Programming assignment, R: Simulating Pathways, Mutual Exclusivity, et al. in Cancer Progression Models

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1 Introduction

1.1 Cancer Progression Models

Cancer is a heterogeneous disease caused by the continuous accumulation of different somatic mutations during the lifetime of an individual (1–3). Identifying mutations leading to cancer progression becomes key to understand cancer development and possible treatment options (4,5). Somatic mutations that affect the cells are classified into two main groups: passenger and driver mutations. Passenger mutations are silent mutations that do not contribute to tumor development (6). On the other hand, driver mutations provide the cells with morphological and metabolic alterations that ultimately lead to a selective growth advantage (7). Driver mutations can affect both oncogenes and tumor suppressor genes, but conversely (8).

Although cancer progression is a dynamic process, tumor data is usually obtained as cross-sectional samples. This sort of data is a combination of single-time snapshots taken from different tumors at different stages of cancer progression (2). However, a longitudinal dataset consisting of the same individual tumor samples from different time points is preferred. Not all possible order of mutations seem to be equally responsible for cancer progression. Therefore, it is necessary to know which are the restrictions leading to cancer development. Models explaining those dependencies are called Cancer Progression Models (CPMs) (9). CPMs are depicted as Directed Acyclic Graphs (DAG), where nodes represent genes and arrows dependencies between them (5).

1.2 Evolutionary Models

Previous studies have inferred the alterations (passenger and driver genes) and the order in which they occur during cancer progression using generative probabilistic models (1,10,11). Those methods use Oncogenic Trees (OT) and Conjunctive Bayesian Networks (CBN) to impose restrictions in the occurrence of mutations. As discussed in (9), in such methods a mutation in a driver gene can occur only if the preceding parent mutations have occurred, this is known as ***monotonicity***. Nevertheless, it is not realistic to have a single set of restrictions for all genotypes since genotypes can follow different paths during disease progression (9). Thus, OTs and CBNs cannot be used to address deviations from monotonicity (9). However, evolutionary tumor progression models can incorporate the order restrictions from OTs and CBNs and allow us to analyze the consequences of deviations from monotonicity and the genetic context in which a mutation appears (9). Moreover, fitness landscapes can be used to understands the consequences of different evolutionary scenarios in CPMs, such as the possible paths of tumor progression and identification of genes that can block those paths (5).

1.3 Order of Effects

The order in which somatic mutations are acquired influence clonal evolution since mutations may behave as driver or passenger depending on the genetic context (9,12). Three mechanisms may contribute to the influence of the order of effects (12). First, the initial mutation can alter the cellular environment of a neoplastic clone. Then, as a consequence, the second mutation will arise in a cellular environment determined by the first mutation (12). Second, the initial mutation can alter cellular pathways as targets for subsequent mutations (12). Third, the initial mutation can modify the epigenetic program of cells and thus alter the consequences of the second mutation (12). Therefore, the fitness of mutations depends on which mutations were acquired previously. It is important to mention the order of effects is different from the restrictions imposed in a DAG since, in restrictions, the fitness of a double mutant does not depend on which mutation was acquired first (13).

1.4 Epistatic Interactions

Epistasis is defined as a deviation from the expected phenotype when combining two alleles (14). Cancer progression is driven by the accumulation of somatic mutations that interact epistatically, that is their effect is non-additive to the tumor fitness as a phenotype (14,15). For example, combinations of mutations that show positive epistasis result in a stronger fitness increase (stronger than the additive effects of individual mutations) (15). On the other hand, mutations that show negative epistasis result in a fitness decrease (less than expected from their additive effects) (15). Therefore, mutations that show positive fitness are more likely to co-occur, whereas mutations that show negative fitness are rarely observed together resulting in mutual exclusivity (15). Moreover, reciprocal sign epistasis (see below) affects the ruggedness and leads to multiple peaks (a signature of epistasis) in the fitness landscape (5,16).

1.4.1 Synthtetic Viability

Synthetic viability is the combination of two mutations that rescue the lethal effects of individual mutation (17). The idea of synthetic viability has been recently applied to identify genomic markers for drug resistance prediction and drug-combination for anti-cancer therapy (17).

1.4.2 Mutual Exclusivity

Mutual exclusivity is a common phenomenon in cancer progression (2,15) and occurs by synthetic lethality (described below) and null effect. This phenomenon is common in cancer signaling pathways (2,15). Synthetic lethality (or reciprocal sign epistasis) occurs when the combination of two mutations is detrimental for the viability of the cell, whereas individual mutation is not (5). On the other hand, the null effect states that a mutation that occurs first involves most of the selective advantage and thus decreases the selective pressure for other mutations to arise (2,15).

1.5 Frequency Dependent Fitness

One of cancer's key features is intratumor heterogeneity, which is the coexistence of multiple malignant clones in time. In an evolutionary context, different cells compete for niche resources, establishing relationships that impact each clone's fitness. Several authors state this behavior can be explained by **evolutionary game theory** (18), in which the "survival game" takes place between cells with different strategies to keep replicating. These strategies are the phenotypes acquired by a certain accumulation of mutations, whose effect is also dependent on the temporary space in which they happen. Ancestor clones and their effect on modulating the tumor microenvironment (TME) would have conditioned the fitness of current phenotypes, which also impact each other while coexisting. Thus, it is reasonable to consider that clones affect one another in a frequency-dependent manner, meaning fitness is actually a function of the relative frequency of other clones (18).

In this work, we mapped DAGs inferred from three different generative probabilistic models to actual tumor evolutionary models by allowing deviations from monotonicity using functions of the **OncoSimulR** package. Moreover, we simulated other relevant scenarios in cancer progression, such as order of effects, epistatic interactions (synthetic lethality, synthetic viability), and frequency-dependent fitness. In addition, we mapped the genotypes of our evolutionary models to fitness landscapes in order to gain a better knowledge of mutational paths during tumor progression. Similarly, we did simulations of tumor progression to understand the effect of fitness associated with each genotype.

2 Methods

Complete fitness specification of each model was obtained from the function allFitnessEffects. Each evolutionary model was specified by a data frame where dependencies between genes inferred in each model were

indicated as parent-child relationships. Parent gene mutations are mandatory for child gene mutations to occur (monotonicity). Parents and children relationships were introduced into the model by two different vectors, "parent" and "child", respectively. Mutations do not require a previous mutation derived from the "Root" node (non-altered genotype) to arise. Moreover, two additional vectors, "s" and "sh", were used to specify fitness effects associated with each genotype (evolutionary model, not just a generative model). Vector "s" specifies the fitness values if the restrictions defined in the CPMs model are satisfied. On the other hand, vector "sh" defines the fitness values if restrictions are not satisfied. The fitness value of mutations against imposed constraints can be set to 0. However, we wanted to allow deviations from the monotonicity by setting a penalization when those situations occur (negative value in "sh").

Additionally, the type of dependency between mutations can also be specified in the data frame. There are three different possible dependencies in OncoSimulR: monotone relationship, where the relationship between specific genes is fully respected; semimonotone relationship, where two or more parents are connected to the same child, but only one parent mutation is enough for the child mutation to appear; and XOR relationship, child mutation will occur only if one parent has already mutated. These relationships were defined in a new vector called "typeDep". Nomenclature for the three possible genes relationship is "MN", "SM", and "XMPN" for monotone, semimonotone, and XOR relationship, respectively.

The data frame with the defined restrictions was used as an argument for the function allFitnessEffects. Restrictions from the data frame can also be applied to a set of genes (i.e. module) (13). This situation was defined in the vector "geneToModule". OncoSimulR allows to specify driver or passenger genes in the models. In this work, all genes/modules implemented are driver genes/modules, defined in the vector "drvNames". A fitnessEffects object is returned from the function allFitnessEffects and was used as input for the function that plots the DAG. This is possible because the OncoSimulR package implements the method plot.fitnessEffects for fitnessEffects objects. Genes names from modules were shown using the argument "expandModules = TRUE". Wild type fitness were displayed using "addwt = TRUE". Also, the function evalAllGenotypes returned the fitness values of each genotype using the fitnessEffects object as input. Finally, the fitness landscape of each evolutionary model was plotted using plot or PlotFitnessLanscape functions.

Fitness effects associated with each genotype were used to simulate tumor progression using the function oncoSimulIndiv or oncoSimulPop. These functions simulate a single evolutionary trajectory or a set of evolutionary trajectories in the same conditions, respectively. The McFarland model (continuous-time, logistic-like, and death rate depends on population size) was used for simulation of tumor progression since it leads to better performance (9). Initial population size, mutation rate, and final time of simulation were set for each evolutionary model. The argument "sampleEvery" informs about how often the whole population is sampled. Since we used the McFarland model, a very small value was set for "sampleEvery". The "keepEvery" value was set larger than the "sampleEvery" value to obtain high-quality plots. "keepPhylog = TRUE" was used to store the parent-child relationships that arise in the simulation as well as their frequency. These relationships were plot using the "plotClonePhylog" function. The argument "onlyCancer" was set to TRUE, so simulations end only if cancer is reached, or FALSE otherwise. Simulations were plotted using the function "plot". Plot styles were set to "stacked" or "line" with the argument "type".

