",
                     mu = 5e-5, finalTime = 500,
                     detectionDrivers = 3,
                     onlyCancer = TRUE,
                     initSize = 10,
                     keepPhylog = TRUE,
                     initMutant = c("d > m > z"),
                     mc.cores = 2
                     )
op <- par(mar = rep(0, 4), mfrow = c(1, 2))
plotClonePhylog(ospI[[1]])
plotClonePhylog(ospI[[2]])
```



```
par(op)

ossI <- oncoSimulSample(2,
                        o3init, model = "Exp",
                        mu = 5e-5, finalTime = 500,
                        detectionDrivers = 2,
                        onlyCancer = TRUE,
                        initSize = 10,
                        initMutant = c("z > d"),
                        ## check presence of initMutant:
                        thresholdWhole = 1
                    )
## Successfully sampled 2 individuals
##
##  Subjects by Genes matrix of 2 subjects and 9 genes.

## No phylogeny is kept with oncoSimulSample, but look at the
## OcurringDrivers and the sample
ossI$popSample
##      d f m u v w x y z
## [1,] 1 0 0 0 0 0 0 0 1
## [2,] 1 0 0 0 0 0 0 0 1
ossI$popSummary[, "OccurringDrivers", drop = FALSE]
##   OccurringDrivers
## 1
## 2
```

5.3 Ending the simulations

OncoSimulR provides very flexible ways to decide when to stop a simulation. Here we focus on a single simulation; see further options with multiple simulations in 6.

- **`onlyCancer = TRUE`**. A simulation will be repeated until any one of the "reach cancer" conditions is met, if this happens before the simulation reaches `finalTime`[6]. These conditions are:
    - Total population size becomes larger than `detectionSize`.
    - The number of drivers in any one genotype or clone becomes equal to, or larger than, `detectionDrivers`; note that this allows you to stop the simulation as soon as a **specific genotype** is found, by using exactly and only the genes that make that genotype as the drivers.
    - The tumor is detected according to a stochastic detection mechanism, where the probability of "detecting the tumor" increases with population size; this is explained below (5.4) and is controlled by argument `detectionProb`.
    - A gene or gene combination among those listed in `fixation` becomes fixed in the population (i.e., has a frequency is 1) (see details in (5.6).

  As we exit as soon as any of the exiting conditions is reached, if you only care about one condition, set the other to `NA` (see also section @ref(and_drvprob)).

- **`onlyCancer = FALSE`**. A simulation will run only once, and will exit as soon as any of the above conditions are met or as soon as the total population size becomes zero or we reach `finalTime`.

As an example of `onlyCancer = TRUE`, focusing on the first two mechanisms, suppose you give `detectionSize = 1e4` and `detectionDrivers =3` (and you have `detectionProb = NA`). A simulation will exit as soon as it reaches a total population size of $1e4$ or any clone has four drivers, whichever comes first (if any of these happen before `finalTime`).

In the `onlyCancer = TRUE` case, what happens if we reach `finalTime` (or the population size becomes zero) before any of the "reach cancer" conditions have been fulfilled? The simulation will be repeated again, within the following limits:

- `max.wall.time`: the total wall time we allow an individual simulation to run;
- `max.num.tries`: the maximum number of times we allow a simulation to be repeated to reach cancer;
- `max.wall.time.total` and `max.num.tries.total`, similar to the above but over a set of simulations in function *`oncoSimulSample`*.

Incidentally, we keep track of the number of attempts used (the component `other$attemptsUsed$`) before we reach cancer, so you can estimate (as from a negative binomial sampling) the probability of reaching your desired end point under different scenarios.

The `onlyCancer = FALSE` case might be what you want to do when you examine general population genetics scenarios without focusing on possible sampling issues. To do this, set `finalTime` to the value you want and set `onlyCancer = FALSE`; in addition, set `detectionProb` to "NA" and `detectionDrivers` and `detectionSize`

[6] Of course, the "reach cancer" idea and the `onlyCancer` argument are generic names; this could have been labeled "reach whatever interests me".

to “NA” or to huge numbers[7]. In this scenario you simply collect the simulation output at the end of the run, regardless of what happened with the population (it became extinct, it did not reach a large size, it did not accumulate drivers, etc).

5.4 Stochastic detection mechanism: “detectionProb”

This is the process that is controlled by the argument `detectionProb`. Here the probability of tumor detection increases with the total population size. This is biologically a reasonable assumption: the larger the tumor, the more likely it is it will be detected.

At regularly spaced times during the simulation, we compute the probability of detection as a function of size and determine (by comparing against a random uniform number) if the simulation should finish. For simplicity, and to make sure the probability is bounded between 0 and 1, we use the function

$$P(N) = \begin{cases} 1 - e^{-cPDetect(N - PDBaseline)} & \text{if } N > PDBaseline \\ 0 & \text{if } N \leq PDBaseline \end{cases} \tag{1}$$

where $P(N)$ is the probability that a tumor with a population size N will be detected, and *cPDetect* controls the increase in $P(N)$ with population size; with $PDBaseline$ we both control the minimal population size at which this mechanism stats operating (because we will rarely want detection unless there is some meaningful increase of population size over `initSize`) and we model the increase in $P(N)$ as a function of differences with respect to $PDBaseline$.

The $P(N)$ refers to the probability of detection at each one of the occasions when we assess the probability of exiting. When, or how often, do we do that? When we assess probability of exiting is controlled by `checkSizePEvery`, which will often be much larger than `sampleEvery`[8]. Biologically, a way to think of `checkSizePEvery` is “time between doctor appointments”.

Finally, you can specify *cPDetect* directly (you will need to set `n2` and `p2` to NA). However, it might be more intuitive to specify the pair `n2`, `p2`, such that $P(n2) = p2$ (and from that pair we solve for the value of `cPDetect`).

You can get a feeling for the effects of these arguments by playing with the following code, that we do not execute here for the sake of speed. Here no mutation has any effect, but there is a non-zero probability of exiting as soon as the total population

[7] Setting `detectionDrivers` and `detectionSize` to “NA” is in fact equivalent to setting them to the largest possible numbers for these variables: $2^{32} - 1$ and ∞, respectively.

[8] We assess probability of exiting at every sampling time, as given by `sampleEvery`, that is the smallest possible sampling time that is separated from the previous time of assessment by at least `checkSizePEvery`. In other words, the interval between successive assessments will be the smallest multiple integer of `sampleEvery` that is larger than `checkSizePEvery`. For example, suppose `sampleEvery = 2` and `checkSizePEvery = 3`: we will assess exiting at times $4, 8, 12, 16, \ldots$. If `sampleEvery = 3` and `checkSizePEvery = 3`: we will assess exiting at times $6, 12, 18, \ldots$.

size becomes larger than the initial population size. So, eventually, all simulations will exit and, as we are using the McFarland model, population size will vary slightly around the initial population size.

```
gi2 <- rep(0, 5)
names(gi2) <- letters[1:5]
oi2 <- allFitnessEffects(noIntGenes = gi2)
s5 <- oncoSimulPop(200,
                   oi2,
                   model = "McFL",
                   initSize = 1000,
                   detectionProb = c(p2 = 0.1,
                                     n2 = 2000,
                                     PDBaseline = 1000,
                                     checkSizePEvery = 2),
                   detectionSize = NA,
                   finalTime = NA,
                   keepEvery = NA,
                   detectionDrivers = NA)
s5
hist(unlist(lapply(s5, function(x) x$FinalTime)))
```

As you decrease `checkSizePEvery` the distribution of “FinalTime” will resemble more and more an exponential distribution.

In this vignette, there are some further examples of using this mechanism in 5.11 and 5.8, with the default arguments.

5.5 Stochastic detection mechanism and minimum number of drivers

We said above that we exit as soon as any of the conditions is reached (i.e., we use an OR operation over the exit conditions). There is a special exception to this porcedure: if you set `AND_DrvProbExit = TRUE`, both the number of drivers and the `detectionProb` mechanism condition must fulfilled. This means that the `detectionProb` mechanism not assessed unless the `detectionDrivers` condition is. Using `AND_DrvProbExit = TRUE` allows to run simulations and ensure that all of the returned simulations will have at least some cells with the number of drivers as specified by `detectionDrivers`. Note, though, that this does not guarantee that when you sample the population, all those drivers will be detected (as this depends on the actual proportion of cells with the drivers and the settings of `samplePop`).

5.6 Fixation of genes/gene combinations with Ochs and Desai example

In some cases we might be interested in running simulations until a particular set of genes, or gene combinations, reaches fixation. This exit condition might be more relevant than some of the above in many non-cancer-related evolutionary genetics scenarios.

Simulations will stop as soon as any of the genes or gene combinations in the vector (or list) `fixation` reaches a frequency of 1. These gene combinations might have non-zero intersection (i.e., they might share genes), and those genes need not be drivers. If we want simulations to only stop when fixation of those genes/gene combinations is reached, we will set all other stopping conditions to `NA`. It is, of course, up to you to ensure that those stopping conditions are reasonable (that they can be reached) and to use, or not, `finalTime`; otherwise, simulations will eventually abort (e.g., when `max.wall.time` or `max.num.tries` are reached). Since we are asking for fixation, the `Exp` or `Bozic` models will often not be appropriate here; instead, models with competition and limited population size such as `McFL` are more appropriate.

We return here to the example from section @(ochsdesai).

```
u <- 0.2; i <- -0.02; vi <- 0.6; ui <- uv <- -Inf
od2 <- allFitnessEffects(
    epistasis = c("u" = u,  "u:i" = ui,
                  "u:v" = uv, "i" = i,
                  "v:-i" = -Inf, "v:i" = vi))
```

Ochs and Desai explain that “Each simulated population was evolved until either the uphill genotype or valley-crossing genotype fixed.” (see Ochs and Desai (2015), p.2, section “Simulations”). We will do the same here. We specify that we want to end the simulation when either the “u” or the “v, i” genotypes have reached fixation, by passing those genotype combinations as the `fixation` argument (in this example using `fixation = c("u", "v")` would have been equivalent, since the “v” genotype has fitness of 0).

We want to be explicit that fixation will be the one and only condition for ending the simulations, and thus we set arguments `detectionDrivers`, `finalTime`, `detectionSize` and `detectionProb` explicitly to `NA`. (We set the number of repetitions only to 50 for the sake of speed when creating the vignette).

```
initS <- 20
od100 <- oncoSimulPop(50, od2,
                      fixation = c("u", "v, i"),
                      model = "McFL",
                      mu = 1e-4,
                      detectionDrivers = NA,
                      finalTime = NA,
                      detectionSize = NA,
                      detectionProb = NA,
                      onlyCancer = TRUE,
                      initSize = initS,
                      mc.cores = 2)
```

What is the frequency of each genotype among the simulations? (or, what is the frequency of fixation of each genotype?)

```
sampledGenotypes(samplePop(od100))
##
##  Subjects by Genes matrix of 50 subjects and 3 genes.
##   Genotype Freq
## 1     i, v    9
## 2        u   41
```

Note the very large variability in reaching fixation (we show here the summary from only 20 simulations)

```
head(summary(od100)[, c(1:3, 8:9)], n = 20)
##    NumClones TotalPopSize LargestClone FinalTime NumIter
## 1          4           33           33    5300.8  212054
## 2          4           36           36    2586.2  103457
## 3          3           19           19    6971.0  278890
## 4          3           16           16    2018.4   80747
## 5          2           20           20     215.8    8634
## 6          3           21           21    1305.9   52242
## 7          3            6            6    2347.6   93925
## 8          3            8            8    1825.8   73049
## 9          3           11           11    3523.1  140946
## 10         2           32           32     529.0   21160
## 11         4           42           42    1065.6   42629
## 12         3           20           20    1356.6   54272
## 13         3           29           29    1021.2   40856
## 14         4           29           29    6026.7  241083
## 15         3           25           25    6187.3  247527
## 16         3           21           21     219.6    8787
## 17         3           36           36    1861.6   74473
## 18         3           23           23     754.9   30206
## 19         3           22           22    3072.0  122894
## 20         3           20           20    1341.7   53675
```

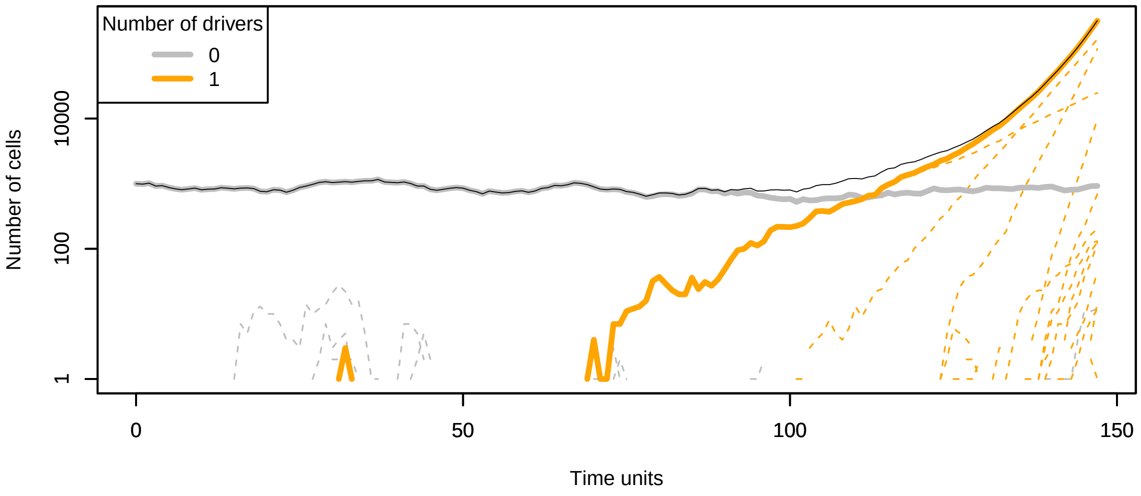
5.7 Bauer's example again

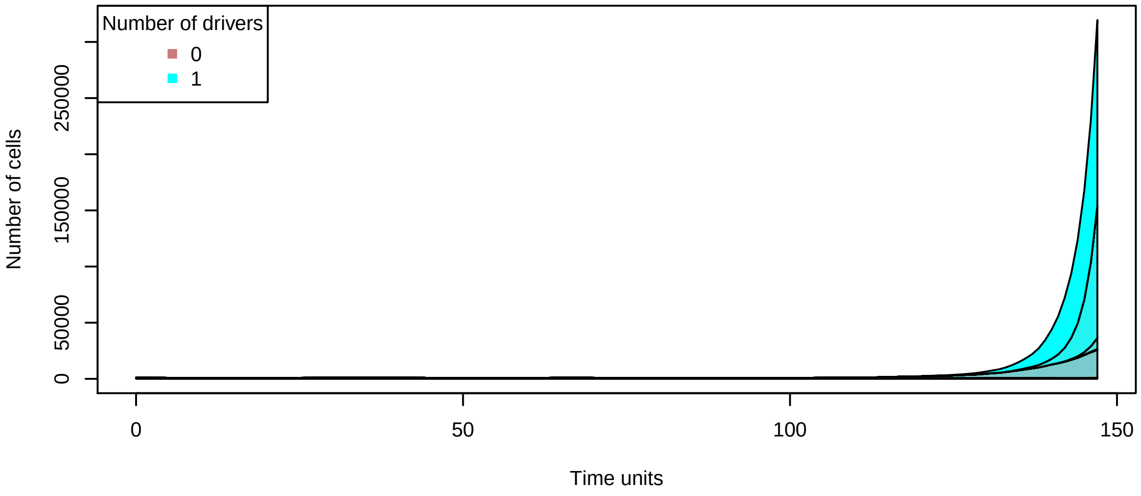
We will use the model of Bauer, Siebert, and Traulsen (2014) that we saw in section 4.1.

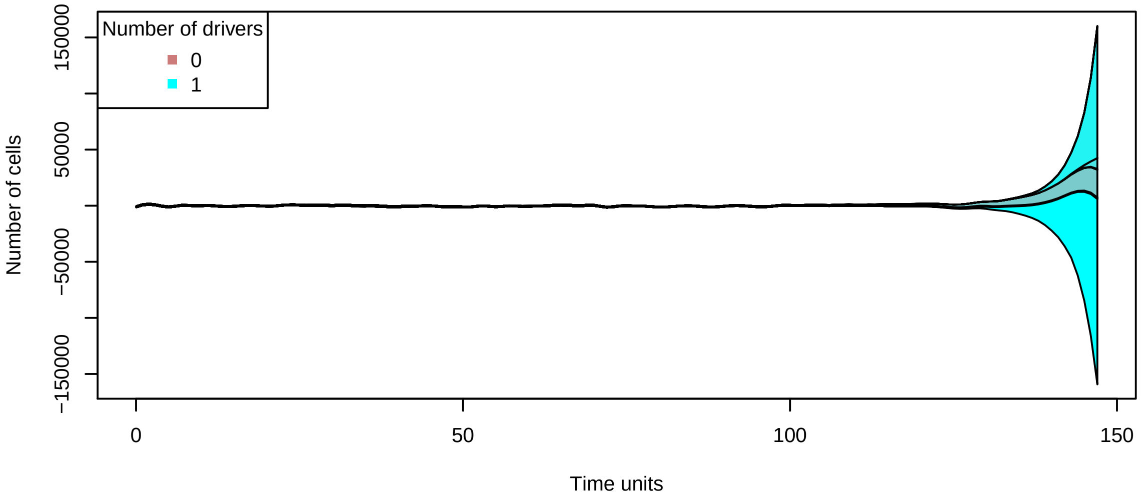
```r
K <- 5
sd <- 0.1
sdp <- 0.15
sp <- 0.05
bauer <- data.frame(parent = c("Root", rep("p", K)),
                    child = c("p", paste0("s", 1:K)),
                    s = c(sd, rep(sdp, K)),
                    sh = c(0, rep(sp, K)),
                    typeDep = "MN")
fbauer <- allFitnessEffects(bauer, drvNames = "p")
set.seed(1)
## Use fairly large mutation rate
b1 <- oncoSimulIndiv(fbauer, mu = 5e-5, initSize = 1000,
                     finalTime = NA,
                     onlyCancer = TRUE,
                     detectionProb = "default")
```

We will now use a variety of plots

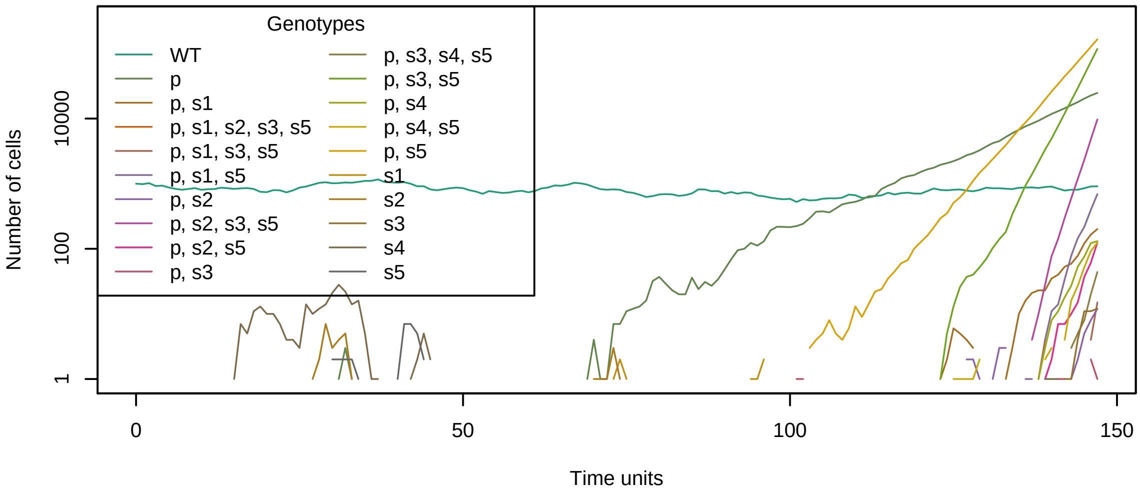
```r
par(mfrow = c(3, 1))
## First, drivers
plot(b1, type = "line", addtot = TRUE)
plot(b1, type = "stacked")
plot(b1, type = "stream")
```

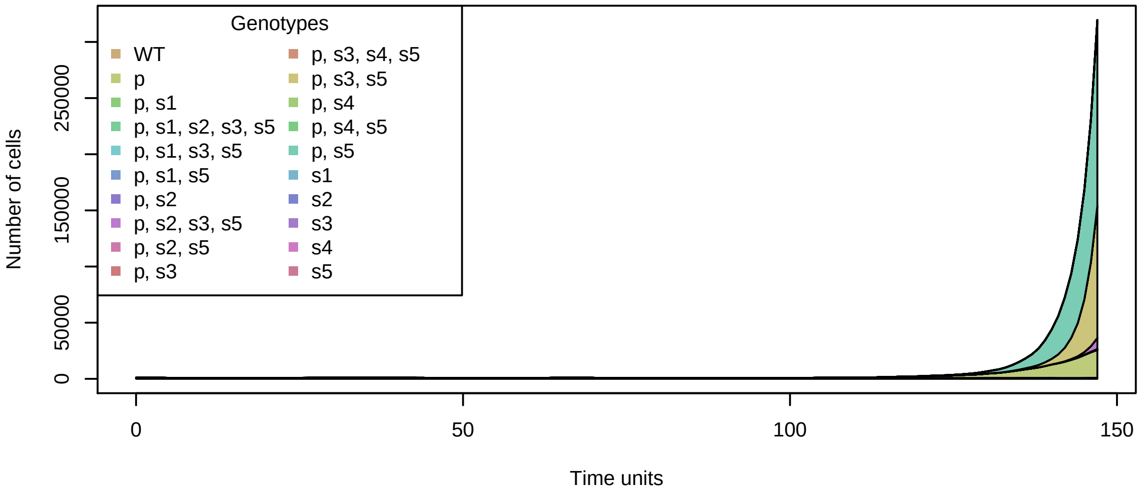

Number of drivers
0
1
Number of cells
1
100
10000
0
50
100
150
Time units

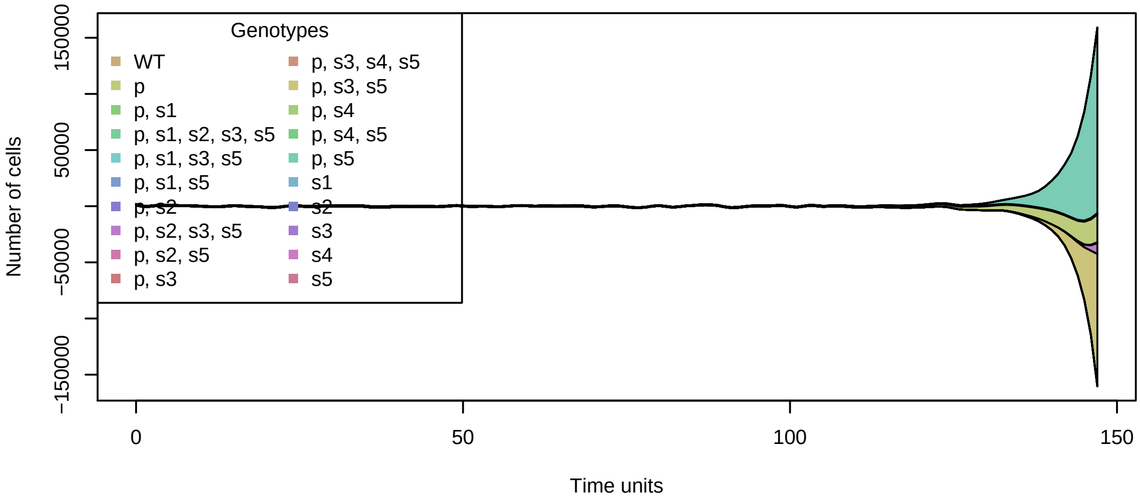

Number of drivers
■ 0
■ 1
Number of cells
0
50000
150000
250000
0
50
100
150
Time units


Number of drivers
■ 0
■ 1
Number of cells
−150000
−50000
50000
150000
0
50
100
150
Time units