We also derived simpler models that maintain all the restrictions imposed in the original models. These simpler models focus only on the important genes in colorectal cancer onset (19) and were used to easily interpret and visualize the different utilities available in OncoSimulR, such as order effects, epistasis, and frequency-dependent fitness.

3 PathTiMEx, a Generative Probabilistic Graphical Model of Cancer Progression

Cristea et al., 2017 (10) introduced a generative probabilistic graphical model of cancer progression called pathTiMEx. pathTiMEx is both, a waiting time model for independent mutually exclusive pathways and a waiting time model for cancer progression among single genes. The colorectal cancer model depicted in Figure 3.A from (10) is composed of 8 genes (APC, KRAS, TP53, EVC2, PIK3CA, EPHA, FBXW7, and

TCF7L2) and is organized into 3 mutually exclusive modules and 2 individual genes. Mutually exclusive modules represent a set of genes participating in the same pathway that do not mutate at the same time (20). The colorectal cancer dataset used to build that model was obtained from (21). The generative model was mapped into an evolutionary model, where deviations from monotonicity are allowed.

The poset restrictions proposed in (10), were implemented using the function allFitnessEffects. Some parameters are mandatory when the function is used (restriction table). s and sh values were not specified in (10) since they were not interested in fitness. Values gave to both parameters derived from the waiting time rate parameter λ defined in the model. Early events in cancer progression are important to cancer onset and, therefore, will get greater λ values, whereas late events will have a lower value (values for all genes or modules are showed in Table 1). It has been previously proposed that alterations associated with the onset of colorectal cancer (such as APC, KRAS, and TP53) may provide a larger fitness advantage than late alterations associated with tumor progression (22). sh was set to a constant value for all possible deviations from restrictions. Dependencies between genes were set as monotonic (MN), and the model was represented as a DAG.

Table 1: Waiting time rate parameter (λ) for each gene/module

Gene/module	Waiting time rate parameter (λ)
APC	9.5
KRAS	2.89
TP53, EVC2	1.92
PIK3CA, EPHA3	0.17
FBXW7, TCF7L2	0.08

```
## First, it is necessary to load OncoSimulR and igraph package
library(OncoSimulR)


## Restriction table (extended version of the poset)
colcancer <- data.frame(
                 parent = c(rep("Root",3), "A", "B", "C"), # Parent nodes
                 child = c("A", "B", "D", "C", "E", "E"), ## Child nodes
                 s = c(0.5, 0.2, 0.05, 0.1, rep(0.05, 2)),

                 sh = -0.3,

                 typeDep = "MN" ## Type of dependency
                  )

## Fitness specification of the poset
colcancer_efec <- allFitnessEffects(
                  colcancer, # Poset

                  geneToModule = c( ## Specification of the modules
                                "Root" = "Root",
                                "A" = "APC",
                                "B" = "TP53, EVC2",
                                "C" = "KRAS",
```

```
                                "D" = "PI3KCA, EPHA",
                                "E" = "FBXW7, TCF7L2"),

                    drvNames = c( ## Specification of drivers
                                "APC", "TP53", "EVC2", "KRAS",
                                "PI3KCA", "EPHA", "FBXW7", "TCF7L2")
                    )



## DAG representation
plot(colcancer_efec, expandModules = TRUE, autofit = TRUE, lwdf = 2)
```

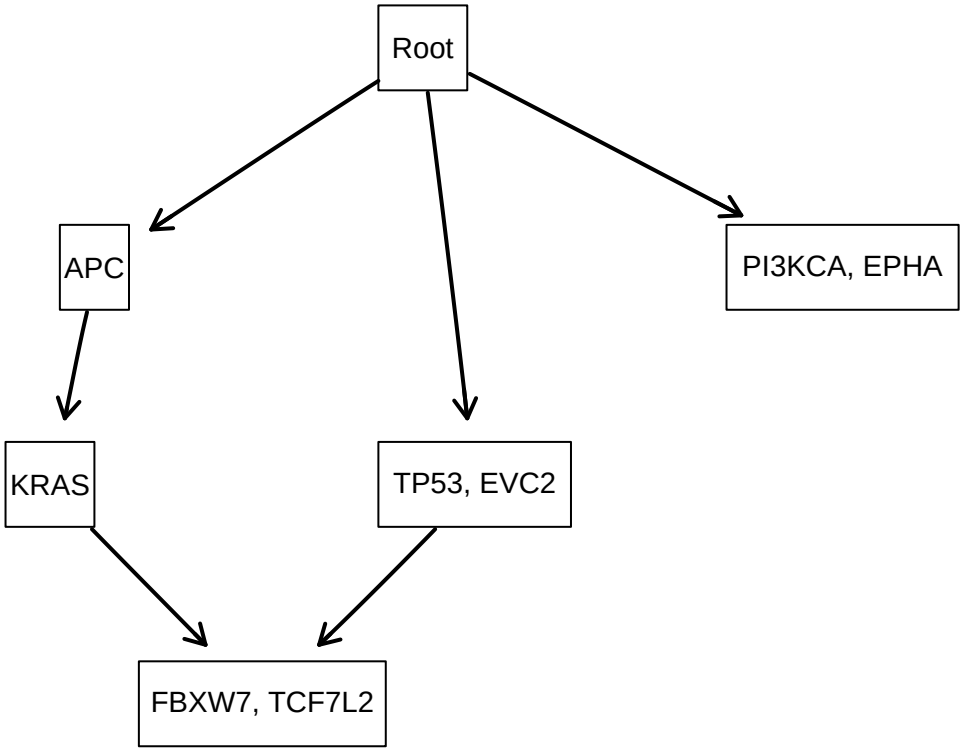

Figure 1: DAG from colorectal cancer dataset

Figure 1 shows the DAG derived from the generative model proposed in (10). Different branches appear out from the non-altered genotype ("Root" node), each of them mimicking the dependencies inferred in (10). The function evalAllGenotypes was used to map genotypes to fitness values. Figure 2 shows the fitness landscape derived from the DAG (see Figure 1). All possible genotypes obtained from the DAG were labeled in the fitness landscape. A busy combination of mountains and valleys can be observed in the fitness landscape due to the huge amount of possible genotypes. The highest peaks represent local maximum (green box), whereas the lowest peaks represent local minimum (red box). Genotypes climb or go down hills depending on whether acquired mutation increase or decrease fitness, respectively. The evolutionary model allows mapping the fitness associated with each genotype beyond a simple set of restrictions.

```
colcancer_efec_FL <- evalAllGenotypes(colcancer_efec, max = 110000)
## Output is not shown due to size of the table.

## Plot of fitness landscape
plotFitnessLandscape(colcancer_efec_FL)
```

Figure 2: Fitness landscape from colorectal cancer

3.1 Simplified Cancer Progression Model

In order to properly visualize the fitness landscape, a simplified version of the model in section 3 was generated. This model does not use modules, just individual genes. This approach will lead to a clear fitness landscape and to proper identification of events that may occur.

There is a phenomenon of mutual exclusivity between genes of the modules (10). Mutations of certain genes may not be present in the genotype if another gene of the same pathway is already mutated. Dependencies between genes of the same module are defined by default as semimonotone (OR relationship) in OncosimulR. Hence, only one mutation in the genes of the pathway is enough to provide all fitness contribution to the genotype. Mutations in other genes of the same module will constitute a null effect on genotype fitness.

```
## Fitness specification of the simplified poset
Scolcancer <- allFitnessEffects(colcancer,

                    geneToModule = c( ## Specification of the modules
```

```
                                "Root" = "Root",
                                "A" = "APC",
                                "B" = "TP53",
                                "C" = "KRAS",
                                "D" = "PI3KCA",
                                "E" = "FBXW7"),

                    drvNames = c( ## Specification of drivers
                                "APC", "TP53", "KRAS",
                                "PI3KCA", "FBXW7")
                    )
```

```
plot(Scolcancer, expandModules = TRUE, autofit = TRUE, lwdf = 2)
```

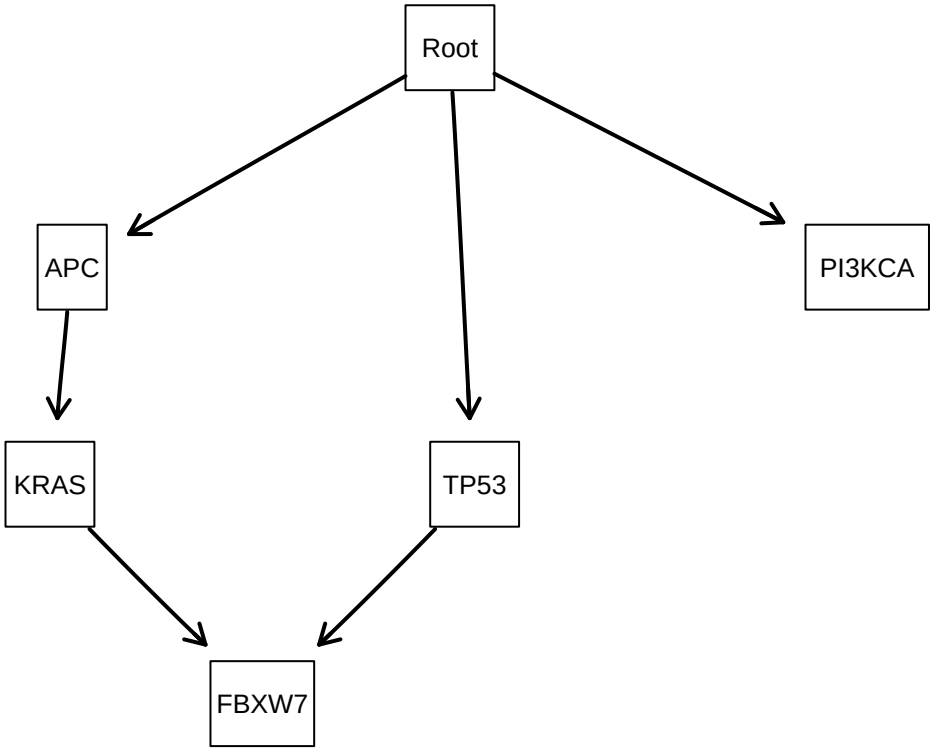

Figure 3: DAG from a simplified model of colorectal cancer

```
## Obtain all genotypes from the fitnessEffect
(Scolcancer_ge <- evalAllGenotypes(Scolcancer))
```

```
##                          Genotype Fitness
## 1                             APC 1.50000
## 2                           FBXW7 0.70000
## 3                            KRAS 0.70000
```

```
## 4                          PI3KCA 1.05000
## 5                            TP53 1.20000
## 6                       APC, FBXW7 1.05000
## 7                        APC, KRAS 1.65000
## 8                      APC, PI3KCA 1.57500
## 9                        APC, TP53 1.80000
## 10                     FBXW7, KRAS 0.49000
## 11                   FBXW7, PI3KCA 0.73500
## 12                     FBXW7, TP53 0.84000
## 13                    KRAS, PI3KCA 0.73500
## 14                      KRAS, TP53 0.84000
## 15                    PI3KCA, TP53 1.26000
## 16                APC, FBXW7, KRAS 1.15500
## 17              APC, FBXW7, PI3KCA 1.10250
## 18                APC, FBXW7, TP53 1.26000
## 19               APC, KRAS, PI3KCA 1.73250
## 20                 APC, KRAS, TP53 1.98000
## 21               APC, PI3KCA, TP53 1.89000
## 22             FBXW7, KRAS, PI3KCA 0.51450
## 23               FBXW7, KRAS, TP53 0.88200
## 24             FBXW7, PI3KCA, TP53 0.88200
## 25              KRAS, PI3KCA, TP53 0.88200
## 26       APC, FBXW7, KRAS, PI3KCA 1.21275
## 27         APC, FBXW7, KRAS, TP53 2.07900
## 28       APC, FBXW7, PI3KCA, TP53 1.32300
## 29        APC, KRAS, PI3KCA, TP53 2.07900
## 30      FBXW7, KRAS, PI3KCA, TP53 0.92610
## 31 APC, FBXW7, KRAS, PI3KCA, TP53 2.18295
```

```
## Plot the fitness landscape.
plotFitnessLandscape(Scolcancer_ge,
                     use_ggrepel = TRUE)
```

Figure 3 and Figure 4 show the DAG graph and fitness landscape of the simplified model, respectively. DAG shown in Figure 3 is the same as the DAG depicted in Figure 1 but without expanding modules (a gene from each module was selected for this simplification). Only one local maximum is depicted in the fitness landscape (see Figure 4). It corresponds to the genotype carrying mutations in the five genes. Note that the fitness value associated with the local maximum does not differ much from the fitness value associated with the genotype carrying mutations in APC-PI3KCA-TP53. This reflects the idea that mutations related to cancer onset contribute more to fitness than late mutations related to cancer progression. Hence, fitness contributions of mutations in genes FBXW7 or PI3KCA are minimal. On the other hand, only one local minimum is depicted. It corresponds to the genotype more deviated from monotonicity and it refers to the genotype carrying mutations in both KRAS and FBXW7 genes. Both mutations are expected to occur after mutations in genes APC and TP53, respectively.

3.2 Simulating Data from a Simplified Model

DAG was used as a guideline to built the fitness landscape (see Figure 4). This fitness landscape shows each possible genotype as well as its fitness. The function oncoSimulIndiv was used to simulate colorectal tumor progression. Poset with the order restrictions defined for the simplified model (see subsection 3.1) was used. The McFarland model was used for the simulation of cancer progression. The initial population size was set to 500. Only one mutation rate was used 5e-5. The parameter keepPhylog was set TRUE to store the

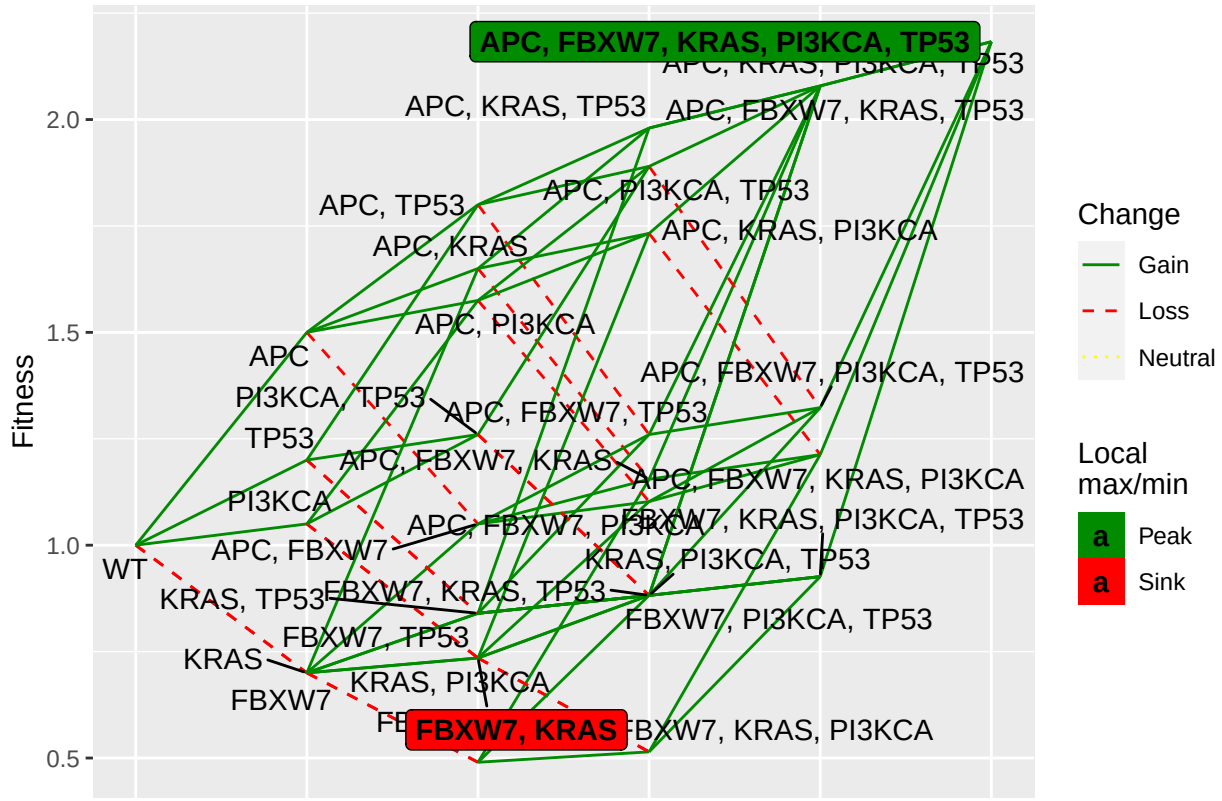

Figure 4: Fitness landscape from a simplified model of colorectal cancer

parent-child relationships occurring in the simulation as well as its frequencies (plotClonePhylog function). The onlyCancer parameter was set True to stop the simulation when cancer is reached.

```
set.seed(35) ## Fix the seed for reproducibility

Simul <- oncoSimulIndiv(Scolcancer, ## A fitnessEffects object
                        model = "McFL", ## Model used
                        mu = 5e-5, ## Mutation rate
                        sampleEvery = 0.03, ## How often the whole population is sampled
                        keepEvery = 1,
                        initSize = 500, ## Initial population size
                        keepPhylog = TRUE, ## Allow to see parent-child relationships
                        onlyCancer = TRUE,
                        detectionSize = NA
)


## Plot of simulation
plot(Simul, ## OncoSimulIndv model
     show = "genotypes",
     type = "stacked"

)
```