```r
par(mfrow = c(3, 1))
## Next, genotypes
plot(b1, show = "genotypes", type = "line")
plot(b1, show = "genotypes", type = "stacked")
plot(b1, show = "genotypes", type = "stream")
```





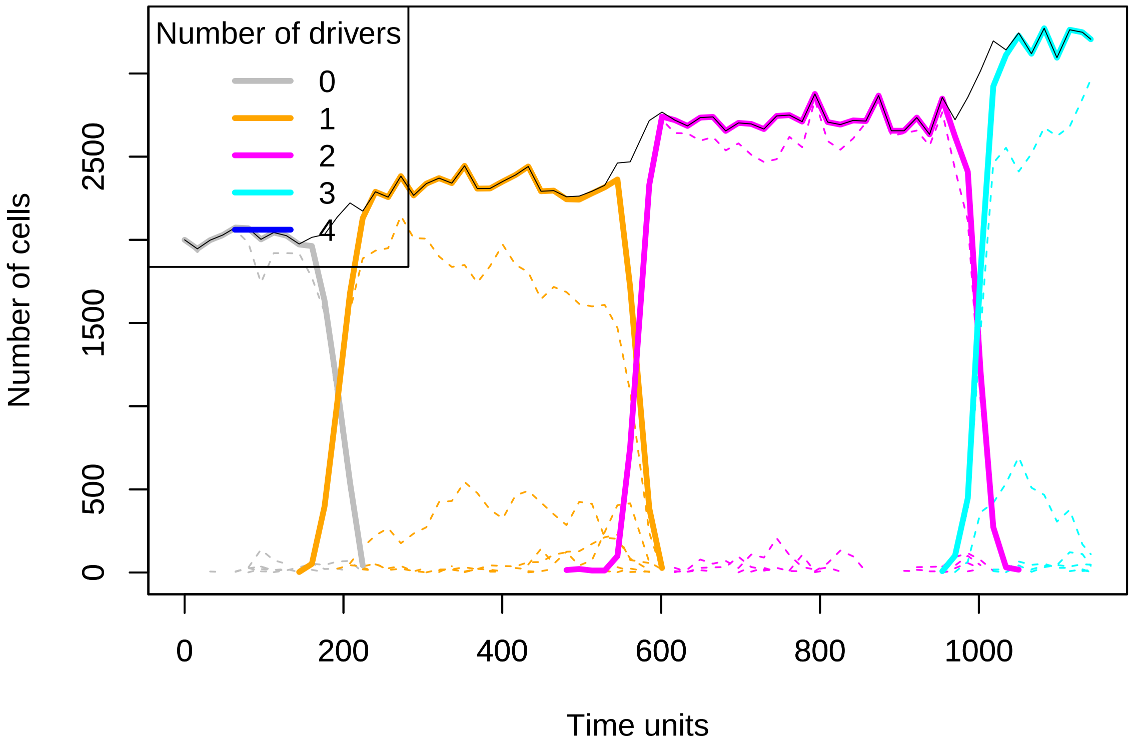


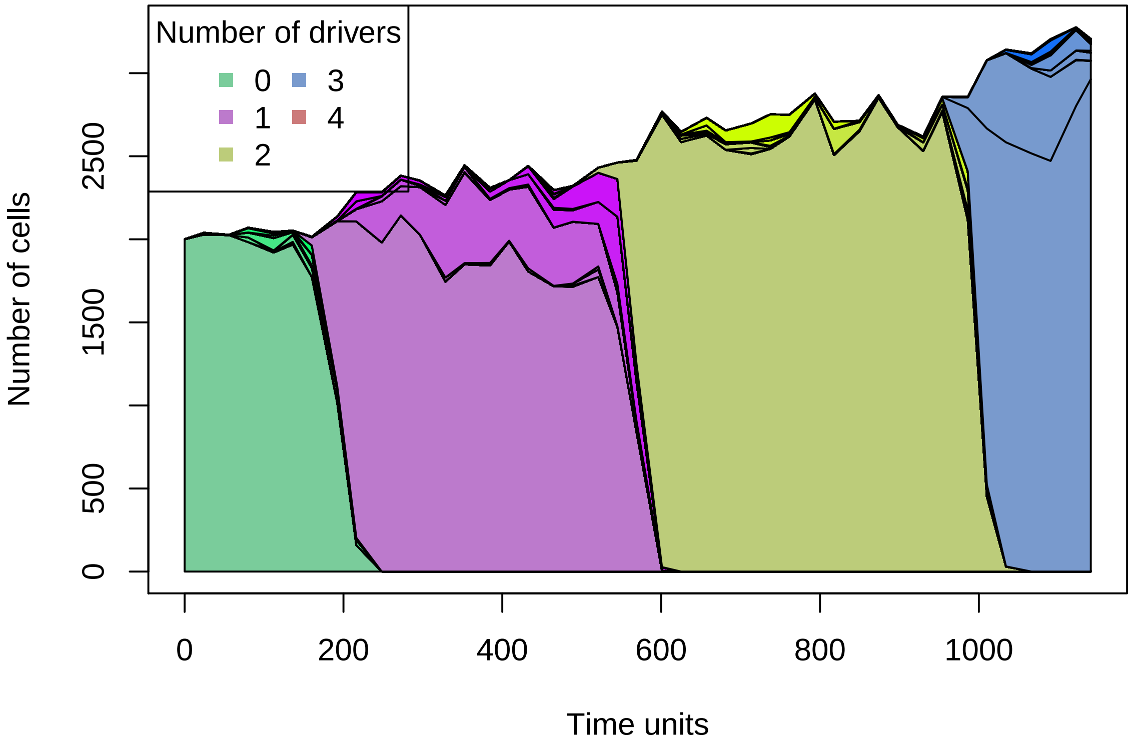
In this case, probably the stream plots are most helpful. Note, however, that (in contrast to some figures in the literature showing models of clonal expansion) the stream plot (or the stacked plot) does not try to explicitly show parent-descendant relationships, which would hardly be realistically possible in these plots (although the plots of phylogenies in section 8 could be of help).

5.8 McFarland model with 5000 passengers and 70 drivers

```
set.seed(678)
nd <- 70
np <- 5000
s <- 0.1
sp <- 1e-3
spp <- -sp/(1 + sp)
mcf1 <- allFitnessEffects(noIntGenes = c(rep(s, nd), rep(spp, np)),
                          drvNames = seq.int(nd))
mcf1s <-  oncoSimulIndiv(mcf1,
                         model = "McFL",
                         mu = 1e-7,
                         detectionProb = "default",
                         detectionSize = NA,
                         detectionDrivers = NA,
                         sampleEvery = 0.02,
                         keepEvery = 8,
                         initSize = 2000,
                         finalTime = 4000,
                         onlyCancer = FALSE)
summary(mcf1s)
##   NumClones TotalPopSize LargestClone MaxNumDrivers MaxDriversLast
## 1       425         3206         2964             4              3
##   NumDriversLargestPop TotalPresentDrivers FinalTime NumIter
## 1                    3                   6      1141   58762
##   HittedWallTime errorMF minDMratio minBMratio
## 1          FALSE 0.01365       1735       1972
##         OccurringDrivers
## 1 10, 18, 35, 60, 62, 66
```

```
par(mfrow  = c(2, 1))
## I use thinData to make figures smaller and faster
plot(mcf1s, addtot = TRUE, lwdClone = 0.9, log = "",
     thinData = TRUE, thinData.keep = 0.5)
plot(mcf1s, show = "drivers", type = "stacked",
     thinData = TRUE, thinData.keep = 0.3,
     legend.ncols = 2)
```





With the above output (where we see there are over 500 different genotypes) trying to represent the genotypes makes no sense.

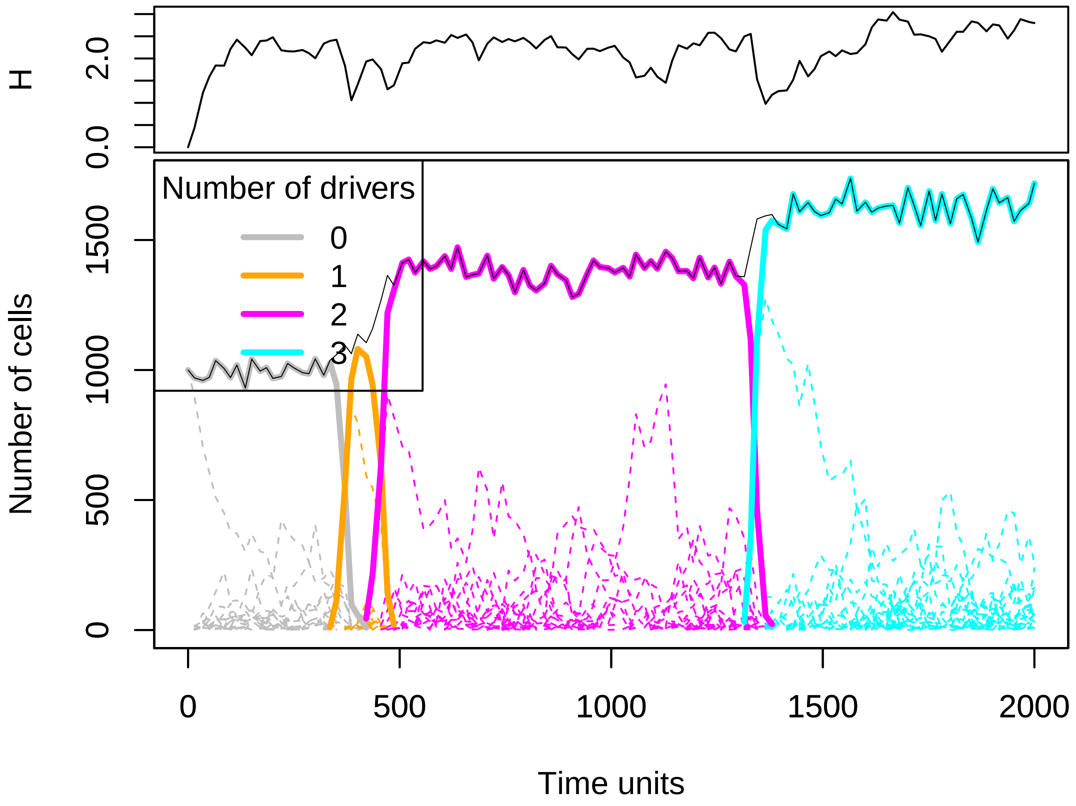
5.9 McFarland model with 50000 passengers and 70 drivers: clonal competition

The next is too slow (takes a couple of minutes in an i5 laptop) and too big to run in a vignette, because we keep track of over 4000 different clones (which leads to a result object of over 800 MB):

```
set.seed(123)
nd <- 70
np <- 50000
s <- 0.1
sp <- 1e-4 ## as we have many more passengers
spp <- -sp/(1 + sp)
mcfL <- allFitnessEffects(noIntGenes = c(rep(s, nd), rep(spp, np)),
                          drvNames = seq.int(nd))
mcfLs <-  oncoSimulIndiv(mcfL,
                         model = "McFL",
                         mu = 1e-7,
                         detectionSize = 1e8,
                         detectionDrivers = 100,
                         sampleEvery = 0.02,
                         keepEvery = 2,
                         initSize = 1000,
                         finalTime = 2000,
                         onlyCancer = FALSE)
```

But you can access the pre-stored results and plot them (beware: this object has been trimmed by removing empty passenger rows in the Genotype matrix)

```
data(mcfLs)
plot(mcfLs, addtot = TRUE, lwdClone = 0.9, log = "",
     thinData = TRUE, thinData.keep = 0.3,
     plotDiversity = TRUE)
```



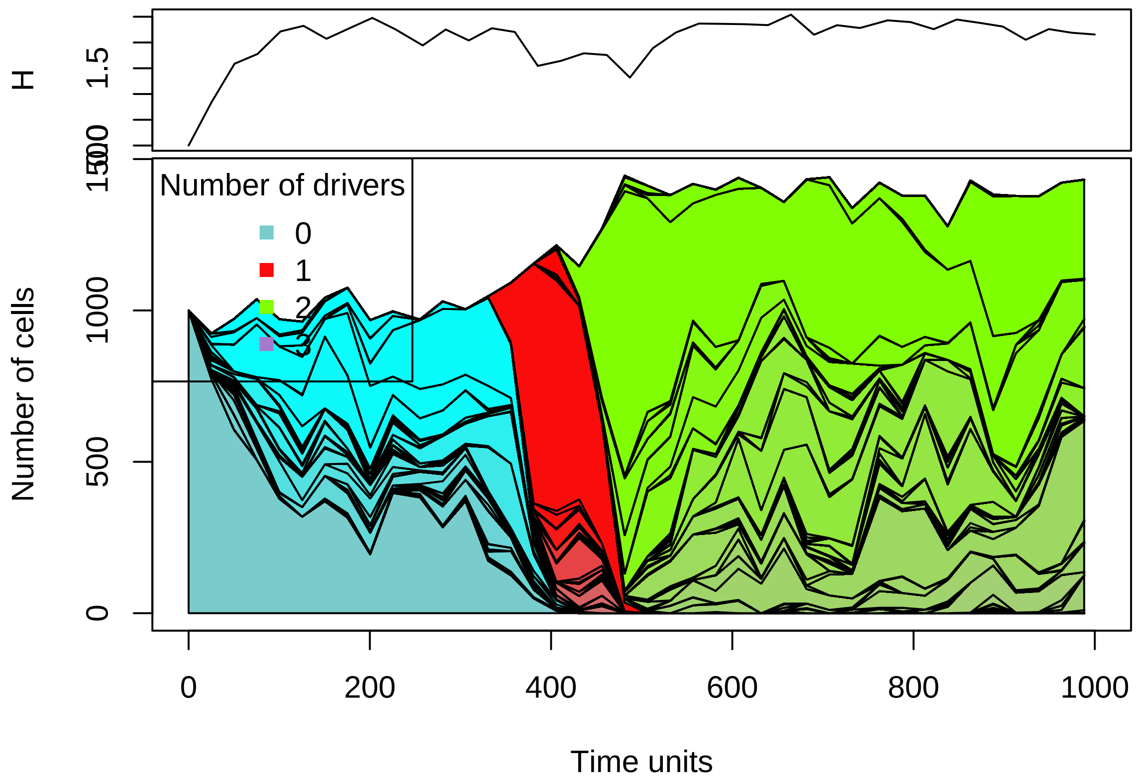
The argument `plotDiversity = TRUE` asks to show a small plot on top with Shannon's diversity index.

```
summary(mcfLs)
##   NumClones TotalPopSize LargestClone MaxNumDrivers MaxDriversLast
## 1      4458         1718          253             3              3
##   NumDriversLargestPop TotalPresentDrivers FinalTime NumIter
## 1                    3                  70      2000  113759
##   HittedWallTime errorMF minDMratio minBMratio OccurringDrivers
## 1          FALSE 0.01922      184.1      199.6   13, 38, 40, 69
## number of passengers per clone
summary(colSums(mcfLs$Genotypes[-(1:70), ]))
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##    0.00    4.00    6.00    5.67    7.75   13.00
```

Note that we see clonal competition between clones with the same number of drivers (and with different drivers, of course). We will return to this (section 5.12).

A stacked plot might be better to show the extent of clonal competition (plotting takes some time —a stream plot reveals similar patterns and is also slower than the line plot). I will aggressively thin the data for this plot so it is faster and smaller (but we miss some of the fine grain, of course):

```
plot(mcfLs, type = "stacked", thinData = TRUE,
     thinData.keep = 0.2,
     plotDiversity = TRUE,
     xlim = c(0, 1000))
```



5.10 Loading fitnessEffects data for simulation examples

We will use several of the previous examples. Most of them are in file `examplesFitnessEffects`, where they are stored inside a list, with named components (names the same as in the examples above):

```
data(examplesFitnessEffects)
names(examplesFitnessEffects)
##  [1] "cbn1"   "cbn2"   "smn1"   "xor1"   "fp3"    "fp4m"   "o3"
##  [8] "ofe1"   "ofe2"   "foi1"   "sv"     "svB"    "svB1"   "sv2"
## [15] "sm1"    "e2"     "E3A"    "em"     "fea"    "fbauer" "w"
## [22] "pancr"
```

5.11 Simulation with a conjunction example

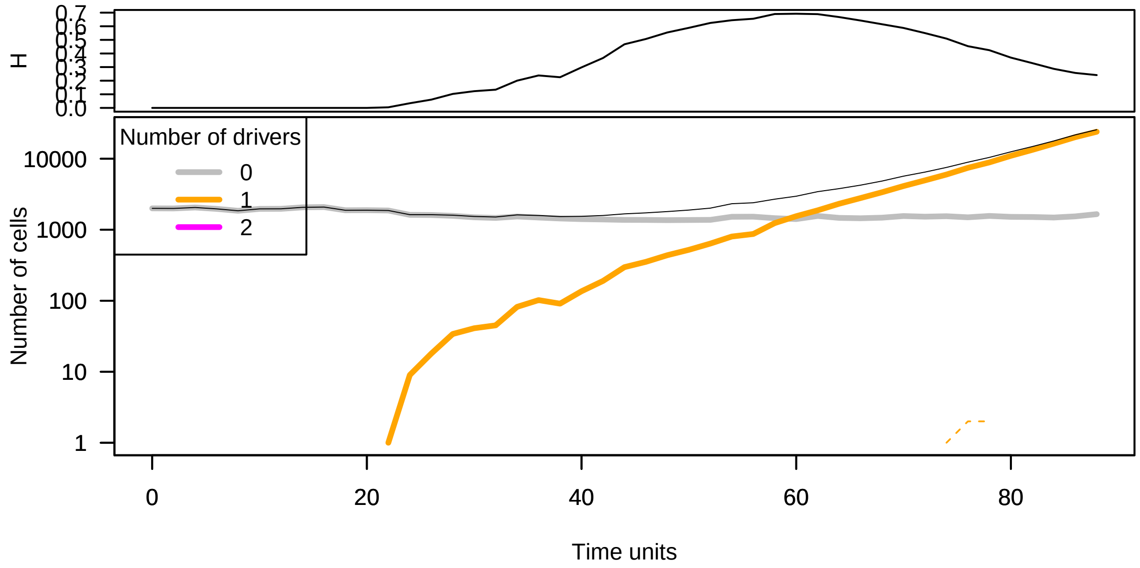
We will simulate using the simple CBN-like restrictions of section 2.4.4 with two different models.

```
data(examplesFitnessEffects)
evalAllGenotypes(examplesFitnessEffects$cbn1, order = FALSE)[1:10, ]
##    Genotype Fitness
## 1         a    1.10
## 2         b    1.10
## 3         c    0.10
## 4         d    1.10
## 5         e    1.10
## 6         g    0.10
## 7      a, b    1.21
## 8      a, c    0.11
## 9      a, d    1.21
## 10     a, e    1.21
sm <-  oncoSimulIndiv(examplesFitnessEffects$cbn1,
                      model = "McFL",
                      mu = 5e-7,
                      detectionSize = 1e8,
                      detectionDrivers = 2,
                      detectionProb = "default",
                      sampleEvery = 0.025,
                      keepEvery = 5,
                      initSize = 2000,
                      onlyCancer = TRUE)
summary(sm)
##   NumClones TotalPopSize LargestClone MaxNumDrivers MaxDriversLast
## 1         4         2613         2008             2              2
##   NumDriversLargestPop TotalPresentDrivers FinalTime NumIter
## 1                    2                   3      1896   75846
##   HittedWallTime errorMF minDMratio minBMratio OccurringDrivers
## 1          FALSE 0.01333     312129     333333          a, d, e
```

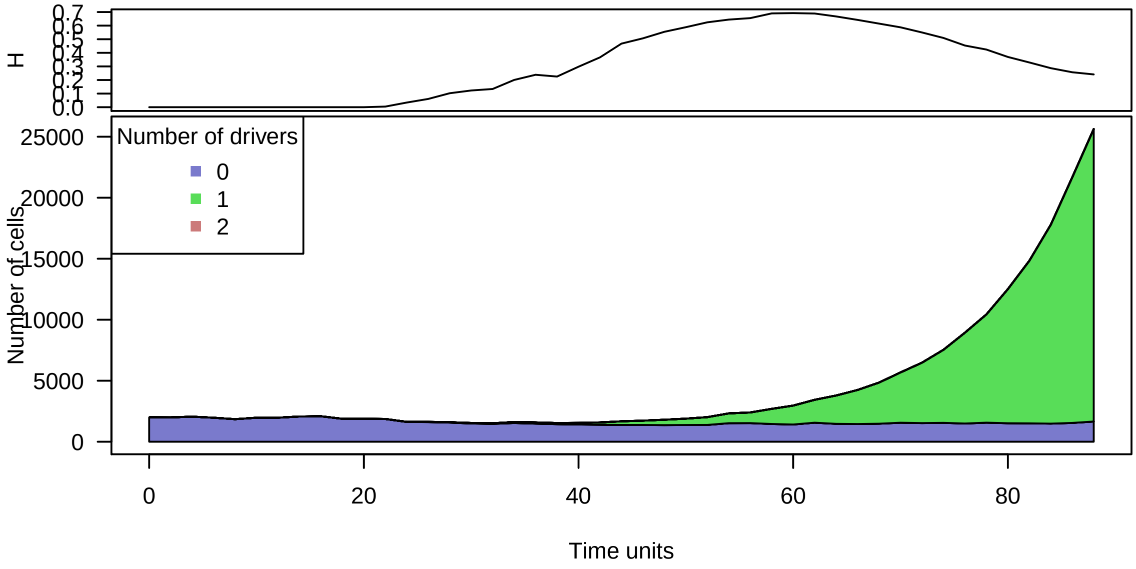
```
set.seed(1234)
evalAllGenotypes(examplesFitnessEffects$cbn1, order = FALSE,
                 model = "Bozic")[1:10, ]
##    Genotype Death_rate
## 1         a       0.90
## 2         b       0.90
## 3         c       1.90
## 4         d       0.90
## 5         e       0.90
## 6         g       1.90
## 7      a, b       0.81
## 8      a, c       1.71
## 9      a, d       0.81
## 10     a, e       0.81
sb <-  oncoSimulIndiv(examplesFitnessEffects$cbn1,
                       model = "Bozic",
                       mu = 5e-6,
                      detectionProb = "default",
                       detectionSize = 1e8,
                       detectionDrivers = 4,
                       sampleEvery = 2,
                       initSize = 2000,
                       onlyCancer = TRUE)
summary(sb)
##   NumClones TotalPopSize LargestClone MaxNumDrivers MaxDriversLast
## 1         7        25636        23975             2              2
##   NumDriversLargestPop TotalPresentDrivers FinalTime NumIter
## 1                    1                   5        88      53
##   HittedWallTime errorMF minDMratio minBMratio OccurringDrivers
## 1          FALSE      NA      33333      33333    a, b, c, e, g
```

As usual, we will use several plots here.

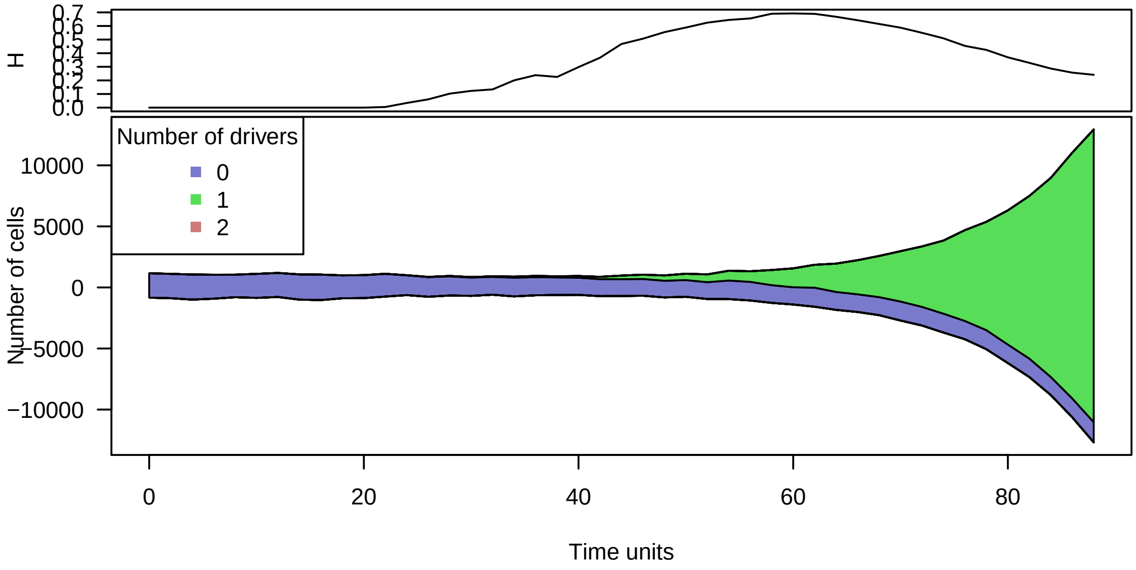
```
## Show drivers, line plot
par(cex = 0.75, las = 1)
plot(sb,show = "drivers", type = "line", addtot = TRUE,
     plotDiversity = TRUE)
```



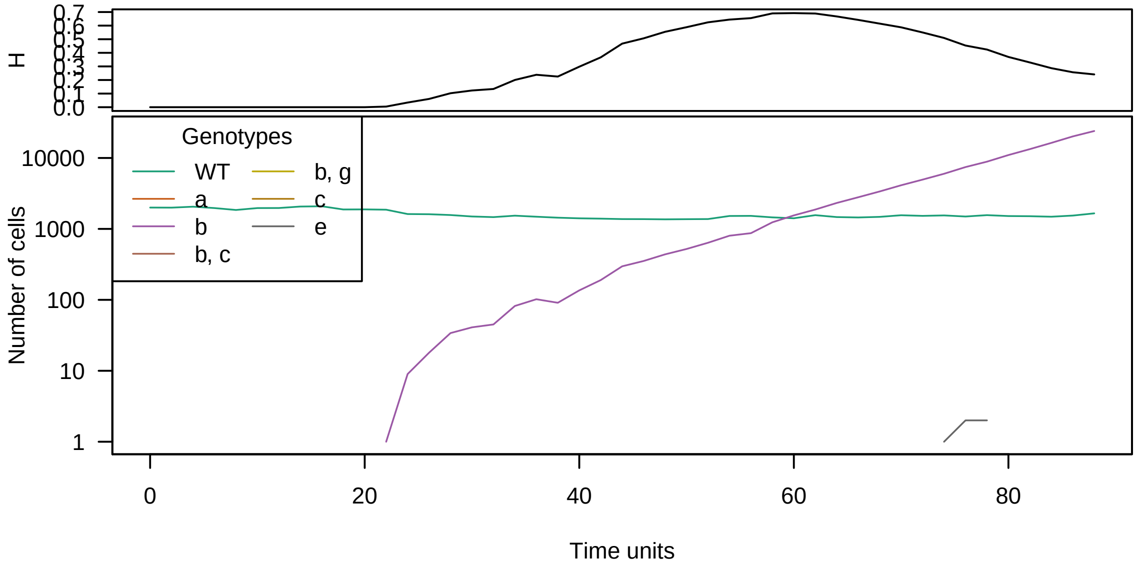
```
## Drivers, stacked
par(cex = 0.75, las = 1)
plot(sb,show = "drivers", type = "stacked", plotDiversity = TRUE)
```



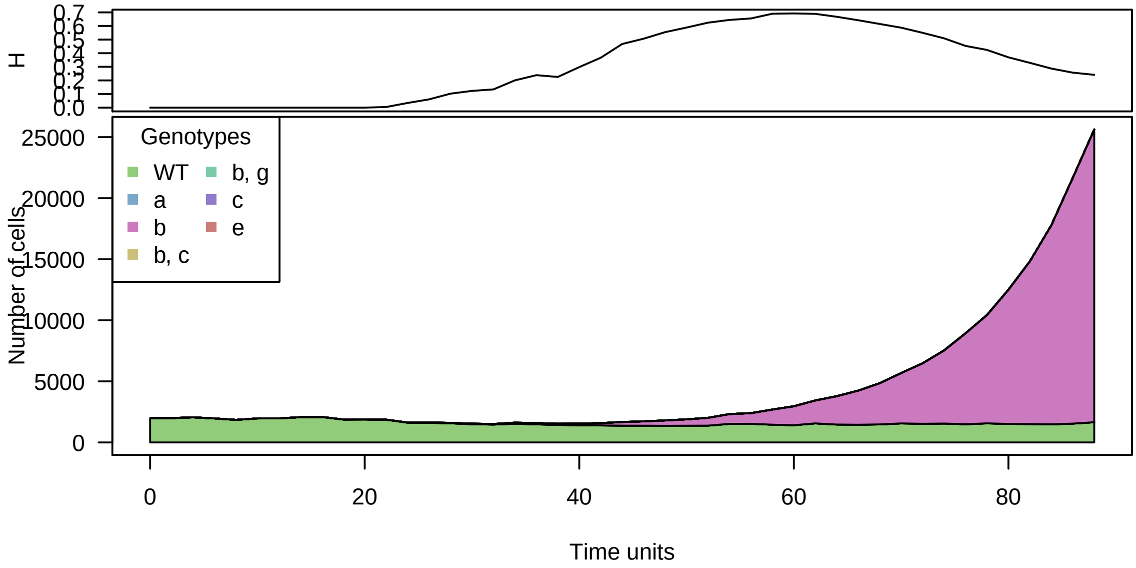
```
## Drivers, stream
par(cex = 0.75, las = 1)
plot(sb,show = "drivers", type = "stream", plotDiversity = TRUE)
```



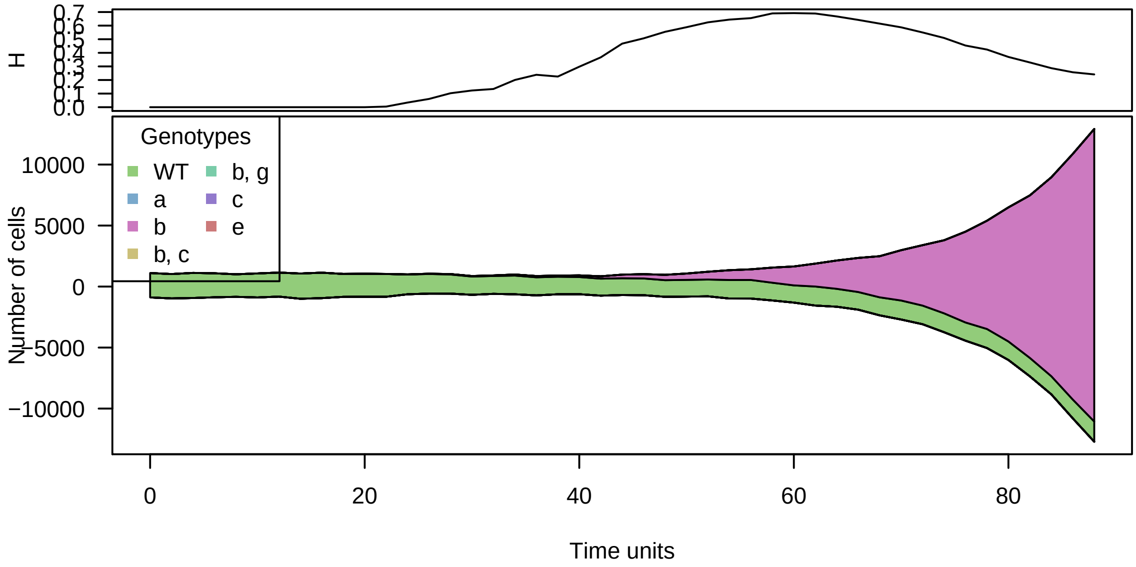
```
## Genotypes, line plot
par(cex = 0.75, las = 1)
plot(sb,show = "genotypes", type = "line", plotDiversity = TRUE)
```



```
## Genotypes, stacked
par(cex = 0.75, las = 1)
plot(sb,show = "genotypes", type = "stacked", plotDiversity = TRUE)
```