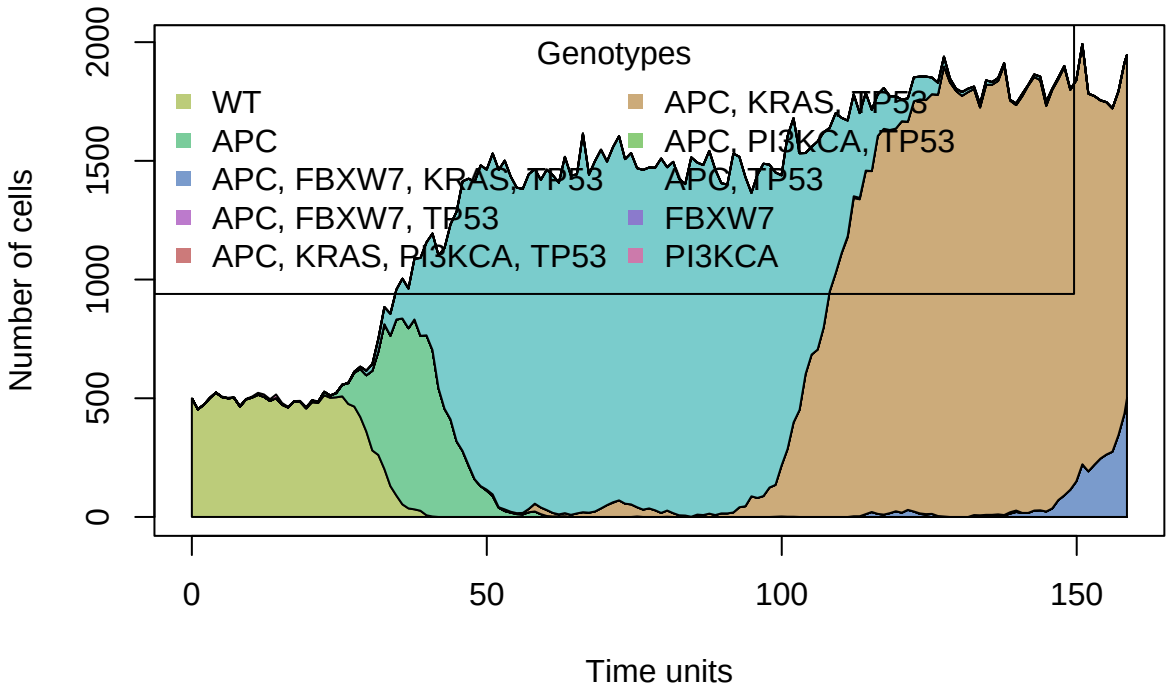

Figure 5: Simulation of cancer progression using the fitness landscape of the simplied model (stacked plot)

```r
## Plot of simulation
plot(Simul, ## OncoSimulIndv model
     show = "genotypes",
     type = "line"
)
```

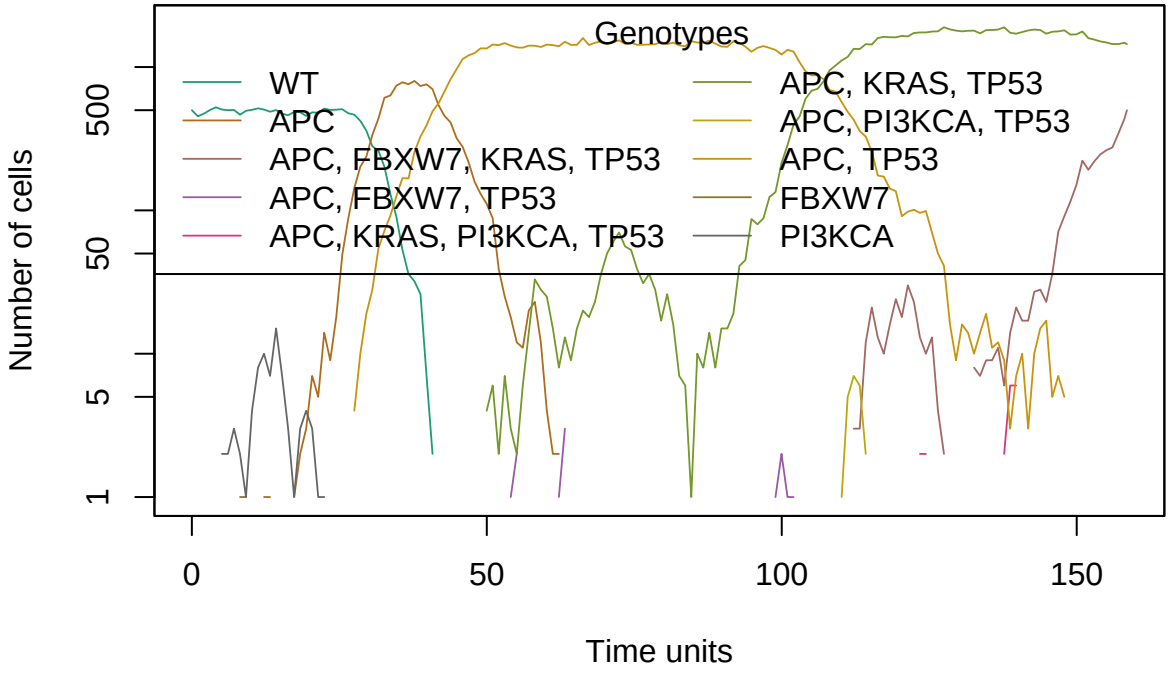

Figure 6: Simulation of cancer progression using the fitness landscape of the simplied model (line plot)

```r
## Parent-child relationship derived from simulation
plotClonePhylog(Simul, fixOverlap = TRUE,
                N = 0, ## Specify clones that exist
                keepEvents = TRUE ## Arrows showing how many times each clones appeared


)
```

A stacked and line plot of the simulation is depicted in Figure 5 and Figure 6, respectively. Both plots show the evolution of the cell population's genotype with time. Note that cancer is reached, and thus simulation is stopped when a genotype other from the local maximum genotype is fixed (genotype carrying all mutations, see Figure 4). Interestingly, the local maximum genotype does not appear in the simulation when cancer is reached. Although different genotypes coexist when cancer is reached, APC-KRAS-TP53 is the predominant genotype fixed in the simulation. This genotype carries mutations in the three genes yielding cancer onset. It was previously discussed that those three genes were responsible for cancer onset, and thus they have associated a higher fitness contribution than late mutations responsible for cancer progression. Moreover,

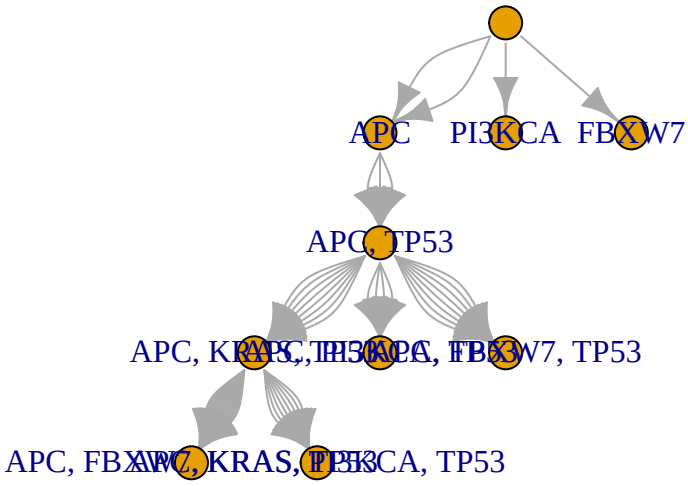

Figure 7: Parent-child relationship derived from simulation

recall that the fitness value of the APC-KRAS-TP53 genotype was close to the local maximum peak in the fitness landscape (see Figure 4). In this context, it is not surprising that cancer is reached when the genotype carrying those three mutations is fixed in simulation. The genotype carrying an additional mutation in the FBXW7 gene starts to arise, but this genotype is responsible for cancer progression, the next step in cancer.

Figure 6 shows a better perspective of genotypes in the simulation. Wild type genotype coexists with clones carrying mutations in APC or PI3KCA. However, APC genotype's increase triggers wild type extinction. PI3KCA genotype is not able to compete with other genotypes and ends goes extinct (deviation from monotonicity). Interestingly, when the APC-TP53-KRAS genotype first shows up, it cannot compete with the predominant genotype at that time. In fact, it goes to en=xtinction, but when it shows up for the second time, is able to fix in simulation and leads to the APC-TP53 genotype's extinction.

Figure 7 shows the genealogical evolution of genotypes in the simulation. Arrow width represents the frequency of clone appearance. Wider arrows indicate a higher frequency of change from the parent genotype to the child genotype. Although Wild Type genotype mutates into APC, PI3KCA or FBXW7, only genotypes carrying APC mutation remain. The highest frequency between parent and child genotypes is located between genotypes APC-KRAS-TP53 and APC-FBXW7-KRAS-TP53. Thus, cancer progression is led by the APC-FBXW7-KRAS-TP53 genotype that comes from the APC-KRAS-TP53 genotype.

3.3 Order Effects

To explore order effects in cancer progression, another evolutionary model derived from the generative model inferred in (10) was created. This simplified model just contains 4 genes: APC, TP53, FBWX7, and KRAS. The relationships between those genes were previously depicted in subsection 3.1. Both, APC and TP53 genes have as "parent" the non-altered genotype ("Root"). APC gene has as "parent" the KRAS gene. On the other hand, a mutation in the FBWX7 gene requires KRAS and TP53 genes already mutated. Figure 8 shows the DAG of the simplified model just described. s and sh parameters are the same as in section 3. Dependency between genes is set as monotonic ("MN").

Based on the waiting time rate parameter λ, the fitness effect of each possible order is given (see Table 1). APC is associated with the highest λ value, which means that it seems to mutate early in the cancer progression. λ for FBWX7 is the lowest between the four, meaning that it mutates the last. TP53 mutation occurs between APC and KRAS. The order effect favored is: APC > TP53 > KRAS > FBWX7. This order in mutation acquisition is consistent with the time rate parameter λ and was given the highest fitness. Other possible combinations of mutation's acquisition are not consistent with restrictions inferred in (10), and therefore a lower fitness value was given.

Order effects between genes were introduced in the argument orderEffects of the allFitnessEffects function and were defined with ">" symbols. For instance, A > B indicates that order effects were satisfied only when gene A is mutated before gen B. Fitness-genotype association was plotted using the evalAllGenotypes function. A table containing all possible mutation acquisitions and the fitness associated with each order of mutations (for each genotype) was obtained. In this approach, each possible genotype was associated with multiple fitness values (e.g. APC-FBWX7 genotype is mapped to a fitness value of 1 or 1.2 depending on gene order mutations), except for the genotypes only carrying one mutation. Different fitness values associated with each possible genotype depend on the mutation's path followed by the genotype. Mutations consistent with restrictions defined in the DAG are associated with higher fitness values. However, paths violating those restrictions (deviations from monotonicity) are penalized and fitness decreases. In total, there is 64 possible order of mutations for the all the genotypes. As expected, the highest fitness value corresponds to the genotype that suffered the mutations in an order that does not deviate from monotonicity.

In section 3 and subsection 3.1 order effect is not considered, and the final fitness value is the same for genotypes carrying the same mutations. Nevertheless, if an order of effect is assumed, multiple fitness values are associated with each genotype yielding to a complex fitness landscape where a single genotype is multidepicted **(NOT SURE IF THAT WORD WORKS)**. An error raised when we try to plot the DAG and the fitness landscape. Order effects implemented in OncoSimulR allow to evaluate all possible gene mutational paths (genotype-fitness table), but it does not allow to generate neither a DAG nor a fitness

landscape from restrictions specified as order effects. This is one limitation of the OncoSimulR package, it does not yet allow to visualize those evolutionary scenarios.

```
cc <- data.frame(parent = c(rep("Root", 2), "A", "B", "C"),
                 child = c("A", "C", "B", "D", "D"),
                 s = c(0.5, 0.2, 0.1, rep(0.05,2)),
                 sh = -0.3,
                 typeDep = "MN")

cc_visuali <- allFitnessEffects(cc,
                                  geneToModule =
    c("Root" = "Root",
      "A" = "APC",
      "B" = "KRAS",
      "C" = "TP53",
      "D" = "FBXW7") )
## DAG
plot(cc_visuali, expandModules = TRUE, autofit = TRUE, lwdf = 2)
```

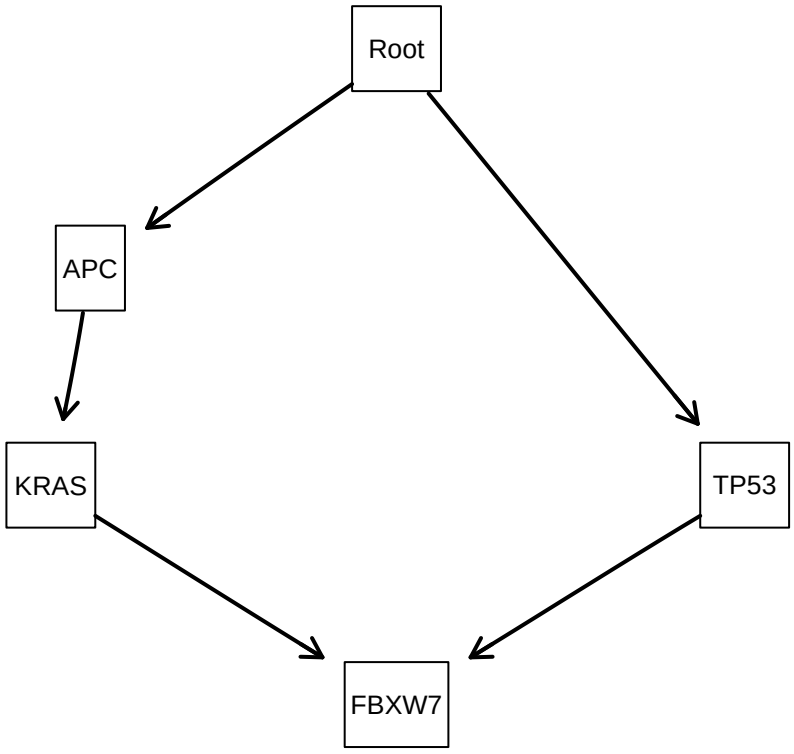

Figure 8: DAG from a simplified model of colorectal cancer

```
cc_order <- allFitnessEffects(
  orderEffects = c("A > B > C > D" = 0.5,
                   "B > A > C > D" = 0.2,
                   "B > C > A > D" = 0.1,
```

```
                  "B > C > D > A" = 0.05,
                  "A > C" = 0.2,
                  "C > A" = 0.05,
                  "D > A" = 0.05,
                  "A > D" = 0.2,
                  "B > D" = 0.2,
                  "C > D" = 0.2,
                  "B > C" = 0.2,
                  "C > B" = 0.1,
                  "B > A" = 0.1,
                  "A > B" = 0.3),

  geneToModule =
    c("A" = "APC",
      "B" = "KRAS",
      "C" = "TP53",
      "D" = "FBXW7") )

(cc_order_geno <- evalAllGenotypes(cc_order, order = TRUE))
```

```
##                     Genotype  Fitness
## 1                        APC 1.000000
## 2                      FBXW7 1.000000
## 3                       KRAS 1.000000
## 4                       TP53 1.000000
## 5                  APC > FBXW7 1.200000
## 6                   APC > KRAS 1.300000
## 7                   APC > TP53 1.200000
## 8                  FBXW7 > APC 1.050000
## 9                 FBXW7 > KRAS 1.000000
## 10                FBXW7 > TP53 1.000000
## 11                  KRAS > APC 1.100000
## 12                KRAS > FBXW7 1.200000
## 13                 KRAS > TP53 1.200000
## 14                  TP53 > APC 1.050000
## 15                TP53 > FBXW7 1.200000
## 16                 TP53 > KRAS 1.100000
## 17         APC > FBXW7 > KRAS 1.560000
## 18         APC > FBXW7 > TP53 1.440000
## 19         APC > KRAS > FBXW7 1.872000
## 20          APC > KRAS > TP53 1.872000
## 21         APC > TP53 > FBXW7 1.728000
## 22          APC > TP53 > KRAS 1.716000
## 23         FBXW7 > APC > KRAS 1.365000
## 24         FBXW7 > APC > TP53 1.260000
## 25         FBXW7 > KRAS > APC 1.155000
## 26        FBXW7 > KRAS > TP53 1.200000
## 27         FBXW7 > TP53 > APC 1.102500
## 28        FBXW7 > TP53 > KRAS 1.100000
## 29         KRAS > APC > FBXW7 1.584000
## 30          KRAS > APC > TP53 1.584000
## 31         KRAS > FBXW7 > APC 1.386000
## 32        KRAS > FBXW7 > TP53 1.440000
```

```
## 33         KRAS > TP53 > APC 1.386000
## 34       KRAS > TP53 > FBXW7 1.728000
## 35        TP53 > APC > FBXW7 1.512000
## 36         TP53 > APC > KRAS 1.501500
## 37        TP53 > FBXW7 > APC 1.323000
## 38       TP53 > FBXW7 > KRAS 1.320000
## 39         TP53 > KRAS > APC 1.270500
## 40       TP53 > KRAS > FBXW7 1.584000
## 41 APC > FBXW7 > KRAS > TP53 2.246400
## 42 APC > FBXW7 > TP53 > KRAS 2.059200
## 43 APC > KRAS > FBXW7 > TP53 2.695680
## 44 APC > KRAS > TP53 > FBXW7 4.852224
## 45 APC > TP53 > FBXW7 > KRAS 2.471040
## 46 APC > TP53 > KRAS > FBXW7 2.965248
## 47 FBXW7 > APC > KRAS > TP53 1.965600
## 48 FBXW7 > APC > TP53 > KRAS 1.801800
## 49 FBXW7 > KRAS > APC > TP53 1.663200
## 50 FBXW7 > KRAS > TP53 > APC 1.455300
## 51 FBXW7 > TP53 > APC > KRAS 1.576575
## 52 FBXW7 > TP53 > KRAS > APC 1.334025
## 53 KRAS > APC > FBXW7 > TP53 2.280960
## 54 KRAS > APC > TP53 > FBXW7 3.284582
## 55 KRAS > FBXW7 > APC > TP53 1.995840
## 56 KRAS > FBXW7 > TP53 > APC 1.746360
## 57 KRAS > TP53 > APC > FBXW7 2.634509
## 58 KRAS > TP53 > FBXW7 > APC 2.200414
## 59 TP53 > APC > FBXW7 > KRAS 2.162160
## 60 TP53 > APC > KRAS > FBXW7 2.594592
## 61 TP53 > FBXW7 > APC > KRAS 1.891890
## 62 TP53 > FBXW7 > KRAS > APC 1.600830
## 63 TP53 > KRAS > APC > FBXW7 2.195424
## 64 TP53 > KRAS > FBXW7 > APC 1.920996
```

```
#DAG
plot(cc_order)
```

```
## Error in `*tmp*`[[i]]: subíndice fuera de  los límites
```

```
# Fitness landscape
plotFitnessLandscape(cc_order_geno)
```

```
## Error in to_Fitness_Matrix(x, max_num_genotypes = max_num_genotypes): We cannot deal with order effe
```

4 Pathway Linear Progression Model: Raphael & Vanding, 2015

The Pathway Linear Progression Model (PLPM) described in (1) introduces the idea that driver mutations target pathways. This is an important concept since different individuals have driver mutations in different genes that affect the same pathway (1). Therefore, the order in which mutations arise is better described at the pathway level instead of a gene-level (1).