```
## Genotypes, stream
par(cex = 0.75, las = 1)
plot(sb,show = "genotypes", type = "stream", plotDiversity = TRUE)
```



The above illustrates again that different types of plots can be useful to reveal different patterns in the data. For instance, here, because of the huge relative frequency of one of the clones/genotypes, the stacked and stream plots do not reveal the other clones/genotypes as we cannot use a log-transformed y-axis, even if there are other clones/genotypes present.

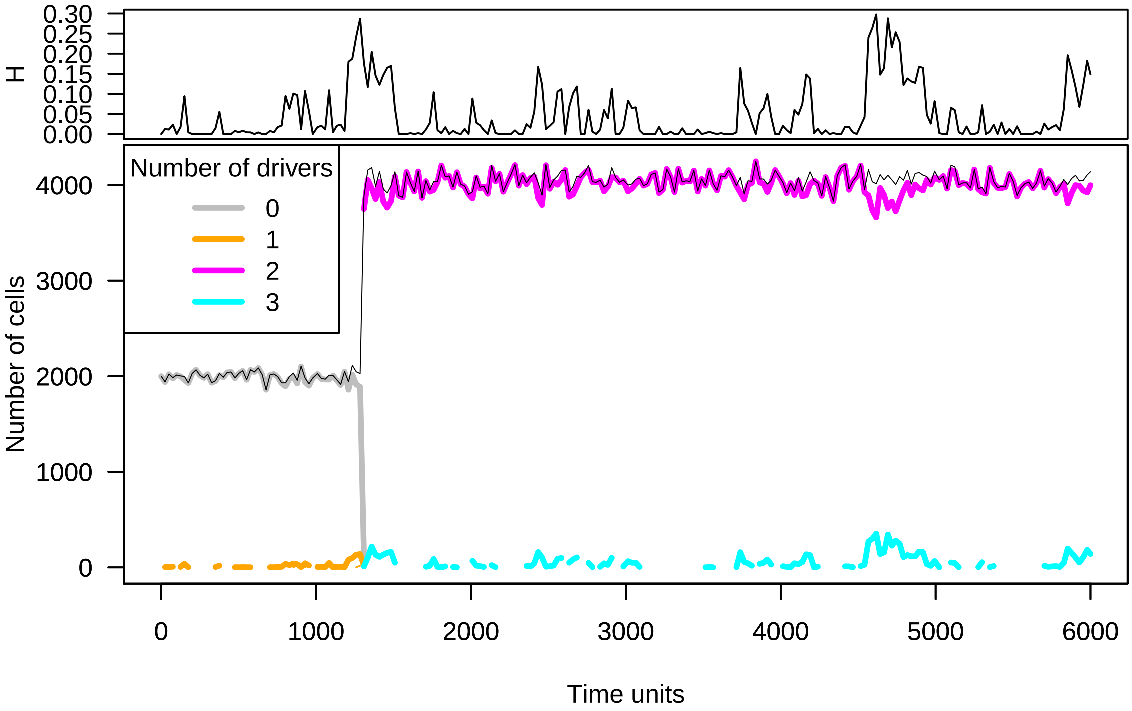
5.12 Simulation with order effects and McFL model

(We use a somewhat large mutation rate than usual, so that the simulation runs quickly.)

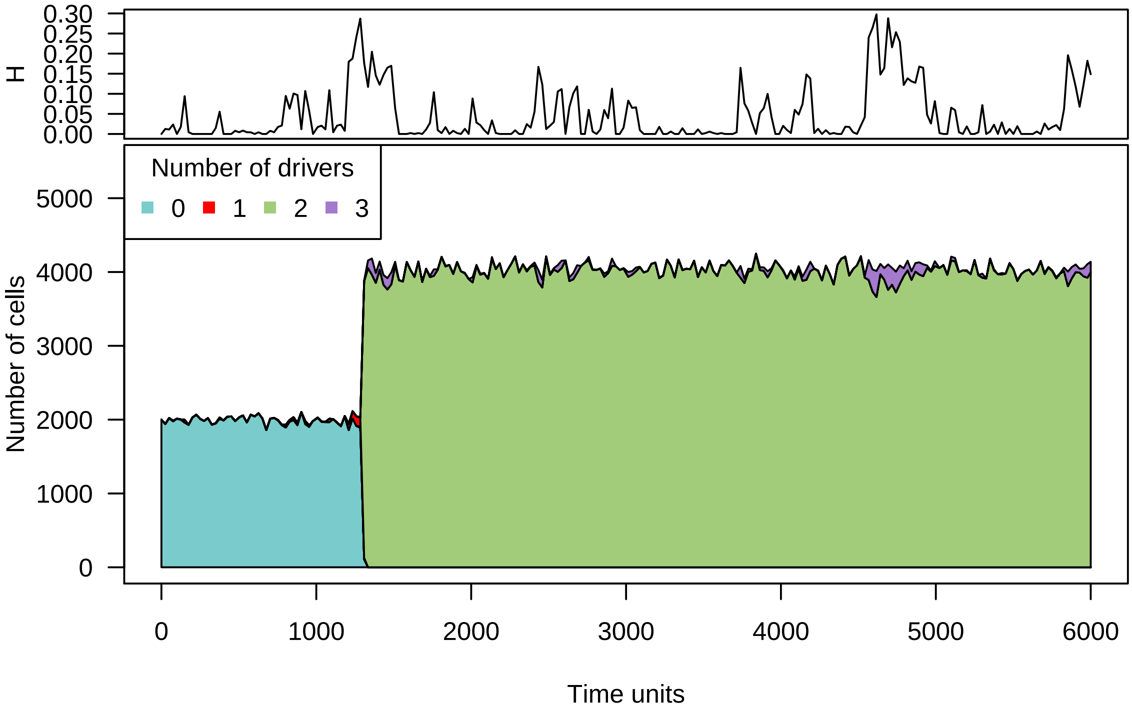
```r
set.seed(4321)
tmp <-  oncoSimulIndiv(examplesFitnessEffects[["o3"]],
                       model = "McFL",
                       mu = 5e-5,
                       detectionSize = 1e8,
                       detectionDrivers = 3,
                       sampleEvery = 0.025,
                       max.num.tries = 10,
                       keepEvery = 5,
                       initSize = 2000,
                       finalTime = 6000,
                       onlyCancer = FALSE)
```

We show a stacked and a line plot of the drivers:

```r
par(las = 1, cex = 0.85)
plot(tmp, addtot = TRUE, log = "", plotDiversity = TRUE,
     thinData = TRUE, thinData.keep = 0.2)
```



```r
par(las = 1, cex = 0.85)
plot(tmp, type = "stacked", plotDiversity = TRUE,
     ylim = c(0, 5500), legend.ncols = 4,
     thinData = TRUE, thinData.keep = 0.2)
```

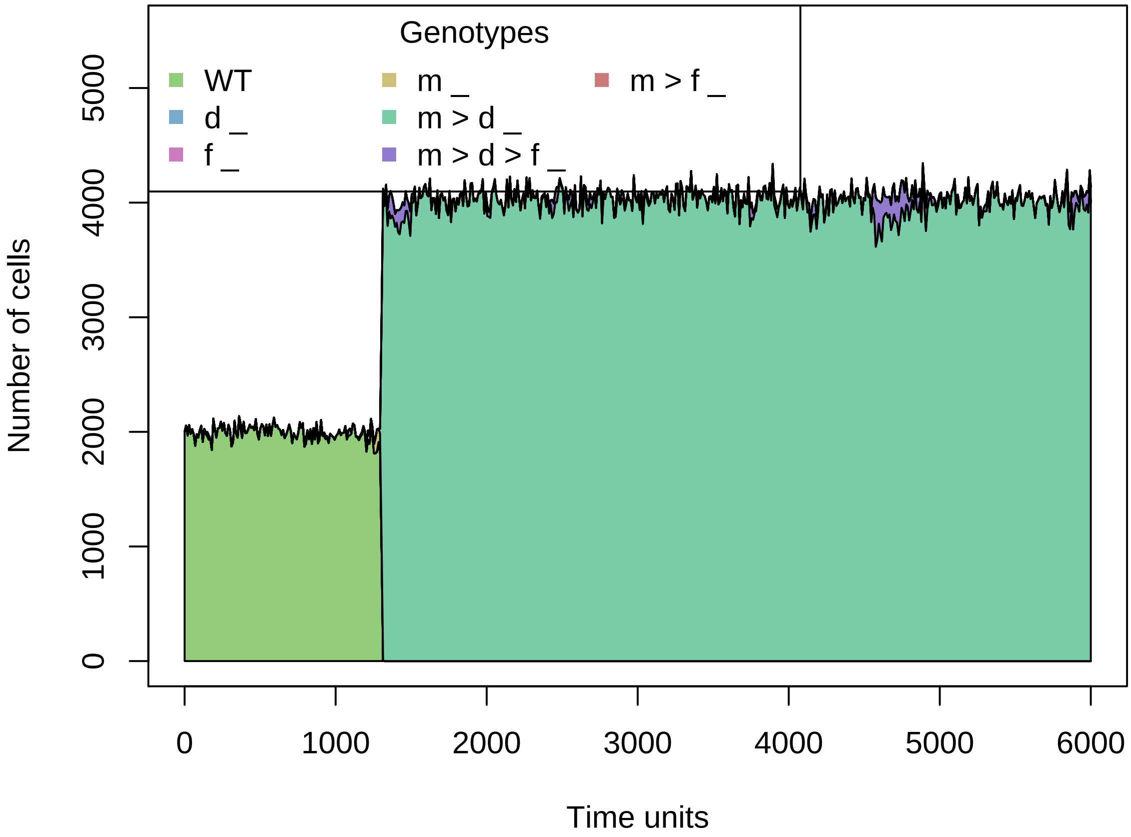


In this example (and at least under Linux, with both GCC and clang), we can see that the mutants with three drivers do not get established when we stop the simulation at time 6000. This is one case where the summary statistics about number of drivers says little of value, as fitness is very different for genotypes with the same number of mutations, and does not increase in a simple way with drivers:

```
evalAllGenotypes(examplesFitnessEffects[["o3"]], addwt = TRUE,
                 order = TRUE)
##      Genotype Fitness
## 1          WT    1.00
## 2           d    1.00
## 3           f    1.00
## 4           m    1.00
## 5       d > f    1.00
## 6       d > m    1.10
## 7       f > d    1.00
## 8       f > m    1.00
## 9       m > d    1.50
## 10      m > f    1.00
## 11 d > f > m    1.54
## 12 d > m > f    1.32
## 13 f > d > m    0.77
## 14 f > m > d    1.50
## 15 m > d > f    1.50
## 16 m > f > d    1.50
```

A few figures could help:

```
plot(tmp, show = "genotypes", ylim = c(0, 5500), legend.ncols = 3,
     thinData = TRUE, thinData.keep = 0.5)
```



(When reading the figure legends, recall that genotype $x > y \_ z$ is one where a mutation in "x" happened before a mutation in "y", and there is also a mutation in "z" for which order does not matter. Here, there are no genes for which order does not matter and thus there is nothing after the "_"").

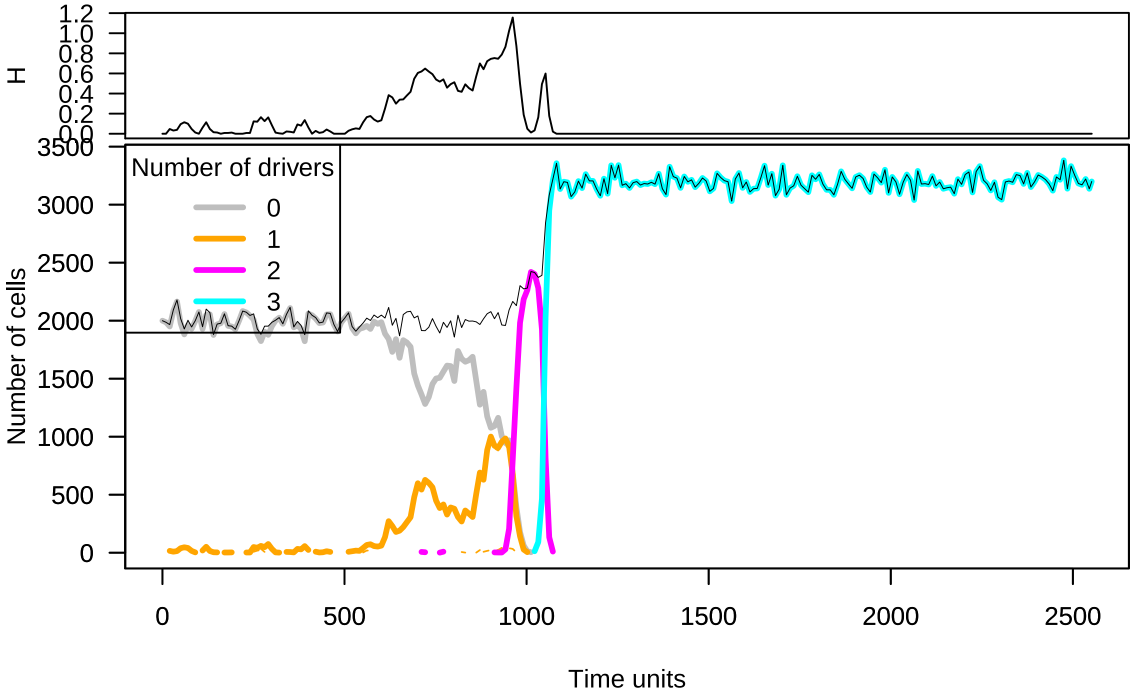
In this case, the clones with three drivers end up displacing those with two by the time we stop; moreover, notice how those with one driver never really grow to a large population size, so we basically go from a population with clones with zero drivers to a population made of clones with two or three drivers:

```
set.seed(15)
tmp <-  oncoSimulIndiv(examplesFitnessEffects[["o3"]],
                       model = "McFL",
                       mu = 5e-5,
                       detectionSize = 1e8,
                       detectionDrivers = 3,
                       sampleEvery = 0.015,
                       max.num.tries = 10,
                       keepEvery = 5,
                       initSize = 2000,
                       finalTime = 20000,
                       onlyCancer = FALSE,
                       extraTime = 1500)
tmp
##
```

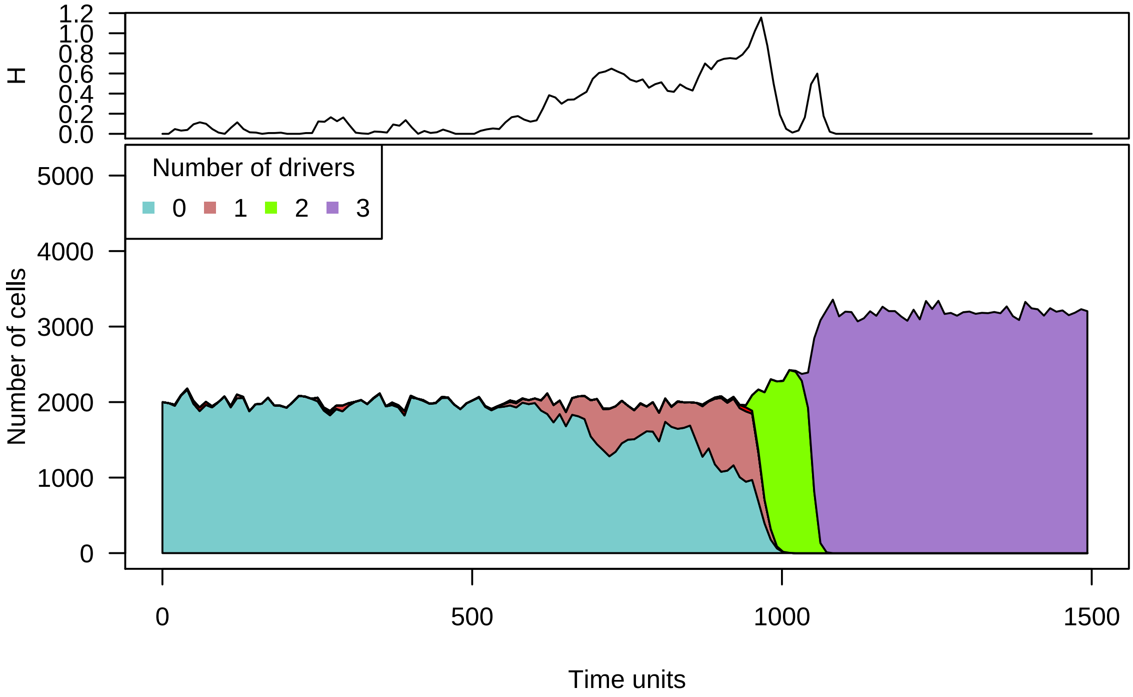
```
## Individual OncoSimul trajectory with call:
##  oncoSimulIndiv(fp = examplesFitnessEffects[["o3"]], model = "McFL",
##     mu = 5e-05, detectionSize = 1e+08, detectionDrivers = 3,
##     sampleEvery = 0.015, initSize = 2000, keepEvery = 5, extraTime = 1500,
##     finalTime = 20000, onlyCancer = FALSE, max.num.tries = 10)
##
##   NumClones TotalPopSize LargestClone MaxNumDrivers MaxDriversLast
## 1         7         3200         3200             3              3
##   NumDriversLargestPop TotalPresentDrivers FinalTime NumIter
## 1                    3                   3      2552  170395
##   HittedWallTime errorMF minDMratio minBMratio OccurringDrivers
## 1          FALSE 0.01218       6276       6667          d, f, m
##
## Final population composition:
##       Genotype    N
## 1            _    0
## 2          d _    0
## 3      d > f _    0
## 4      d > m _    0
## 5  d > m > f _ 3200
## 6          f _    0
## 7          m _    0
```

use a drivers plot:

```
par(las = 1, cex = 0.85)
plot(tmp, addtot = TRUE, log = "", plotDiversity = TRUE,
     thinData = TRUE, thinData.keep = 0.5)
```

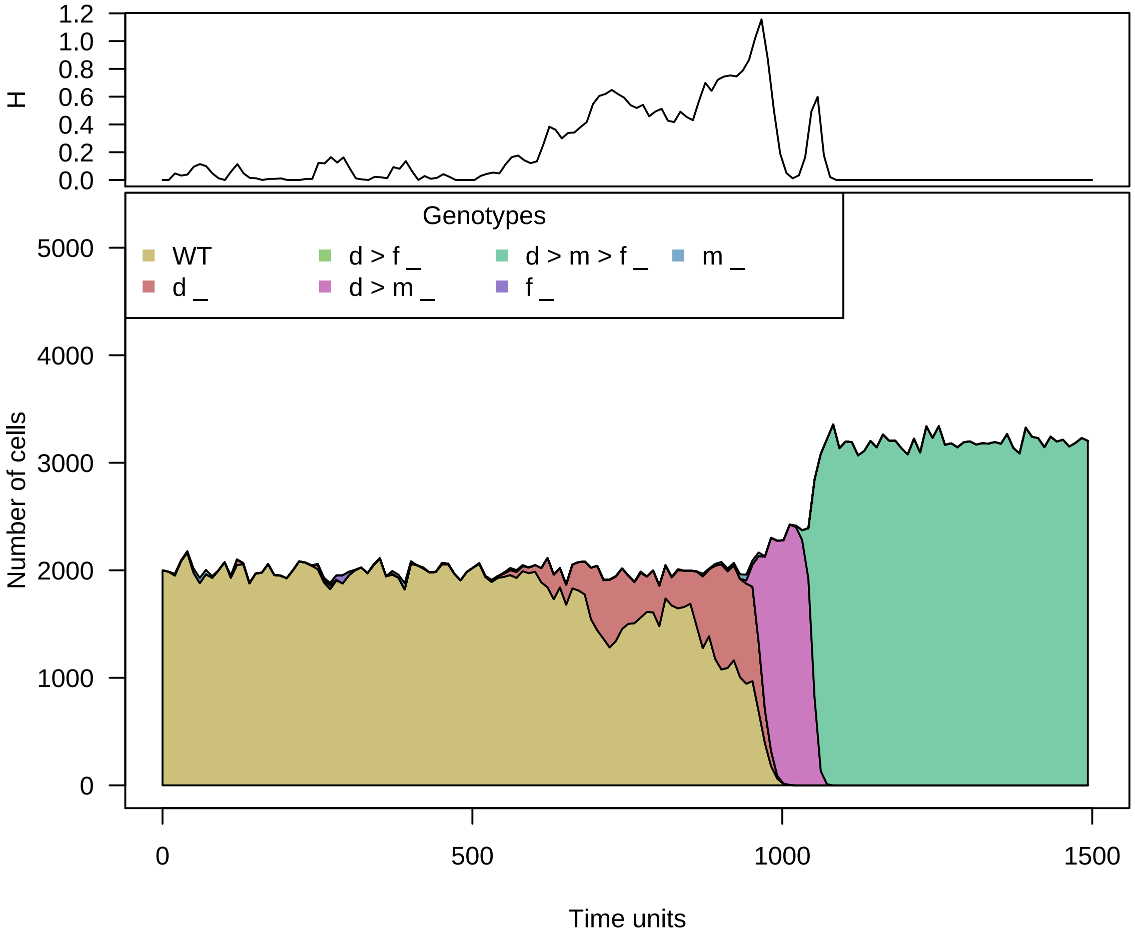