Here, we mapped the progression model from colorectal cancer data inferred by (1) (originally described in (21)) into an evolutionary model, allowing deviations from the restrictions imposed in the DAG. For

this, we used a vector s to indicate the fitness effects when the restrictions are satisfied and a vector sh for deviations. In (1), the authors analyzed eight genes: APC, EPHA3, EVC2, FBXW7, KRAS, PIK3CA, TCF7L2, and TP53. In this model, APC mutation is an early event, followed by mutations in TP53 and PIK3CA (mutually exclusive). KRAS mutations appear after TP53/PIK3CA mutations.

We used the allFitnessEffects function to define the nodes and their relationships. Moreover, we used modules to represent mutually exclusive genes that affect the same pathway. Assigned fitness effects values (s) were higher for earlier mutations and lower for late mutation since an earlier mutation is more prevalent in the clonal population than a later mutation, as explained in (23). A single negative value was set for deviations from restrictions (sh) and a monotonic relationship (MN) was used for relationships between nodes of the DAG since nodes have only one parent.

Figure 9 shows the DAG inferred in (1) mapped to an evolutionary model that allows deviation from restrictions. Note that genes within a module are mutually exclusive, and the restrictions go top-down (i.e. from the root to the last mutation).

```
## Define poset restrictions, mapping of genes to modules, and driver genes
CRC_W <- allFitnessEffects(data.frame(parent = c("Root", "A", "B", "C"),
                                      child = c("A", "B", "C", "D"),
                                      s = c(0.6, 0.4, 0.1, 0.05),
                                      sh = -0.5,
                                      typeDep = "MN"),
                           geneToModule = c("Root" = "Root",
                                            "A" = "APC, EPHA3, TCF7L2",
                                            "B" = "EVC2, PIK3CA, TP53",
                                            "C" = "KRAS",
                                            "D" = "FBXW7"),
                           drvNames = c("APC", "EPHA3", "TCF7L2", "EVC2", "PIK3CA",
                                        "TP53", "KRAS", "FBXW7"))

# DAG representation
plot(CRC_W, expandModules = TRUE, autofit = TRUE, lwdf = 2)
```

The function evalAllGenotypes was used to map genotypes to fitness values. Figure 10 shows the fitness landscape inferred from the DAG of Figure 9. As mentioned before, this fitness landscape with eight genes is difficult to visualize. Nevertheless, we can give a general description of the topology of the landscape. Note that there are multiple peaks and valleys, suggesting a high degree of ruggedness. Moreover, note that KRAS constitutes a local minimum. This result confirms the order of restrictions imposed by the DAG. It is important to mention that some genotypes in the local maxima are composed of genes that belong to the same module. Such genes participate in the same pathway and are mutually exclusive. Nevertheless, modules cannot capture this idea. A combination of order of restrictions (XOR relationships) and epistatic interactions was able to simulate better mutual exclusivity (see below).

```
## Map genotypes to fitness
CRC_F <- evalAllGenotypes(CRC_W, order = FALSE, addwt = TRUE)

## Plot of fitness landscape

plot(CRC_F)
```

4.1 Simplified Model

Given that our initial DAG contains eight genes, then the number of possible genotypes is $2^8 = 256$, which makes it difficult to visualize the fitness landscape. For this, reason a smaller number of genes were used to

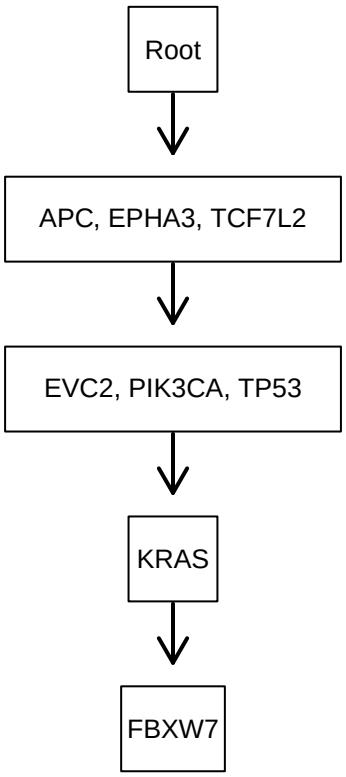

Figure 9: DAG from colorectal cancer dataset

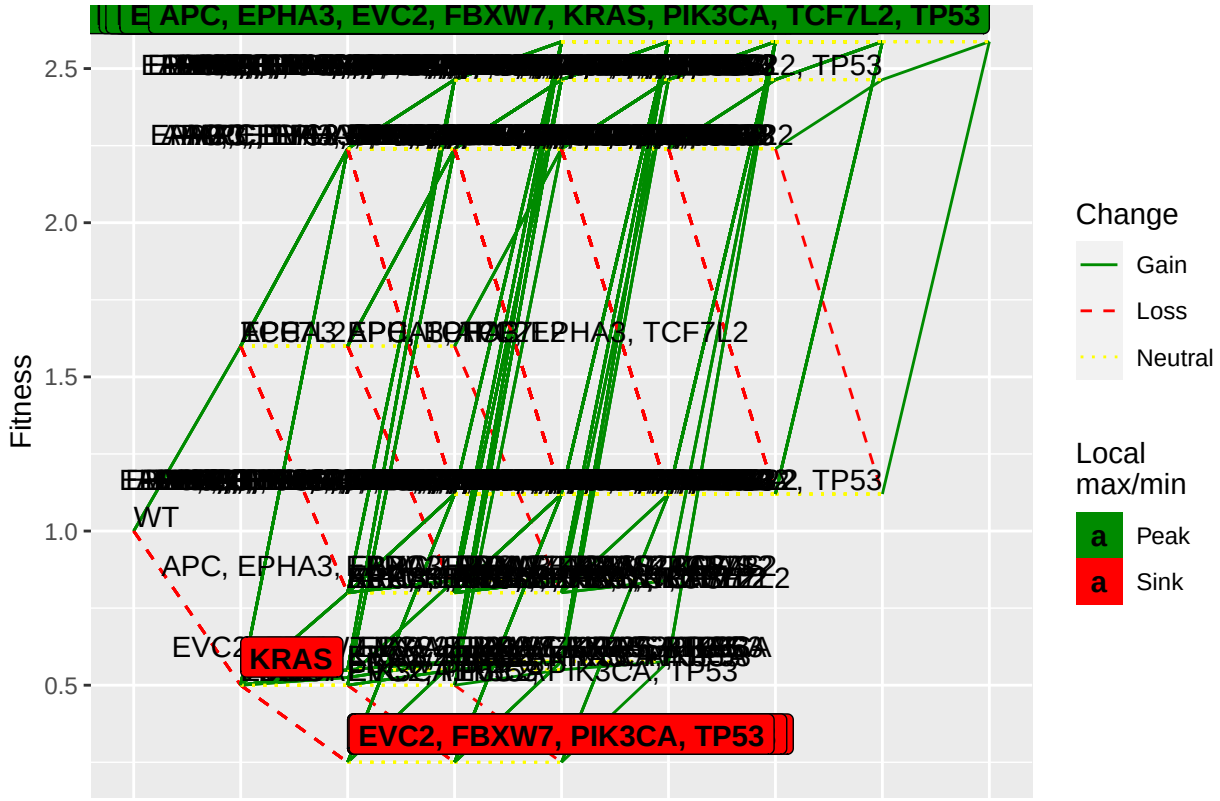

Figure 10: Fitness landscape inferred from colorectal cancer DAG

build a slightly different DAG to model other interesting scenarios (see Figure 11). The idea is to represent mutual exclusivity with an XOR relationship (red edges). Also, note that the fitness value for mutual exclusive genes (APC and TP53) is almost the same (see vector s).

```
## Simplified model
## Define poset restrictions, mapping of genes to modules, and driver genes
CRC_W2 <- allFitnessEffects(data.frame(parent = c(rep("Root", 2), "A", "B", "C"),
                                       child = c("A", "B", rep("C", 2), "D"),
                                       s = c(0.2, 0.1, rep(0.05, 2), 0.01),
                                       sh = -0.5,
                                       typeDep = c(rep("XMPN", 4), "MN")),
                            geneToModule = c("Root" = "Root",
                                             "A" = "APC",
                                             "B" = "TP53",
                                             "C" = "KRAS",
                                             "D" = "FBXW7"),
                            drvNames = c("APC", "TP53", "KRAS", "FBXW7"))

# DAG representation
plot(CRC_W2, expandModules = TRUE, autofit = TRUE, lwdf = 2)
```