```
par(las = 1, cex = 0.85)
plot(tmp, type = "stacked", plotDiversity = TRUE,
     legend.ncols = 4, ylim = c(0, 5200), xlim = c(0, 1500),
     thinData = TRUE, thinData.keep = 0.5)
```



Now show the genotypes explicitly:

```
## Improve telling appart the most abundant
## genotypes by sorting colors
## differently via breakSortColors
## Modify ncols of legend, so it is legible by not overlapping
## with plot
par(las = 1, cex = 0.85)
plot(tmp, show = "genotypes", breakSortColors = "distave",
     plotDiversity = TRUE, legend.ncols = 4,
     ylim = c(0, 5300), xlim = c(0, 1500),
     thinData = TRUE, thinData.keep = 0.5)
```



As before, the argument `plotDiversity = TRUE` asks to show a small plot on top with Shannon's diversity index. Here, as before, the quick clonal expansion of the clone with two drivers leads to a sudden drop in diversity (for a while, the population is made virtually of a single clone). Note, however, that compared to section 5.9, we are modeling here a scenario with very few genes, and correspondingly very few possible genotypes, and thus it is not strange that we observe very little diversity.

(We have used `extraTime` to continue the simulation well past the point of detection, here specified as three drivers. Instead of specifying `extraTime` we can set the `detectionDrivers` value to a number larger than the number of existing possible drivers, and the simulation will run until `finalTime` if `onlyCancer = FALSE`.)

5.13 Numerical issues with death rates of 0 in Bozic model

As we mentioned above (section 2.7.2) death rates of 0 can lead to trouble when using Bozic's model:

```
i1 <- allFitnessEffects(noIntGenes = c(1, 0.5))
evalAllGenotypes(i1, order = FALSE, addwt = TRUE,
                 model = "Bozic")
##   Genotype Death_rate
## 1       WT        1.0
## 2        1        0.0
## 3        2        0.5
## 4     1, 2        0.0
i1_b <- oncoSimulIndiv(i1, model = "Bozic")
## Warning in nr_oncoSimul.internal(rFE = fp, birth = birth, death
## = death, : You are using a Bozic model with the new restriction
## specification, and you have at least one s of 1. If that gene is
## mutated, this will lead to a death rate of 0 and the simulations
## will abort when you get a non finite value.
##
##  DEBUG2: Value of rnb = nan
##
##  DEBUG2: Value of m = 1
##
##  DEBUG2: Value of pe = 0
##
##  DEBUG2: Value of pm = 1
##
##  this is spP
##
##  popSize = 1
##  birth = 1
##  death = 0
##  W = 1
##  R = 1
##  mutation = 1e-10
##  timeLastUpdate = 1080.56
##  absfitness = -inf
##  numMutablePos =0
##
##  Unrecoverable exception: Algo 2: retval not finite. Aborting.
```

Of course, there is no problem in using the above with other models:

```
evalAllGenotypes(i1, order = FALSE, addwt = TRUE,
                 model = "Exp")
##   Genotype Fitness
## 1       WT     1.0
## 2        1     2.0
## 3        2     1.5
## 4     1, 2     3.0
i1_e <- oncoSimulIndiv(i1, model = "Exp")
summary(i1_e)
##   NumClones TotalPopSize LargestClone MaxNumDrivers MaxDriversLast
## 1         3    129193871     74702920             0              0
##   NumDriversLargestPop TotalPresentDrivers FinalTime NumIter
## 1                    0                   0       514     688
##   HittedWallTime errorMF minDMratio minBMratio OccurringDrivers
## 1          FALSE      NA      5e+05      5e+05
```

5.14 Interactive graphics

It is possible to create interactive stacked area and stream plots using the *streamgraph* package, available from https://github.com/hrbrmstr/streamgraph. However, that package is not available as a CRAN or BioConductor package, and thus we cannot depend on it for this vignette (or this package). You can, however, paste the code below and make it run locally.

Before calling the *streamgraph* function, though, we need to convert the data from the original format in which it is stored into "long format". A simple convenience function is provided as *OncoSimulWide2Long* in *OncoSimulR*.

As an example, we will use the data we generated above for section 5.7.

```
## Convert the data
lb1 <- OncoSimulWide2Long(b1)

## Install the streamgraph package from github and load
library(devtools)
devtools::install_github("hrbrmstr/streamgraph")
library(streamgraph)

## Stream plot for Genotypes
sg_legend(streamgraph(lb1, Genotype, Y, Time, scale = "continuous"),
              show=TRUE, label="Genotype: ")

## Staked area plot and we use the pipe
streamgraph(lb1, Genotype, Y, Time, scale = "continuous",
            offset = "zero") %>%
    sg_legend(show=TRUE, label="Genotype: ")
```

6 Sampling multiple simulations

Often, you will want to simulate multiple runs of the same scenario, and then obtain the matrix of runs by mutations (a matrix of individuals/samples by genes or, equivalently, a vector of "genotypes"), and do something with them. OncoSimulR offers several ways of doing this.

The key function here is *samplePop*, either called explicitly after *oncoSimulPop* (or *oncoSimulIndiv*), or implicitly as part of a call to *oncoSimulSample*. With *samplePop* you can use **single cell** or **whole tumor** sampling (for details see the help of *samplePop*). Depending on how the simulations were conducted, you might also sample at different times, or as a function of population sizes. A major difference between procedures has to do with whether or not you want to keep the complete history of the simulations.

You want to keep the complete history of simulations. You will simulate using:

- oncoSimulIndiv repeatedly (maybe within *mclapply*), to parallelize the run).
- oncoSimulPop. oncoSimulPop is basically a thin wrapper around oncoSimulIndiv that uses *mclapply*.

In both cases, you specify the conditions for ending the simulations (as explained in 5.3).

Then, you will use function oncoSimulSample to obtain the matrix of samples by mutations.

You do not want to keep the complete history of the simulations. You will simulate using:

- oncoSimulIndiv repeatedly, with argument keepEvery = NA.
- oncoSimulPop, with argument keepEvery = NA.

In both cases you specify the conditions for ending the simulations (as explained in 5.3). Then, you will use function oncoSimulSample.

- oncoSimulSample, specifying the conditions for ending the simulations (as explained in 5.3). In this case, you will not use oncoSimulSample, as that is implicitly called by oncoSimulSample. The output is directly the matrix (and a little bit of summary from each run), and during the simulation it only stores one time point.

Why the difference between the above cases? If you keep the complete history, you can take samples at any of the times between the beginning and the end of the simulations. If you do not keep the history, you can only sample at the time the simulation exited. Why would you want to use the second route? If we are only interested in the final matrix of individuals by mutations, keeping the complete history the above is wasteful, because we store fully all of the simulations (for example in the call to *oncoSimulPop*) and then sample (in the call to *samplePop*). Other reasons for choosing one over the other might have to do with flexibility (e.g., if you use *oncoSimulPop* the arguments for detectionSize, detectionDrivers must be the same for all simulations but this is not the case for *oncoSimulSample*) and parallelized execution.

Further criteria to use when choosing between sampling procedures is to realize that if you use *oncoSimulPop* the arguments for detectionSize and detectionDrivers must be the same for all simulations. This is not the case for *oncoSimulSample*. See further comments in 6.1.

The following are a few examples. First we run oncoSimulPop to obtain 4 simulations and in the last line we sample from them:

```
pancrPop <- oncoSimulPop(4, pancr,
                         detectionSize = 1e7,
                         keepEvery = 10,
                         mc.cores = 2)

summary(pancrPop)
##   NumClones TotalPopSize LargestClone MaxNumDrivers MaxDriversLast
## 1         9     10566707     10481674             0              0
## 2        10     10837903     10620191             0              0
## 3        13     10487303      8430193             0              0
## 4        13     10673640     10519058             0              0
##   NumDriversLargestPop TotalPresentDrivers FinalTime NumIter
## 1                    0                   0      1842    2565
## 2                    0                   0       204     854
## 3                    0                   0      1309    1930
## 4                    0                   0      1171    1930
##   HittedWallTime errorMF minDMratio minBMratio OccurringDrivers
## 1          FALSE      NA     142857     142857
## 2          FALSE      NA     142857     142857
## 3          FALSE      NA     142857     142857
## 4          FALSE      NA     142857     142857
samplePop(pancrPop)
##
##  Subjects by Genes matrix of 4 subjects and 7 genes.
##      CDNK2A KRAS MLL3 PXDN SMAD4 TGFBR2 TP53
## [1,]      0    1    0    0     0      0    0
## [2,]      0    1    0    0     0      0    0
## [3,]      0    1    0    0     0      0    0
## [4,]      0    1    0    0     0      0    0
```

Now a simple multiple call to oncoSimulIndiv wrapped inside *mclapply*; this is basically the same we just did above. We set the class of the object to allow direct usage of *samplePop*. (Note: in Windows mc.cores > 1 is not supported, so for the vignette to run in Windows, Linux, and Mac we explicitly set it here in the call to *mclapply*. For regular usage, you will not need to do this; just use whatever is appropriate for your operating system and number of cores. As well, we do not need any of this with *oncoSimulPop* because the code inside *oncoSimulPop* already takes care of setting mc.cores to 1 in Windows).

```
library(parallel)

if(.Platform$OS.type == "windows") {
    mc.cores <- 1
} else {
    mc.cores <- 2
}

p2 <- mclapply(1:4, function(x) oncoSimulIndiv(pancr,
                                               detectionSize = 1e7,
                                               keepEvery = 10),
                                               mc.cores = mc.cores)
class(p2) <- "oncosimulpop"
samplePop(p2)
##
##  Subjects by Genes matrix of 4 subjects and 7 genes.
##      CDNK2A KRAS MLL3 PXDN SMAD4 TGFBR2 TP53
## [1,]      0    1    0    0     0      0    0
## [2,]      0    1    0    0     0      0    0
## [3,]      0    1    0    0     0      0    0
## [4,]      0    1    0    0     0      0    0
```

Above, we have kept the complete history of the simulations as you can check by doing, for instance

```
tail(pancrPop[[1]]$pops.by.time)
##          [,1] [,2]  [,3]     [,4] [,5] [,6]  [,7] [,8]  [,9] [,10]
## [181,] 1800 2324     0   155383    1    0     4    0    17     0
## [182,] 1810 2302    17   428633    2    3    14    0    77     0
## [183,] 1820 2085   153  1166073  112    1   415    0   296     0
## [184,] 1830 2006  1354  3162647  777    3  2822    0  1985     0
## [185,] 1840 2116 10738  8577487 6608    8 21287   10 16068     0
## [186,] 1842 2048 15989 10481674 9885   12 32093    6 25000     0
```

If we were not interested in the complete history of simulations we could have done instead (note the argument `keepEvery = NA`)

```
pancrPopNH <- oncoSimulPop(4, pancr,
                           detectionSize = 1e7,
                           keepEvery = NA,
                           mc.cores = 2)

summary(pancrPopNH)
##   NumClones TotalPopSize LargestClone MaxNumDrivers MaxDriversLast
## 1         8     10622184     10614929             0              0
## 2        11     10933126     10763011             0              0
## 3         8     10717307     10640886             0              0
## 4         9     10860203     10439099             0              0
##   NumDriversLargestPop TotalPresentDrivers FinalTime NumIter
## 1                    0                   0       421    1135
## 2                    0                   0       836    1574
## 3                    0                   0       297    1056
## 4                    0                   0       165     872
##   HittedWallTime errorMF minDMratio minBMratio OccurringDrivers
## 1          FALSE      NA     142857     142857
## 2          FALSE      NA     142857     142857
## 3          FALSE      NA     142857     142857
## 4          FALSE      NA     142857     142857
samplePop(pancrPopNH)
##
##  Subjects by Genes matrix of 4 subjects and 7 genes.
##      CDNK2A KRAS MLL3 PXDN SMAD4 TGFBR2 TP53
## [1,]      0    1    0    0     0      0    0
## [2,]      0    1    0    0     0      0    0
## [3,]      0    1    0    0     0      0    0
## [4,]      0    1    0    0     0      0    0
```

which only keeps the very last sample:

```
pancrPopNH[[1]]$pops.by.time
##      [,1] [,2] [,3]     [,4] [,5] [,6] [,7] [,8] [,9]
## [1,]  421 1268  716 10614929 1167    9 2912   14 1169
```

Or we could have used *oncoSimulSample*:

```
pancrSamp <- oncoSimulSample(4, pancr)
## Successfully sampled 4 individuals
##
##  Subjects by Genes matrix of 4 subjects and 7 genes.
pancrSamp$popSamp
##      CDNK2A KRAS MLL3 PXDN SMAD4 TGFBR2 TP53
## [1,]      0    1    0    0     0      0    0
## [2,]      0    1    0    0     0      0    0
## [3,]      0    1    0    0     0      0    0
## [4,]      0    1    0    0     0      0    0
```

Again, why the above differences? If we are only interested in the final matrix of populations by mutations, keeping the complete history the above is wasteful, because we store fully all of the simulations (in the call to *oncoSimulPop*) and then sample (in the call to *samplePop*).

6.1 Differences between "samplePop" and "oncoSimulSample"

samplePop provides two sampling times: "last" and "uniform". It also allows you to sample at the first sample time(s) at which the population(s) reaches a given size, which can be either the same or different for each simulation (with argument `popSizeSample`). "last" means to sample each individual in the very last time period of the simulation. "uniform" means sampling each individual at a time choosen uniformly from all the times recorded in the simulation between the time when the first driver appeared and the final time period. “unif” means that it is almost sure that different individuals will be sampled at different times. “last” does not guarantee that different individuals will be sampled at the same time unit, only that all will be sampled in the last time unit of their simulation.

With *oncoSimulSample* we obtain samples that correspond to `timeSample = "last"` in *samplePop* by specifying a unique value for *detectionSize* and *detectionDrivers*. The data from each simulation will correspond to the time point at which those are reached (analogous to `timeSample = "last"`). How about uniform sampling? We pass a vector of *detectionSize* and *detectionDrivers*, where each value of the vector comes from a uniform distribution. This is not identical to the “uniform” sampling of *oncoSimulSample*, as we are not sampling uniformly over all time periods, but are stopping at uniformly distributed values over the stopping conditions. Arguably, however, the procedure in *samplePop* might be closer to what we mean with “uniformly sampled over the course of the disease” if that course is measured in terms of drivers or size of tumor.

An advantage of *oncoSimulSample* is that we can specify arbitrary sampling schemes, just by passing the appropriate vector *detectionSize* and *detectionDrivers*. A disadvantage is that if we change the stopping conditions we can not just resample the data, but we need to run it again.

There is no difference between *oncoSimulSample* and *oncoSimulPop* + *samplePop* in terms of the `typeSample` argument (whole tumor or single cell).

Finally, there are some additional differences between the two functions. *oncoSimulPop* can run parallelized (it uses *mclapply*). This is not done with *oncoSimulSample* because this function is designed for simulation experiments where you want to examine many different scenarios simultaneously. Thus, we provide additional stopping criteria (`max.wall.time.total` and `max.num.tries.total`) to determine whether to continue running the simulations, that bounds the total running time of all the simulations in a call to *oncoSimulSample*. And, if you are running multiple different scenarios, you might want to make multiple, separate, independent calls (e.g., from different R processes) to *oncoSimulSample*, instead of relying in *mclapply*, since this is likely to lead to better usage of multiple cores/CPUs if you are examining a large number of different scenarios.

7 Runing simulations: odds and ends

7.1 Dealing with errors in “oncoSimulPop”

When running OncoSimulR under Windows *mclapply* does not use multiple cores, and errors from *oncoSimulPop* are reported directly. For example:

```
## This code will only be evaluated under Windows
if(.Platform$OS.type == "windows")
```

```
    try(pancrError <- oncoSimulPop(10, pancr,
                               initSize = 1e-5,
                               detectionSize = 1e7,
                               keepEvery = 10,
                               mc.cores = 2))
```

Under POSIX operating systems (e.g., GNU/Linux or Mac OSX) *oncoSimulPop* can ran parallelized by calling *mclapply*. Now, suppose you did something like

```
## Do not run under Windows
if(.Platform$OS.type != "windows")
    pancrError <- oncoSimulPop(10, pancr,
                               initSize = 1e-5,
                               detectionSize = 1e7,
                               keepEvery = 10,
                               mc.cores = 2)
## Warning in mclapply(seq.int(Nindiv), function(x) oncoSimulIndiv(fp =
## fp, : all scheduled cores encountered errors in user code
```

The warning you are seeing tells you there was an error in the functions called by *mclapply*. If you check the help for *mclpapply* you'll see that it returns a try-error object, so we can inspect it. For instance, we could do:

```
pancrError[[1]]
```

But the output of this call might be easier to read:

```
pancrError[[1]][1]
```

And from here you could see the error that was returned by *oncoSimulIndiv*: `initSize < 1` (which is indeed true: we pass `initSize = 1e-5`).

7.2 What can you do with the simulations?

This is up to you. Later (section 11.2) we show an example where we infer an oncogenetic tree from simulated data.

7.3 Whole tumor sampling, genotypes, and allele counts: what gives?

You are obtaining genotypes, regardless of order. When we use "whole tumor sampling", it is the frequency of the mutations in each gene that counts, not the order. So, for instance, "c, d" and "c, d" both contribute to the counts of "c" and "d". Similarly, when we use single cell sampling, we obtain a genotype defined in terms of mutations, but there might be multiple orders that give this genotype. For example, $d > c$ and $c > d$ both give you a genotype with “c” and “d” mutated, and thus in the output you can have two columns with both genes mutated.

7.4 Doesn’t the BNB algorithm require small mutation rates for it to be advantageous?

As discussed in the original paper by Mather, Hasty, and Tsimring (2012) (see also their supplementary material), the BNB algorithm can achieve considerable speed advantages relative to other algorithms especially when mutation events are rare relative to birth and death events; the larger the mutation rate, the smaller the gains compared to other algorithms. As mentioned in their supplementary material (see p.5) “Note that the ‘cost’ of each step in BNB is somewhat higher than in SSA [SSA is the original Gillespie Stochastic Simulation Algorithm] since it requires generation of several random numbers as compared to only two uniform random numbers for SSA. However this cost increase is small compared with significant benefits of jumping over birth and death reactions for the case of rare mutations.”

Since the earlier versions, OncoSimulR has provided information to assess these issues. The output of function `oncoSimulIndiv` includes a list called *“other”* that itself includes two lists named *“minDMratio”* and *“minBMratio”*, the smallest ratio, over all simulations, of death rate to mutation rate or birth rate to mutation rate, respectively. As explained above, the BNB algorithm thrives when those are large.

8 Showing the relationships of clones

If you run simulations with the `keepPhylog = TRUE` argument, the simulations keep track of when every clone is generated, and that will allow us to see the parent-child relationships between clones. (This is disabled by default: the code runs a little bit slower and the result is larger.)

In an abuse of terminology, we will use functions with the “phylog” term, but note these are not proper phylogenies. (Though you could construct proper phylogenies from the information kept).

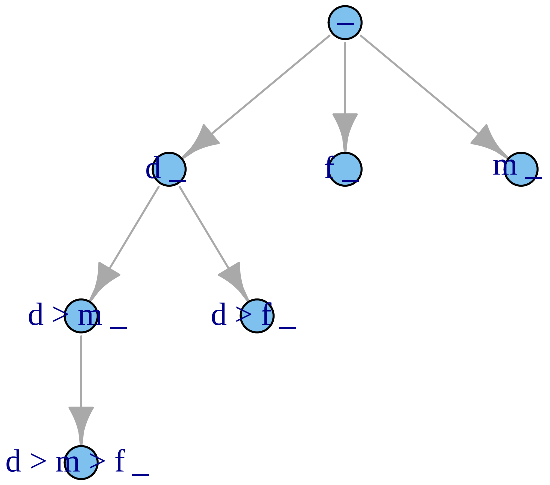
Let us re-run a previous example:

```r
set.seed(15)
tmp <-  oncoSimulIndiv(examplesFitnessEffects[["o3"]],
                       model = "McFL",
                       mu = 5e-5,
                       detectionSize = 1e8,
                       detectionDrivers = 3,
                       sampleEvery = 0.015,
                       max.num.tries = 10,
                       keepEvery = 5,
                       initSize = 2000,
                       finalTime = 20000,
                       onlyCancer = FALSE,
                       extraTime = 1500,
                       keepPhylog = TRUE)
tmp
##
## Individual OncoSimul trajectory with call:
##  oncoSimulIndiv(fp = examplesFitnessEffects[["o3"]], model = "McFL",
##     mu = 5e-05, detectionSize = 1e+08, detectionDrivers = 3,
##     sampleEvery = 0.015, initSize = 2000, keepEvery = 5, extraTime = 1500,
##     finalTime = 20000, onlyCancer = FALSE, keepPhylog = TRUE,
##     max.num.tries = 10)
##
##   NumClones TotalPopSize LargestClone MaxNumDrivers MaxDriversLast
## 1         7         3200         3200             3              3
##   NumDriversLargestPop TotalPresentDrivers FinalTime NumIter
## 1                    3                   3      2552  170395
##   HittedWallTime errorMF minDMratio minBMratio OccurringDrivers
## 1          FALSE 0.01218       6276       6667          d, f, m
##
## Final population composition:
##       Genotype    N
## 1            _    0
## 2          d _    0
## 3      d > f _    0
## 4      d > m _    0
## 5  d > m > f _ 3200
## 6          f _    0
## 7          m _    0
```

We can plot the parent-child relationships[9] of every clone ever created (with fitness larger than 0 —clones without viability are never shown):

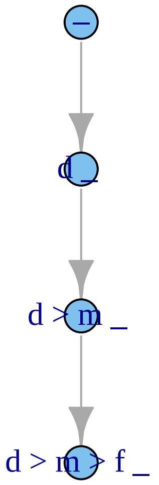
```
plotClonePhylog(tmp, N = 0)
```

[9] There are several packages in R devoted to phylogenetic inference and related issues. For instance, *ape*. I have not used that infrastructure because of our very specific needs and circumstances; for instance, internal nodes are observed, we can have networks instead of trees, and we have no uncertainty about when events occurred.



However, we often only want to show clones that exist (have number of cells > 0) at a certain time (while of course showing all of their ancestors, even if those are now extinct —i.e., regardless of their current numbers).

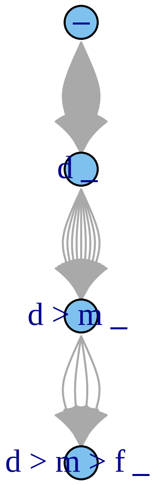
```r
plotClonePhylog(tmp, N = 1)
```



If we set `keepEvents = TRUE` the arrows show how many times each clone appeared:

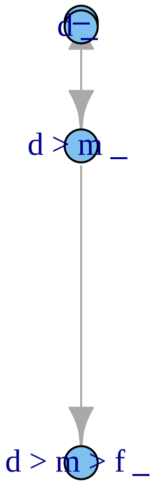
(The next can take a while)

```r
plotClonePhylog(tmp, N = 1, keepEvents = TRUE)
```



And we can show the plot so that the vertical axis is proportional to time (though you might see overlap of nodes if a child node appeared shortly after the parent):

```
plotClonePhylog(tmp, N = 1, timeEvents = TRUE)
```



We can obtain the adjacency matrix doing

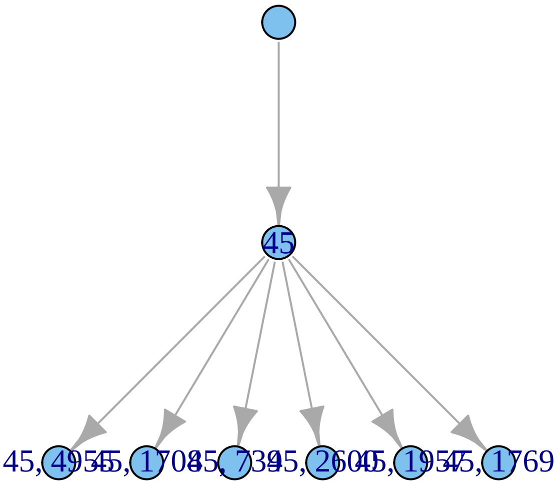
```
get.adjacency(plotClonePhylog(tmp, N = 1, returnGraph = TRUE))
## 4 x 4 sparse Matrix of class "dgCMatrix"
##                _  d _  d > m _  d > m > f _
##   _            .    1        .            .
## d _            .    .        1            .
## d > m _        .    .        .            1
## d > m > f _    .    .        .            .
```

We can see another example here:

```
set.seed(456)
mcf1s <-  oncoSimulIndiv(mcf1,
                         model = "McFL",
                         mu = 1e-7,
                         detectionSize = 1e8,
                         detectionDrivers = 100,
                         sampleEvery = 0.02,
                         keepEvery = 2,
                         initSize = 2000,
                         finalTime = 1000,
                         onlyCancer = FALSE,
                         keepPhylog = TRUE)
```

Showing only clones that exist at the end of the simulation (and all their parents):

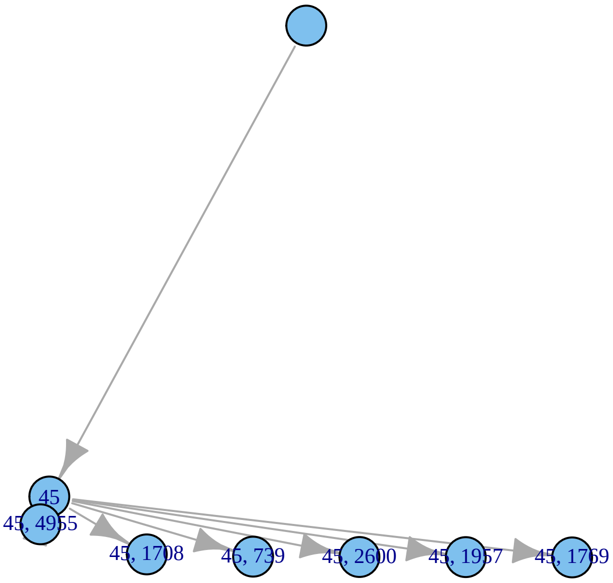
```
plotClonePhylog(mcf1s, N = 1)
```



Notice that the labels here do not have a "_", since there were no order effects in fitness. However, the labels show the genes that are mutated, just as before.

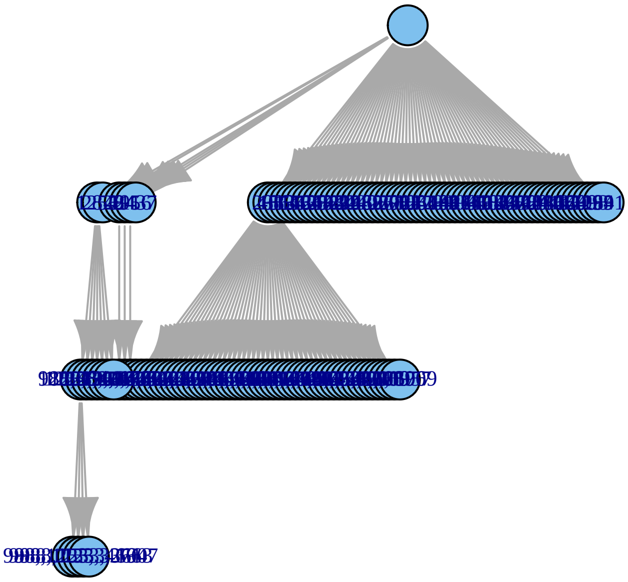
Similar, but with vertical axis proportional to time:

```
par(cex = 0.7)
plotClonePhylog(mcf1s, N = 1, timeEvents = TRUE)
```



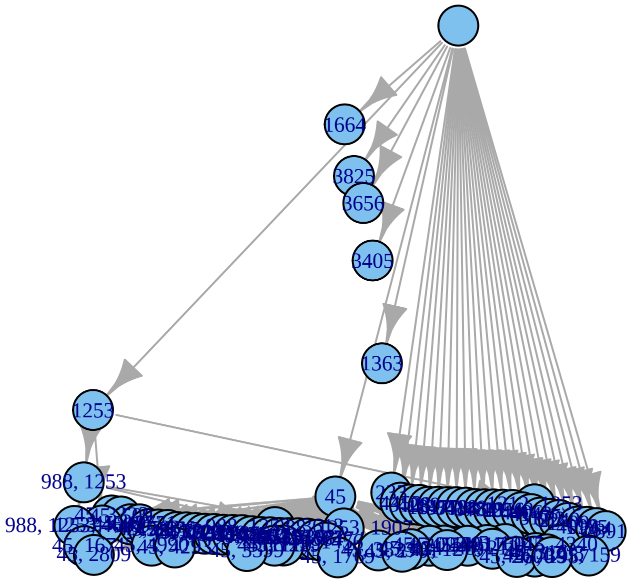
What about those that existed in the last 200 time units?

```r
par(cex = 0.7)
plotClonePhylog(mcf1s, N = 1, t = c(800, 1000))
```



And try now to show also when the clones appeared (we restrict the time to between 900 and 1000, to avoid too much clutter):

```r
par(cex = 0.7)
plotClonePhylog(mcf1s, N = 1, t = c(900, 1000), timeEvents = TRUE)
```

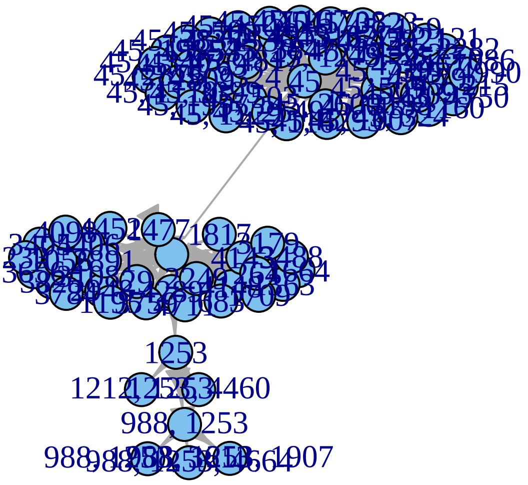


(By playing with t, it should be possible to obtain animations of the phylogeny. We will not pursue it here.)

If the previous graph seems cluttered, we can represent it in a different way by calling *igraph* directly after storing the graph and using the default layout:

```r
g1 <- plotClonePhylog(mcf1s, N = 1, t = c(900, 1000),
                      returnGraph = TRUE)
```

```r
plot(g1)
```



which might be easier to show complex relationships or identify central or key clones.

It is of course quite possible that, especially if we consider few genes, the parent-child relationships will form a network, not a tree, as the same child node can have multiple parents. You can play with this example, modified from one we saw before (section 2.4.6):

```r
op <- par(ask = TRUE)
while(TRUE) {
    tmp <- oncoSimulIndiv(smn1, model = "McFL",
                          mu = 5e-5, finalTime = 500,
                          detectionDrivers = 3,
                          onlyCancer = FALSE,
                          initSize = 1000, keepPhylog = TRUE)
    plotClonePhylog(tmp, N = 0)
}
par(op)
```

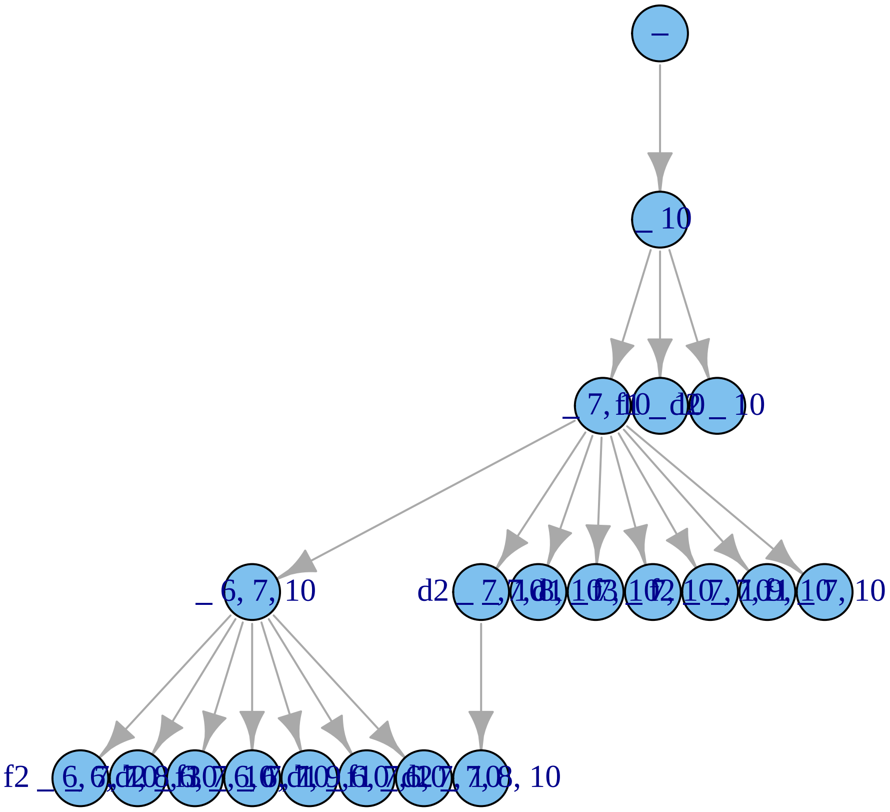
8.1 Parent-child relationships from multiple runs

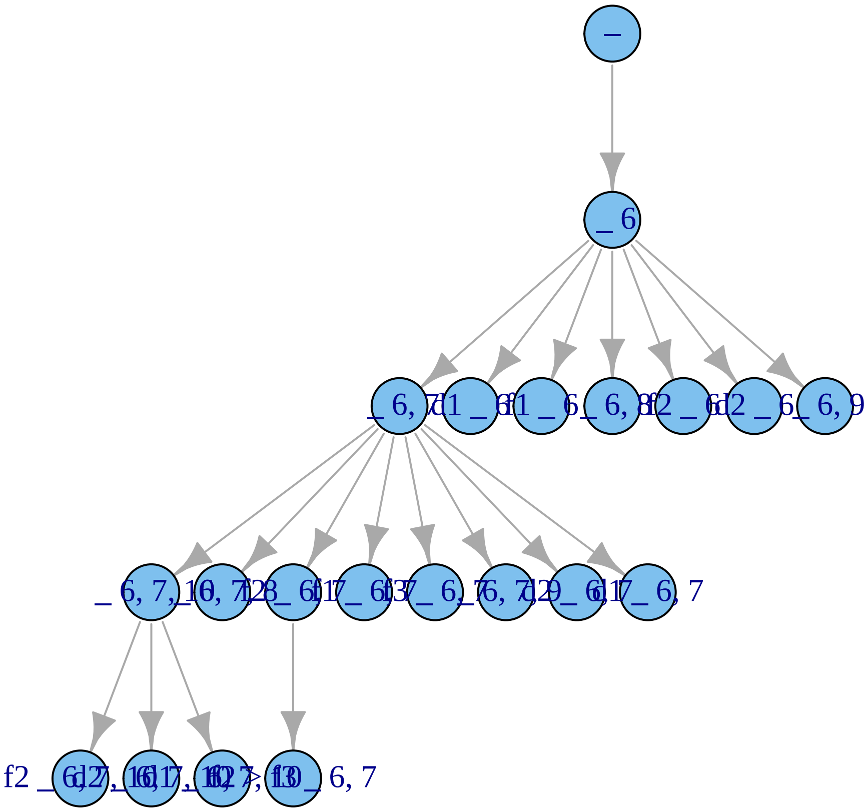
If you use *oncoSimulPop* you can store and plot the “phylogenies" of the different runs:

```r
oi <- allFitnessEffects(orderEffects =
               c("F > D" = -0.3, "D > F" = 0.4),
               noIntGenes = rexp(5, 10),
                          geneToModule =
                              c("F" = "f1, f2, f3",
                                "D" = "d1, d2") )
oiI1 <- oncoSimulIndiv(oi, model = "Exp")
oiP1 <- oncoSimulPop(4, oi,
                     keepEvery = 10,
                     mc.cores = 2,
                     keepPhylog = TRUE)
```

We will plot the first two:

```
op <- par(mar = rep(0, 4), mfrow = c(2, 1))
plotClonePhylog(oiP1[[1]])
plotClonePhylog(oiP1[[2]])
```





```r
par(op)
```

This is so far disabled in function *oncoSimulSample*, since that function is optimized for other uses. This might change in the future.

9 Generating random fitness landscapes

In most of the examples seen above, we have fully specified the fitness of the different genotypes (either by providing directly the full mapping genotyeps to fitness, or by providing that mapping by specifying the effects of the different gene combinations). In some cases, however, we might want to specify a particular model that generates the fitness landscape, and then have fitnesses be random variables obtained under this model. In this random fitness landscape the fitness of the genotypes is a random variable generated under some specific model. Random fitness landscapes are used extensively, for instance, to understand the evolutionary consequences of different types of epistatic interactions (e.g., Szendro et al. (2013), Franke et al. (2011)) and there are especially developed tools for plotting and analyzing random fitness landscapes (e.g., Brouillet et al. (2015)).

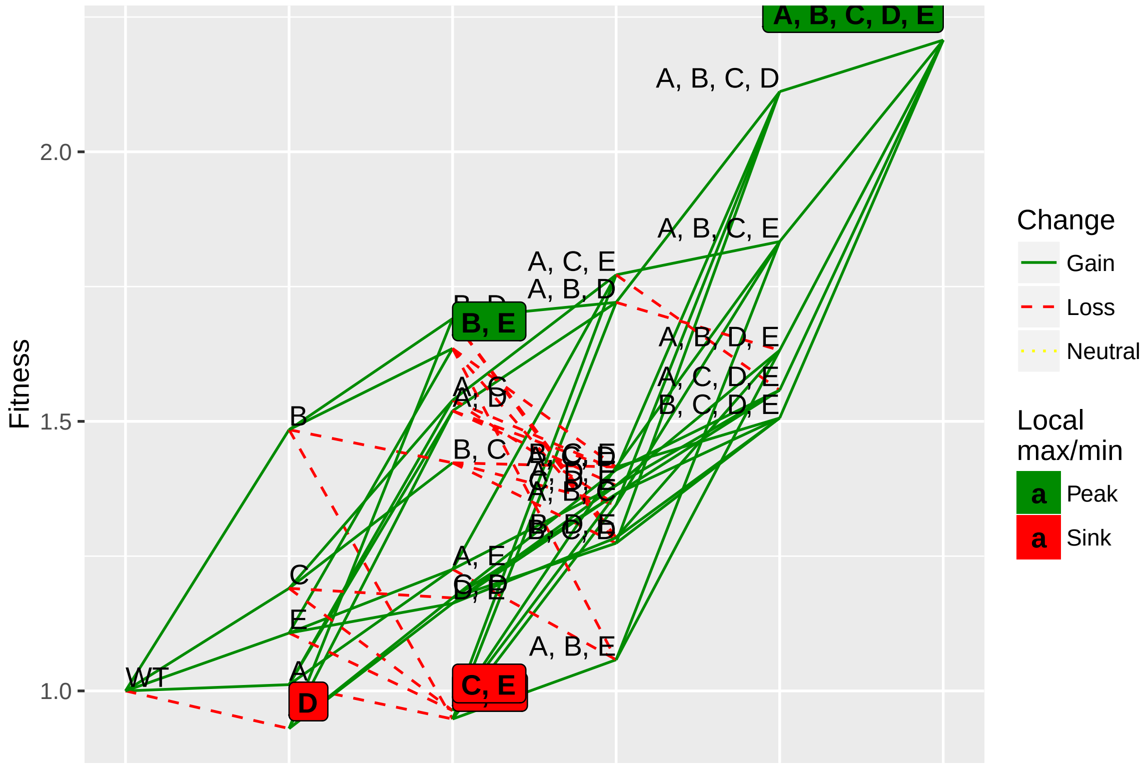
With OncoSimulR it is possible to generate mappings of genotype to fitness using the function *rfitness* that allows you to use from a pure House of Cards model to a purely additive model. I have followed Szendro et al. (2013), Franke et al. (2011) and model fitness as

$$f_i = -c\ d(i, reference) + x_i \tag{2}$$

where $d(i, j)$ is the Hamming distance between genotypes i and j (the number of positions that differ), c is the decrease in fitness of a genotype per each unit increase in Hamming distance from the reference genotype, and x_i is a random variable (in this case, a normal deviate of mean 0 and standard deviation sd). You can change the reference genotype to any of the genotypes: for the deterministic part, you make the fittest genotype be the one with all positions mutated by setting `reference = "max"`, or use the wildtype by using a string of 0s, or randomly select a genotype as a reference by using `reference = "random"`. And by playing with c and sd you can flexibly modify the relative weight of the purely House of Cards vs. additive component. The expression used above is also very similar to the one on Greene and Crona (2014) if you use *rfitness* with the argument `reference = "max"`.

What can you do with these genotype to fitness mappings? You could plot them, you could use them as input for *oncoSimulIndiv* and related functions, or you could export them and plot them externally (e.g., in MAGELLAN: http://wwwabi.snv.jussieu.fr/public/Magellan/, Brouillet et al. (2015)).