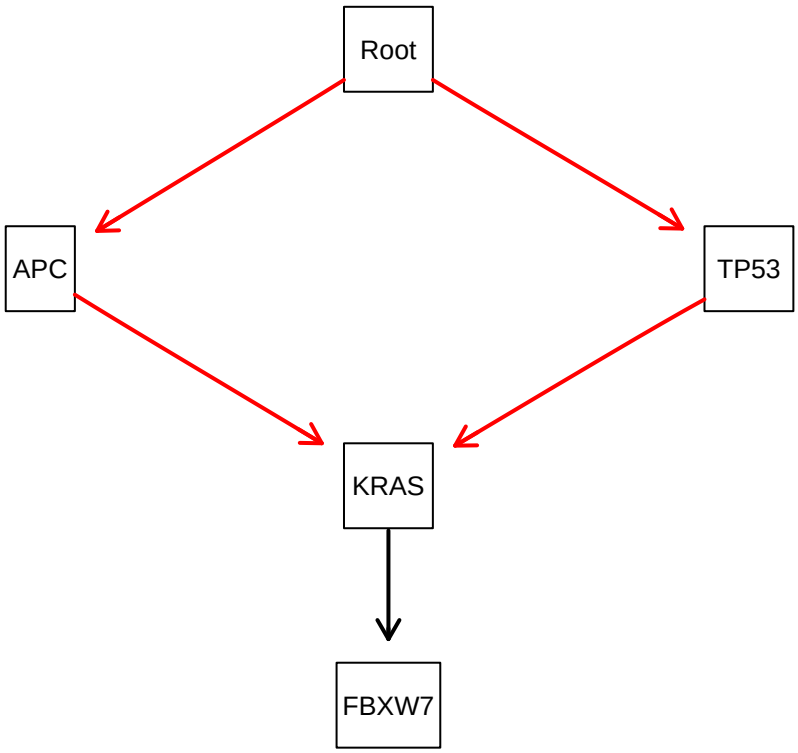

Figure 11: Simplified model from colorectal cancer DAG

Since only four genes are used in the DAG, then the possible number of genotypes is $2^4 = 16$, which are easier to interpret in a fitness landscape (see Figure 12). Now, the genotypes with the highest fitness are the

ones that fulfill the order of restrictions imposed by the DAG (e.g. APC, FBXW7, KRAS - APC, FBXW7, KRAS, TP53). On the other hand, genotypes that deviate from the imposed restrictions have the lowest fitness (e.g. KRAS - FBXW7 - APC, KRAS, TP53). However, specifying mutual exclusivity with XOR relationships cannot capture synthetic lethality between APC and TP53. Also, if an AND relationship is defined from the Root to APC and TP53, there is no change in fitness values.

```
## Simplified Model
## Map genotypes to fitness
(CRC_F2 <- evalAllGenotypes(CRC_W2, order = FALSE, addwt = TRUE))
```

```
##                   Genotype Fitness
## 1                       WT 1.00000
## 2                      APC 1.20000
## 3                    FBXW7 0.50000
## 4                     KRAS 0.50000
## 5                     TP53 1.10000
## 6               APC, FBXW7 0.60000
## 7                APC, KRAS 1.26000
## 8                APC, TP53 1.32000
## 9              FBXW7, KRAS 0.50500
## 10             FBXW7, TP53 0.55000
## 11              KRAS, TP53 1.15500
## 12       APC, FBXW7, KRAS 1.27260
## 13       APC, FBXW7, TP53 0.66000
## 14        APC, KRAS, TP53 0.66000
## 15      FBXW7, KRAS, TP53 1.16655
## 16 APC, FBXW7, KRAS, TP53 0.66660
```

```
## Plot of fitness landscap

plot(CRC_F2, use_ggrepel = TRUE)
```

4.2 Simulating Data from Simplified Model

Fitness effects and restrictions defined in the DAG from Figure 11 from the previous section was used to simulate clonal evolution. The same parameters from subsection 3.2, except initSize and finalTime, were set in the OncoSimulIndiv function. Figure 13 shows the genotypes that arise during clonal evolution. The genotype APC, TP53 fixates quickly in the clonal population. This result supports the fitness value for APC, FBXW7, KRAS depicted in Figure 12 since that genotype is one of the local maxima. A more detailed order of genotype appearances and extinctions is shown in Figure 14. Note that not all the 16 genotypes appear in the simulation because the best-fitted genotype fixates rapidly in the population leading to the extinction of some genotypes, whereas other genotypes cannot even appear. When the simulation was executed with onlyCancer = TRUE, cancer was never reached, although the fixated genotype is the global maxima of the fitness landscape (see Figure 15 and Figure 16).

```
## Fix the seed for reproducibility
set.seed(87)


CRC_W2_S <- oncoSimulIndiv(CRC_W2, ## A fitnessEffects object
  model = "McFL", ## Model used
```

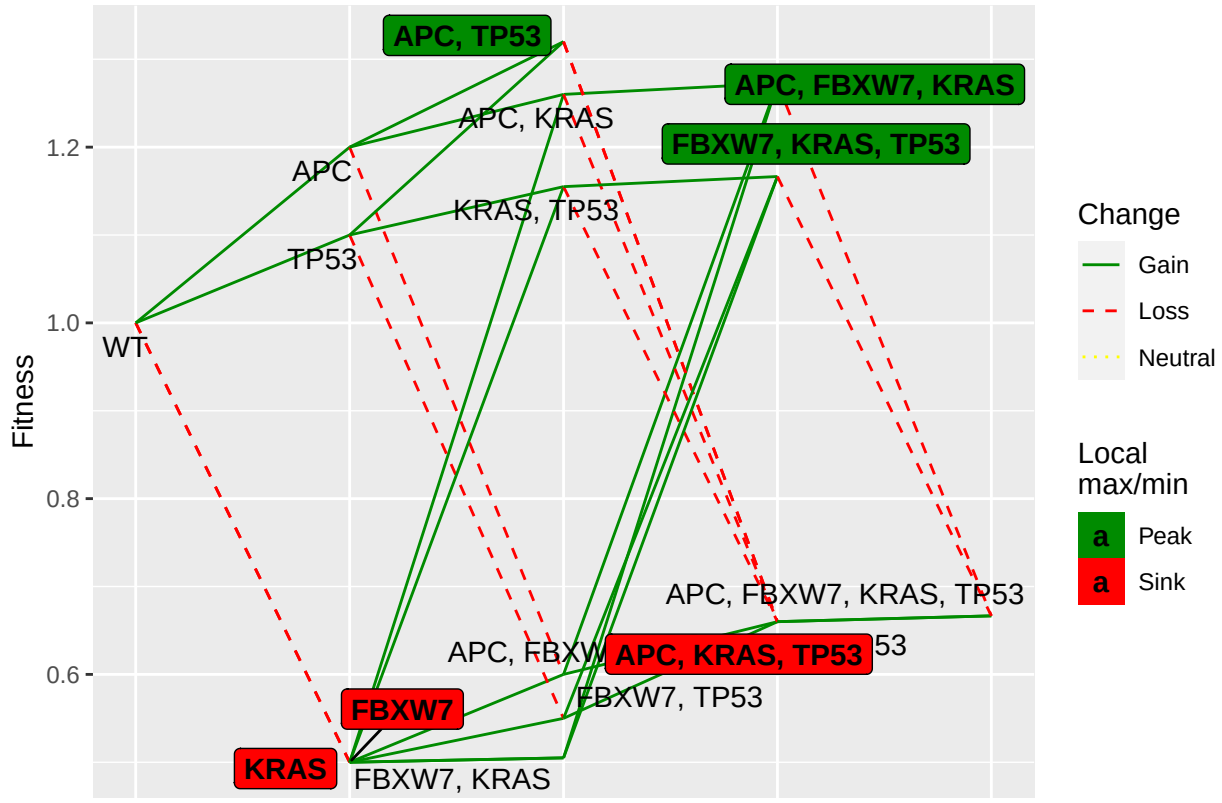

Figure 12: Fitness landscape from simplified model

```
  mu = 1e-4, ## Mutation rate
  sampleEvery = 0.02, ## How often the whole population is sampled
  keepEvery = 1,
  initSize = 2000, ## Initial population size
  finalTime = 2000,
  keepPhylog = TRUE, ## Allow to see parent-child relationships
  onlyCancer = FALSE)

## Plot of simulation for genotypes
plot(CRC_W2_S,
show = "genotypes",
type = "stacked")
```