```
## A small example
rfitness(3)
##      A B C Fitness
## [1,] 0 0 0  1.0000
## [2,] 1 0 0  0.6956
## [3,] 0 1 0  1.3079
## [4,] 0 0 1  1.9800
## [5,] 1 1 0  1.6190
## [6,] 1 0 1  0.9829
## [7,] 0 1 1  0.7574
## [8,] 1 1 1  1.4396
## attr(,"class")
## [1] "matrix"                  "genotype_fitness_matrix"

## A 5-gene example, where the reference genotype is the one the one with
## all positions mutated, similar to Greene and Crona, 2014.  We will plot
## the landscape and use it for simulations We downplay the random
## component with a sd = 0.5

r1 <- rfitness(5, reference = rep(1, 5), sd = 0.6)
plot(r1)
```



```
oncoSimulIndiv(allFitnessEffects(genotFitness = r1))
##
## Individual OncoSimul trajectory with call:
##  oncoSimulIndiv(fp = allFitnessEffects(genotFitness = r1))
##
##   NumClones TotalPopSize LargestClone MaxNumDrivers MaxDriversLast
## 1        15    130949247     69035510             0              0
##   NumDriversLargestPop TotalPresentDrivers FinalTime NumIter
## 1                    0                   0      2106    4156
##   HittedWallTime errorMF minDMratio minBMratio OccurringDrivers
## 1          FALSE      NA     168402      2e+05
##
## Final population composition:
##    Genotype        N
## 1                1392
## 2         A        0
## 3   A, B, C      347
## 4      A, C 69035510
## 5   A, C, D      693
## 6   A, C, E      954
## 7         B        0
## 8      B, C  1104000
## 9   B, C, D       21
## 10  B, C, E      138
## 11        C 60802626
## 12     C, D     3255
## 13     C, E      311
## 14        D        0
## 15        E        0
```

10 Further comments and examples about time, size, and simulation limits

11 Using v.1 posets and simulations

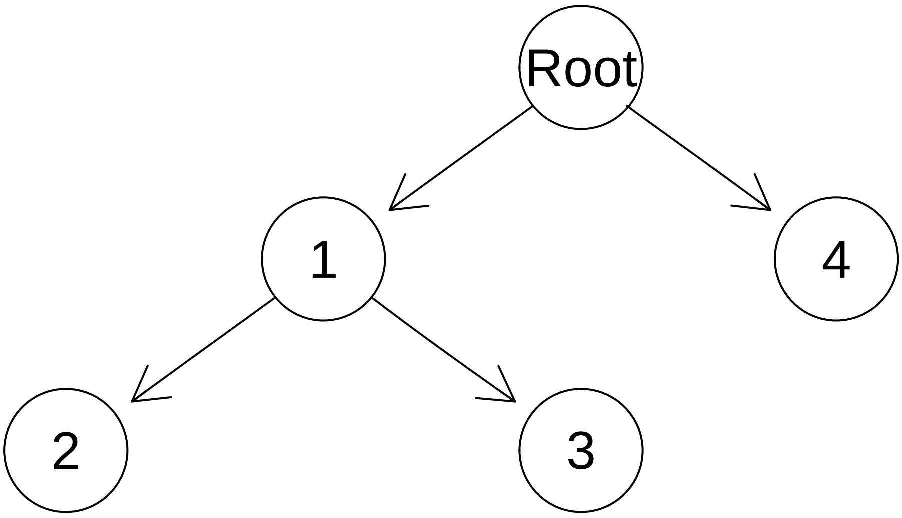
It is strongly recommended that you use the new (v.2) procedures for specifying fitness effects. However, the former v.1 procedures are still available, with only very minor changes to function calls. What follows below is the former vignette. You might want to use v.1 because for certain models (e.g., small number of genes, with restrictions as specified by a simple poset) simulations might be faster with v.1 (fitness evaluation is much simpler —we are working on further improving speed).

11.1 Specifying restrictions: posets

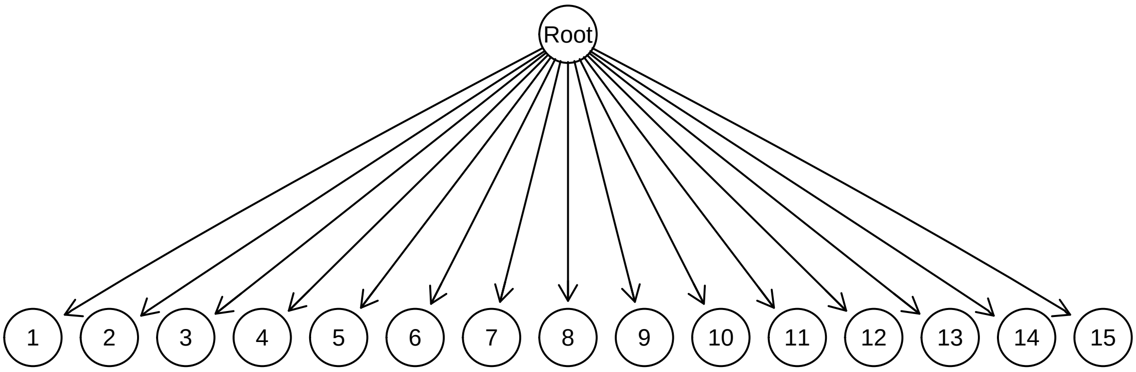
How to specify the restrictions is shown in the help for *poset*. It is often useful, to make sure you did not make any mistakes, to plot the poset. This is from the examples (we use an "L" after a number so that the numbers are integers, not doubles; we could alternatively have modified storage.mode).

```
## Node 2 and 3 depend on 1, and 4 depends on no one
p1 <- cbind(c(1L, 1L, 0L), c(2L, 3L, 4L))
```

```r
plotPoset(p1, addroot = TRUE)
```

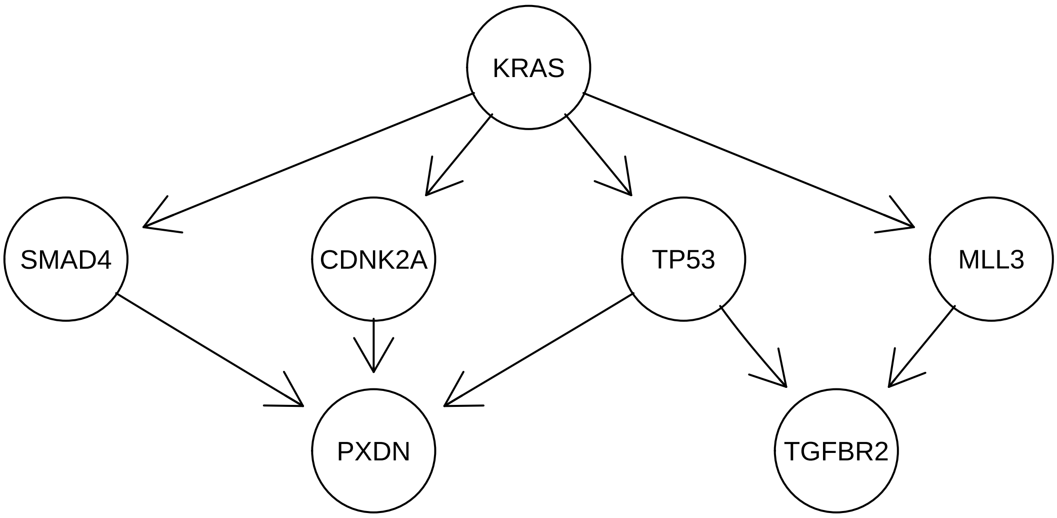


```r
## A simple way to create a poset where no gene (in a set of 15) depends
## on any other.
p4 <- cbind(0L, 15L)
plotPoset(p4, addroot = TRUE)
```



Specifying posets is actually straightforward. For instance, we can specify the pancreatic cancer poset in Gerstung et al. (2011) (their figure 2B, left). We specify the poset using numbers, but for nicer plotting we will use names (KRAS is 1, SMAD4 is 2, etc). This example is also in the help for *poset*:

```r
pancreaticCancerPoset <- cbind(c(1, 1, 1, 1, 2, 3, 4, 4, 5),
                               c(2, 3, 4, 5, 6, 6, 6, 7, 7))
storage.mode(pancreaticCancerPoset) <- "integer"
plotPoset(pancreaticCancerPoset,
          names = c("KRAS", "SMAD4", "CDNK2A", "TP53",
                    "MLL3","PXDN", "TGFBR2"))
```



Simulating cancer progression {#simul1}

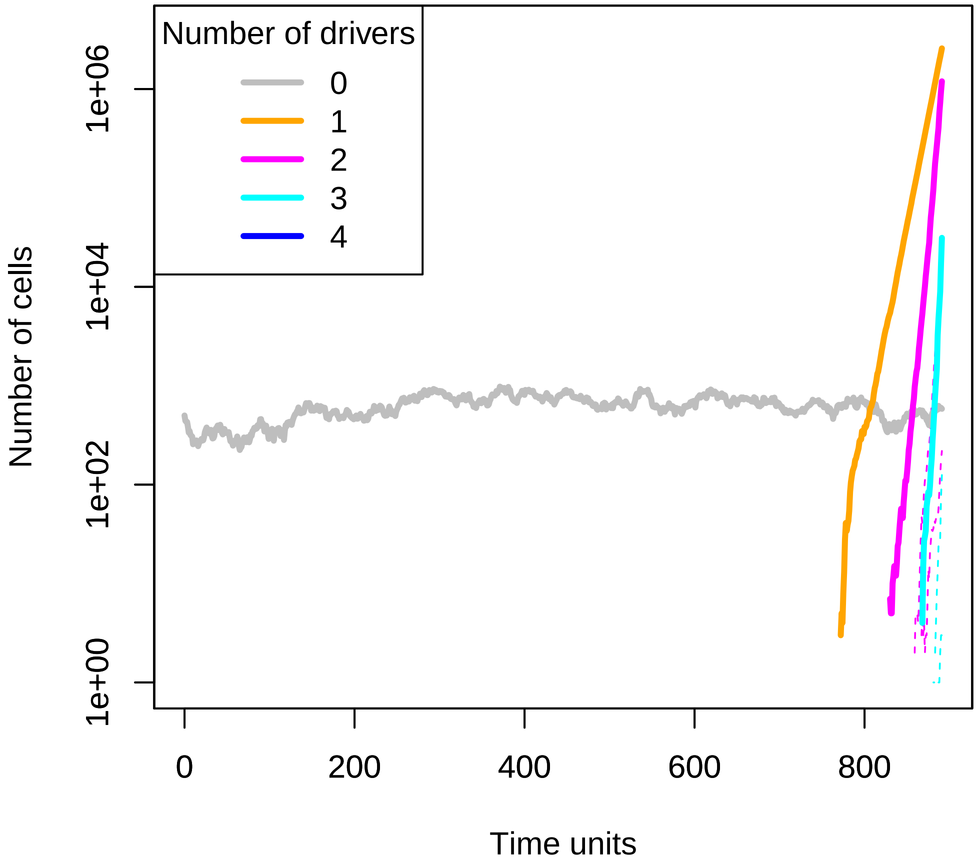
We can simulate the progression in a single subject. Using an example very similar to the one in the help:

```
## use poset p1101
data(examplePosets)
p1101 <- examplePosets[["p1101"]]

## Bozic Model
b1 <- oncoSimulIndiv(p1101, keepEvery = 15)
summary(b1)
##   NumClones TotalPopSize LargestClone MaxNumDrivers
## 1        14     39953745     13268083             4
##   MaxDriversLast NumDriversLargestPop TotalPresentDrivers
## 1              4                    1                   9
##   FinalTime NumIter HittedWallTime errorMF minDMratio
## 1       827    3131          FALSE      NA      90909
##   minBMratio          OccurringDrivers
## 1      90909 1, 2, 3, 4, 5, 6, 7, 8, 9
```

The first thing we do is make it simpler (for future examples) to use a set of restrictions. In this case, those encoded in poset p1101. Then, we run the simulations and look at a simple summary and a plot.

If you want to plot the trajectories, it is better to keep more frequent samples, so you can see when clones appear:

```
b2 <- oncoSimulIndiv(p1101, keepEvery = 1)
summary(b2)
##   NumClones TotalPopSize LargestClone MaxNumDrivers
## 1        10      3806490      2577722             4
##   MaxDriversLast NumDriversLargestPop TotalPresentDrivers
## 1              4                    1                   7
##   FinalTime NumIter HittedWallTime errorMF minDMratio
## 1       891    1206          FALSE      NA      90909
##   minBMratio    OccurringDrivers
## 1      90909 1, 2, 3, 4, 7, 8, 9
plot(b2)
```



As we have seen before, the stacked plot here is less useful and that is why I do not evaluate that code for this vignette.

```
plot(b2, type = "stacked")
```

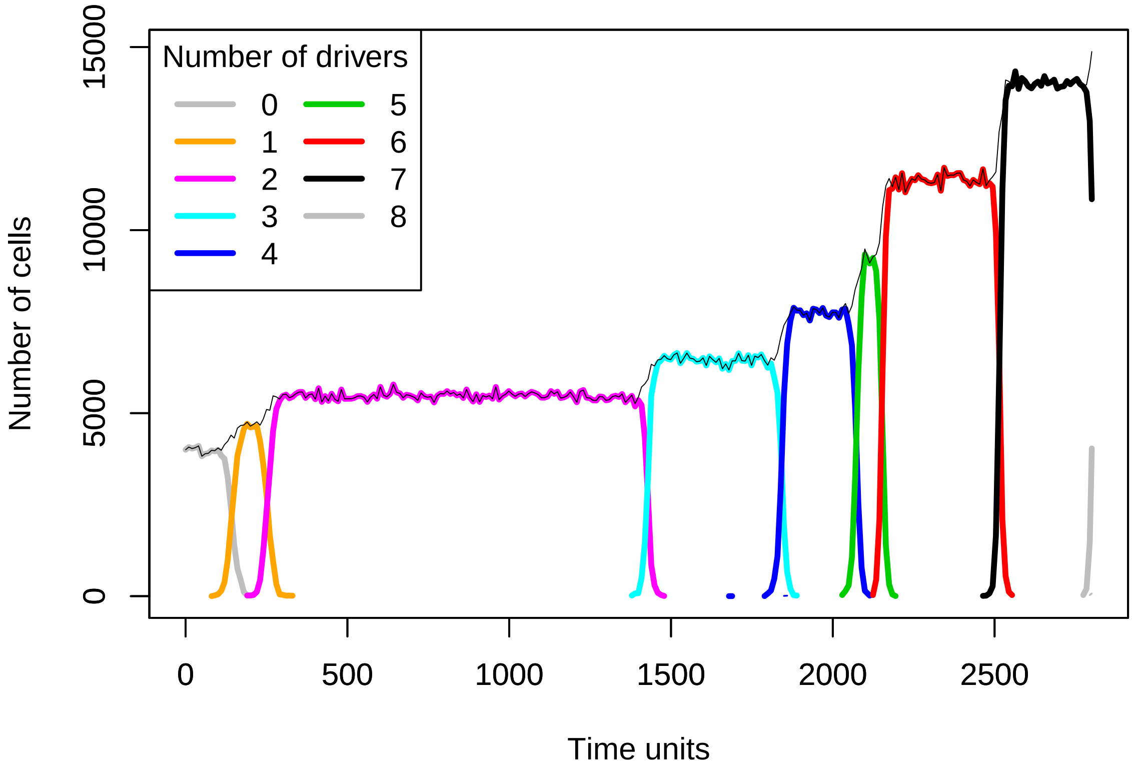
The following is an example where we do not care about passengers, but we want to use a different graph, and we want a few more drivers before considering cancer has been reached. And we allow it to run for longer. Note that in the McF model `detectionSize` really plays no role. Note also how we pass the poset: it is the same as before, but now we directly access the poset in the list of posets.

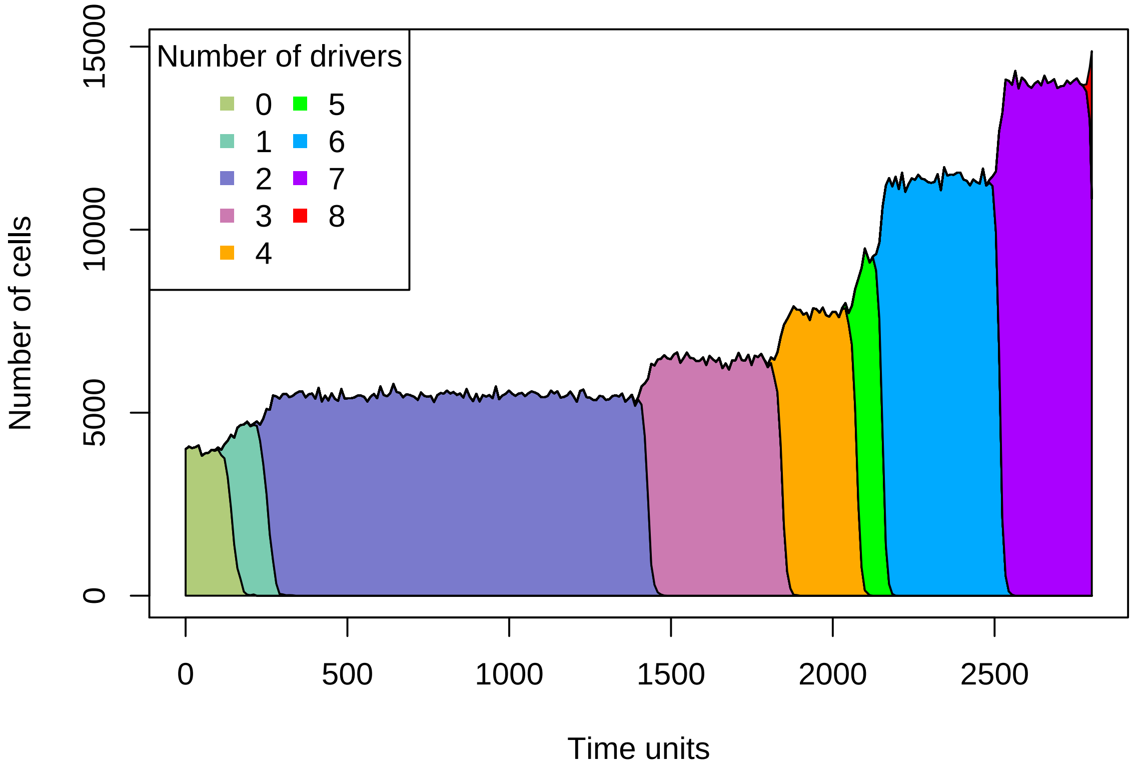
```
m2 <- oncoSimulIndiv(examplePosets[["p1101"]], model = "McFL",
                     numPassengers = 0, detectionDrivers = 8,
                     mu = 5e-7, initSize = 4000,
                     sampleEvery = 0.025,
                     finalTime = 25000, keepEvery = 5,
                     detectionSize = 1e6)
```

(Very rarely the above run will fail to reach cancer. If that happens, execute it again.)

As usual, we will plot using both a line and a stacked plot:

```r
par(mfrow = c(2, 1))
plot(m2, addtot = TRUE, log = "",
     thinData = TRUE, thinData.keep = 0.5)
plot(m2, type = "stacked",
     thinData = TRUE, thinData.keep = 0.5)
```





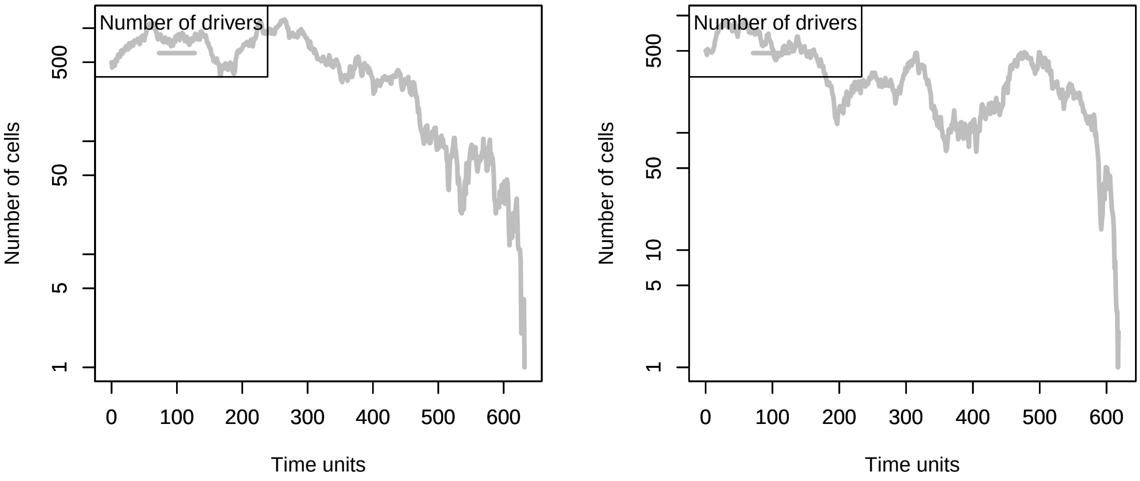
The default is to simulate progression until a simulation reaches cancer (i.e., only simulations that satisfy the detectionDrivers or the detectionSize will be returned).

If you use the McF model with large enough `initSize` this will often be the case but not if you use very small `initSize`. Likewise, most of the Bozic runs do not reach cancer. Lets try a few:

```
b3 <- oncoSimulIndiv(p1101, onlyCancer = FALSE)
summary(b3)
##   NumClones TotalPopSize LargestClone MaxNumDrivers
## 1         1            0            0             0
##   MaxDriversLast NumDriversLargestPop TotalPresentDrivers
## 1              0                    0                   0
##   FinalTime NumIter HittedWallTime errorMF minDMratio
## 1       633     636          FALSE      NA      90909
##   minBMratio OccurringDrivers
## 1      90909               NA

b4 <- oncoSimulIndiv(p1101, onlyCancer = FALSE)
summary(b4)
##   NumClones TotalPopSize LargestClone MaxNumDrivers
## 1         1            0            0             0
##   MaxDriversLast NumDriversLargestPop TotalPresentDrivers
## 1              0                    0                   0
##   FinalTime NumIter HittedWallTime errorMF minDMratio
## 1       619     621          FALSE      NA      90909
##   minBMratio OccurringDrivers
## 1      90909               NA
```

Plot those runs:

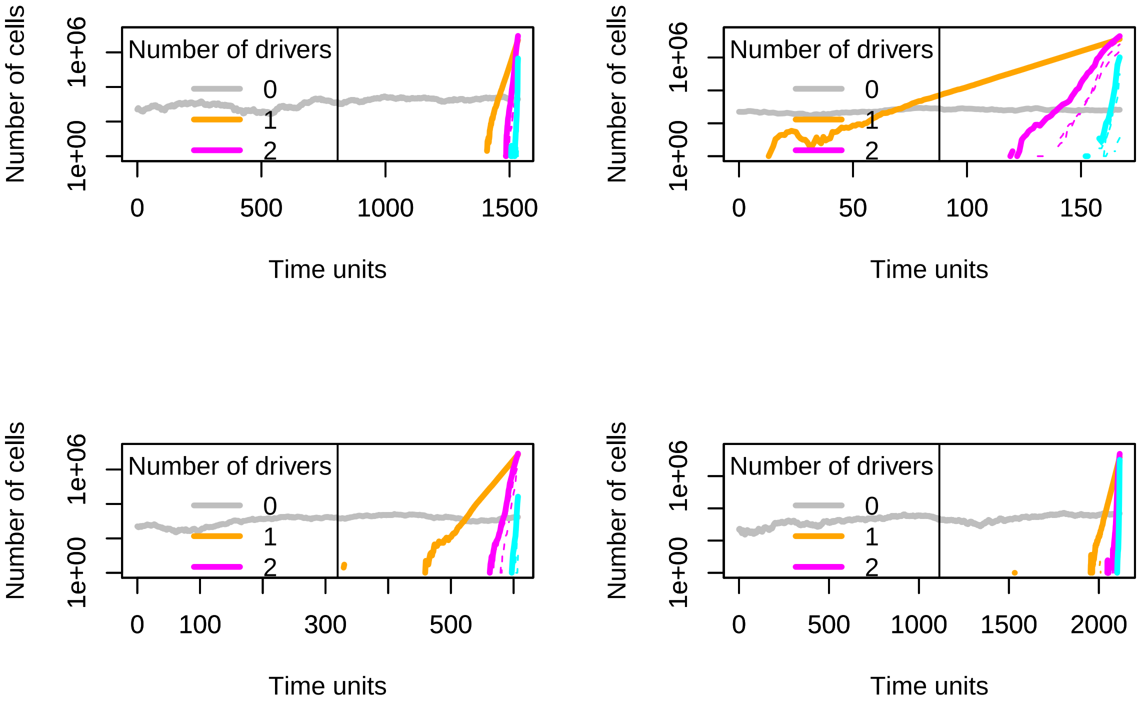
```
par(mfrow = c(1, 2))
par(cex = 0.8) ## smaller font
plot(b3)
plot(b4)
```



11.1.1 Simulating progression in several subjects

To simulate the progression in a bunch of subjects (we will use only four, so as not to fill the vignette with plots) we can do, with the same settings as above:

```r
p1 <- oncoSimulPop(4, p1101, mc.cores = 2)
par(mfrow = c(2, 2))
plot(p1, ask = FALSE)
```



We can also use stream and stacked plots, though they might not be as useful in this case. For the sake of keeping the vignette small, these are commented out.

```r
par(mfrow = c(2, 2))
plot(p1, type = "stream", ask = FALSE)
```

```r
par(mfrow = c(2, 2))
plot(p1, type = "stacked", ask = FALSE)
```

11.2 Sampling from a set of simulated subjects

You will often want to do something with the simulated data. For instance, sample the simulated data. Here we will obtain the trajectories for 100 subjects in a scenario without passengers. Then we will sample with the default options and store that as a vector of genotypes (or a matrix of subjects by genes):

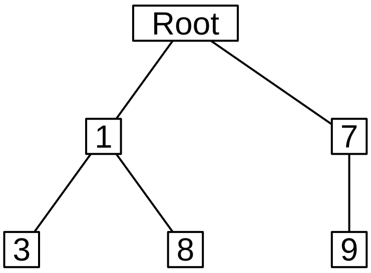
```r
m1 <- oncoSimulPop(100, examplePosets[["p1101"]],
                   numPassengers = 0, mc.cores = 2)
```

The function ***samplePop*** samples that object, and also gives you some information about the output:

```
genotypes <- samplePop(m1)
##
##  Subjects by Genes matrix of 100 subjects and 11 genes.
```

What can you do with it? That is up to you. As an example, let us try to infer an oncogenetic tree (and plot it) using the *Oncotree* package Aniko Szabo and Pappas (2013) after getting a quick look at the marginal frequencies of events:

```
colSums(genotypes)/nrow(genotypes)
##    1    2    3    4    5    6    7    8    9   10   11
## 0.52 0.00 0.01 0.00 0.00 0.00 0.54 0.01 0.02 0.00 0.00

require(Oncotree)
## Loading required package: Oncotree
## Loading required package: boot
ot1 <- oncotree.fit(genotypes)
## The following events had no observed occurances,so they will not be included in
## 2 4 5 6 10 11
plot(ot1)
```

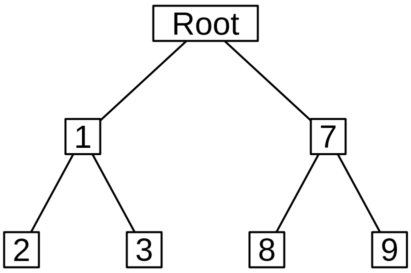


Your run will likely differ from mine, but with the defaults (detection size of 10^8) it is likely that events down the tree will never appear. You can set `detectionSize = 1e9` and you will see that events down the tree are now found in the cross-sectional sample.

Alternatively, you can use single cell sampling and that, sometimes, recovers one or a couple more events.

```
genotypesSC <- samplePop(m1, typeSample = "single")
##
##  Subjects by Genes matrix of 100 subjects and 11 genes.
colSums(genotypesSC)/nrow(genotypesSC)
##    1    2    3    4    5    6    7    8    9   10   11
## 0.57 0.10 0.12 0.00 0.00 0.00 0.63 0.12 0.07 0.00 0.00

ot2 <- oncotree.fit(genotypesSC)
## The following events had no observed occurances,so they will not be included in
## 4 5 6 10 11
```

```
plot(ot2)
```

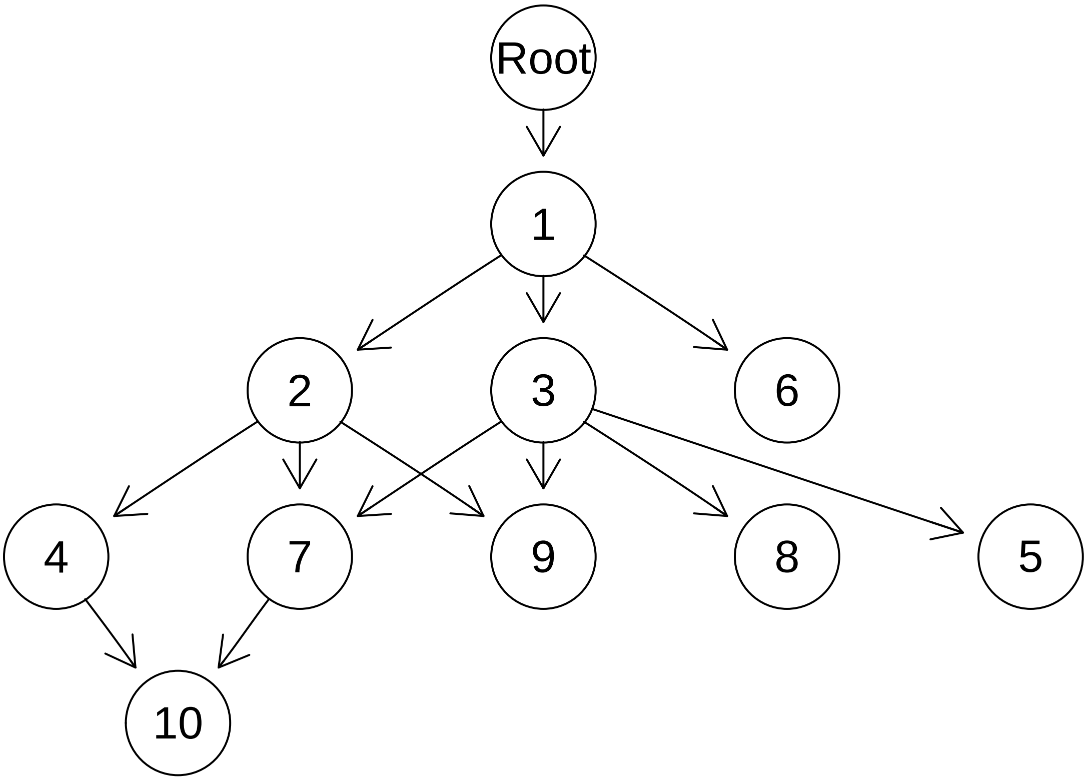


You can of course rename the columns of the output matrix to something else if you want so the names of the nodes will reflect those potentially more meaningful names.

12 Generating random DAGs for restrictions

You might want to randomly generate DAGs like those often found in the literature on oncogenetic trees et al. Function *simOGraph* might help here.

```
## No seed fixed, so reruns will give different DAGs.
(a1 <- simOGraph(10))
##      Root 1 2 3 4 5 6 7 8 9 10
## Root    0 1 0 0 0 0 0 0 0 0  0
## 1       0 0 1 1 0 0 1 0 0 0  0
## 2       0 0 0 0 1 0 0 1 0 1  0
## 3       0 0 0 0 0 1 0 1 1 1  0
## 4       0 0 0 0 0 0 0 0 0 0  1
## 5       0 0 0 0 0 0 0 0 0 0  0
## 6       0 0 0 0 0 0 0 0 0 0  0
## 7       0 0 0 0 0 0 0 0 0 0  1
## 8       0 0 0 0 0 0 0 0 0 0  0
## 9       0 0 0 0 0 0 0 0 0 0  0
## 10      0 0 0 0 0 0 0 0 0 0  0
library(graph) ## for simple plotting
plot(as(a1, "graphNEL"))
```



Once you obtain the adjacency matrices, it is for now up to you to convert them into appropriate posets or fitnessEffects objects.

Why this function? I searched for, and could not find any that did what I wanted, in particular bounding the number of parents, being able to specify the approximate depth[10] of the graph, and optionally being able to have DAGs where no node is connected to another both directly (an edge between the two) and indirectly (there is a path between the two through other nodes). So I wrote my own code. The code is fairly simple to understand (all in file `generate-random-trees.R`). I would not be surprised if this way of generating random graphs has been proposed and named before; please let me know, best if with a reference.

Should we remove direct connections if there are indirect? Or, should we set `removeDirectIndirect = TRUE`? Except for Farahani and Lagergren (2013), none of the DAGs I've seen in the context of CBNs, oncogenetic trees, etc, include both direct and indirect connections between nodes. If these exist, reasoning about the model can be harder. For example, with CBN (AND or CMPN or monotone relationships) adding a direct connection makes no difference iff we assume that the relationships encoded in the DAG are fully respected (e.g., all $s_h = -\infty$). But it can make a difference if we allow for deviations from the monotonicity, specially if we only check for the satisfaction of the presence of the immediate ancestors. And things get even trickier if we combine XOR with AND. The code for computing fitness, however, should deal with all of this just fine.

[10] Where depth is defined in the usual way to mean smallest number of nodes —or edges— to traverse to get from the bottom to the top of the DAG.

13 Session info and packages used

This is the information about the version of R and packages used:

```
sessionInfo()
## R Under development (unstable) (2016-11-03 r71622)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Debian GNU/Linux stretch/sid
##
## locale:
##  [1] LC_CTYPE=en_GB.utf8       LC_NUMERIC=C
##  [3] LC_TIME=en_GB.utf8        LC_COLLATE=en_GB.utf8
##  [5] LC_MONETARY=en_GB.utf8    LC_MESSAGES=en_GB.utf8
##  [7] LC_PAPER=en_GB.utf8       LC_NAME=C
##  [9] LC_ADDRESS=C              LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_GB.utf8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] parallel  methods   stats     graphics  grDevices
## [6] utils     datasets  base
##
## other attached packages:
##  [1] Oncotree_0.3.3      boot_1.3-18
##  [3] igraph_1.0.1        graph_1.53.0
##  [5] BiocGenerics_0.21.0 bookdown_0.1
##  [7] BiocStyle_2.3.3     rmarkdown_1.1
##  [9] testthat_1.0.2      OncoSimulR_2.5.1
##
## loaded via a namespace (and not attached):
##  [1] gtools_3.5.0       splines_3.4.0
##  [3] lattice_0.20-34    colorspace_1.2-7
##  [5] miniUI_0.1.1       htmltools_0.3.5
##  [7] stats4_3.4.0       yaml_2.1.13
##  [9] mgcv_1.8-15        chron_2.3-47
## [11] nloptr_1.0.4       DBI_0.5-1
## [13] Rgraphviz_2.19.0   RColorBrewer_1.1-2
## [15] plyr_1.8.4         stringr_1.1.0
## [17] MatrixModels_0.4-1 munsell_0.4.3
## [19] gtable_0.2.0       evaluate_0.10
## [21] knitr_1.14         SparseM_1.72
## [23] httpuv_1.3.3       quantreg_5.29
## [25] pbkrtest_0.4-6     Rcpp_0.12.7
## [27] xtable_1.8-2       scales_0.4.0
## [29] formatR_1.4        mime_0.5
## [31] lme4_1.1-12        smatr_3.4-3
## [33] ggplot2_2.1.0      digest_0.6.10
## [35] stringi_1.1.2      dplyr_0.5.0
## [37] ggrepel_0.6.3      shiny_0.14.2
## [39] grid_3.4.0         tools_3.4.0
## [41] magrittr_1.5       lazyeval_0.2.0
## [43] tibble_1.2         crayon_1.3.2
## [45] car_2.1-3          MASS_7.3-45
## [47] Matrix_1.2-7.1     data.table_1.9.6
## [49] assertthat_0.1     minqa_1.2.4
## [51] R6_2.2.0           nnet_7.3-12
## [53] nlme_3.1-128
```

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