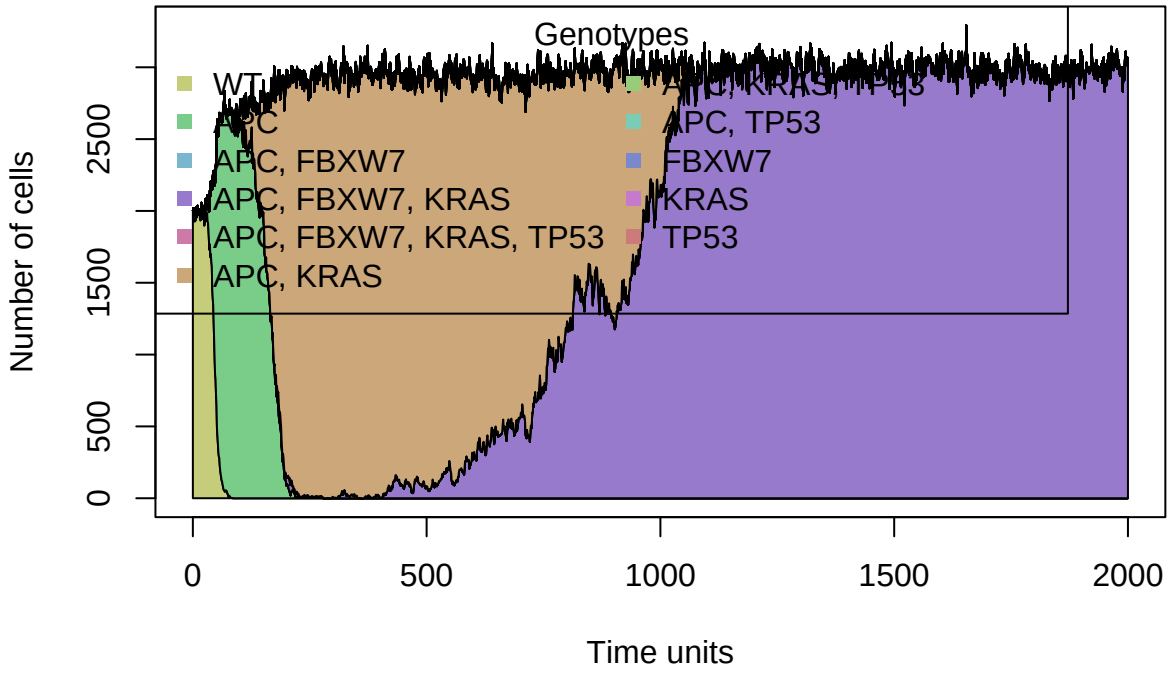

Figure 13: Simulation of cancer progression for the simplified model. Genotypes are shown stacked

```
## Plot of simulation for genotypes
plot(CRC_W2_S,
show = "genotypes",
legend.ncols = 2,
xlim = c(0, 1500),
type = "line")
```

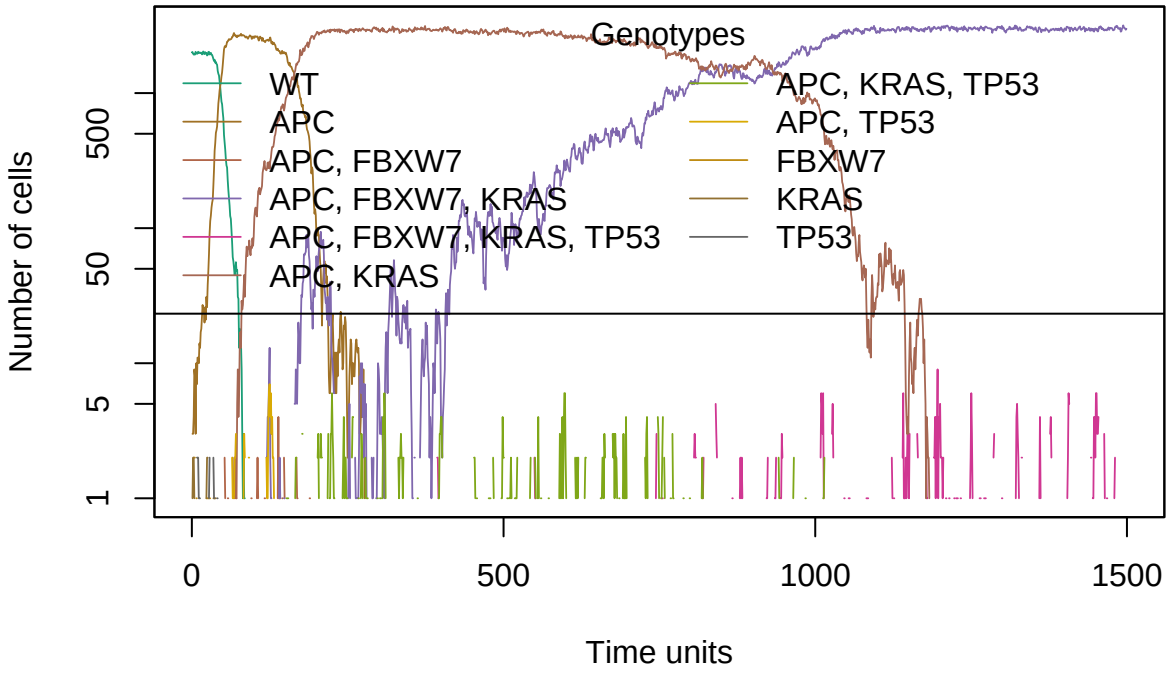

Figure 14: Simulation of cancer progression for the simplified model. Genotypes are shown as lines

```r
## Fix the seed for reproducibility
set.seed(52)


CRC_W2_S1 <- oncoSimulIndiv(CRC_W2, ## A fitnessEffects object
  model = "McFL", ## Model used
  mu = 1e-4, ## Mutation rate
  sampleEvery = 0.02, ## How often the whole population is sampled
  keepEvery = 1,
  initSize = 2000, ## Initial population size
  finalTime = 800,
  keepPhylog = TRUE, ## Allow to see parent-child relationships
  onlyCancer = TRUE,
  errorHitWallTime = FALSE, ## See results even if stopping conditions are not met
  errorHitMaxTries = FALSE)
```

```
##
##  Hitted maxtries. Exiting.
```

```r
## Time to reach cancer
(CRC_W2_S1$FinalTime)
```

```
## [1] 800
```

```r
## Plot of simulation for genotypes
plot(CRC_W2_S1,
show = "genotypes",
legend.ncols = 2,
xlim = c(0, 500),
type = "stacked")
```

```r
## Plot of simulation for genotypes
plot(CRC_W2_S1,
show = "genotypes",
legend.ncols = 1,
xlim = c(0, 300),
type = "line")
```

Figure 17 and Figure 18 shows the genealogical relationships of clones that appeared during the simulations. The number of the arrows represent the times that each clone has appeared. When the simulation was set to reach cancer, the clones that have a genotype belonging to the two local optima appeared (Figure 18). Whereas if the simulation was executed without the cancer parameter, the most represented clone is the one that has the best-fitted genotype (see Figure 17).

```r
## Plot of genealogical relationships
plotClonePhylog(CRC_W2_S, N = 0, keepEvents = TRUE)
```

```r
## Plot of genealogical relationships
plotClonePhylog(CRC_W2_S1, N = 0, keepEvents = TRUE)
```

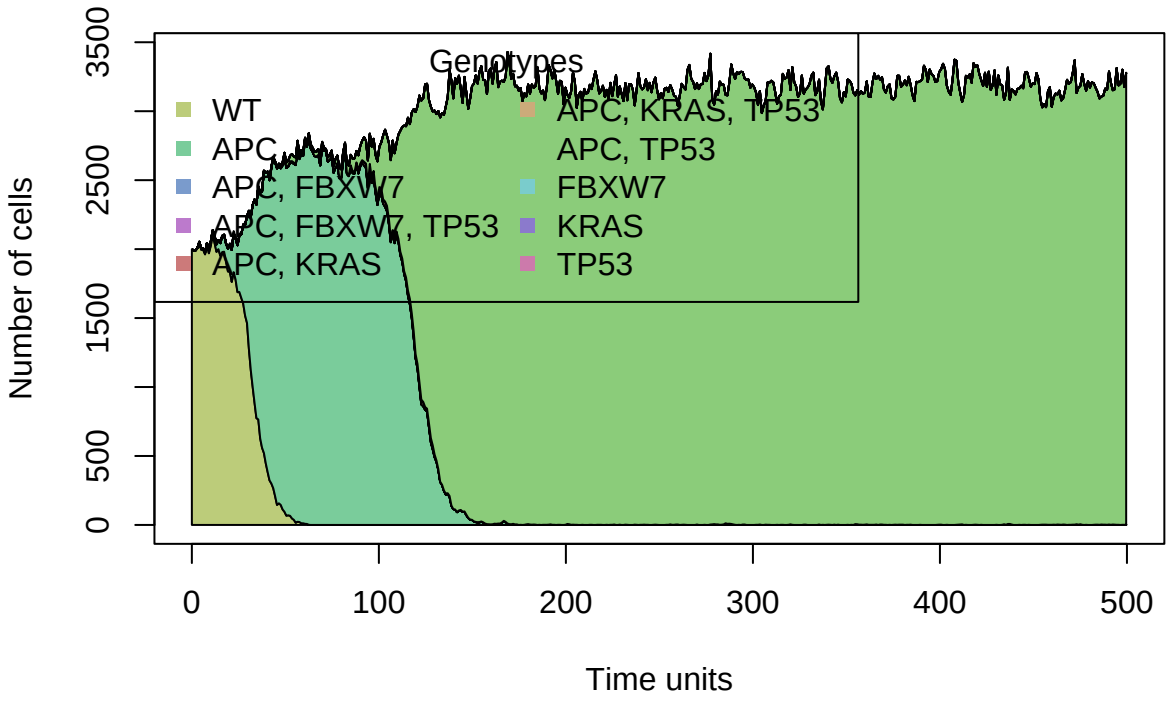

Figure 15: Simulation of cancer progression for the simplified model when onlyCancer = TRUE. Genotypes are shown stacked

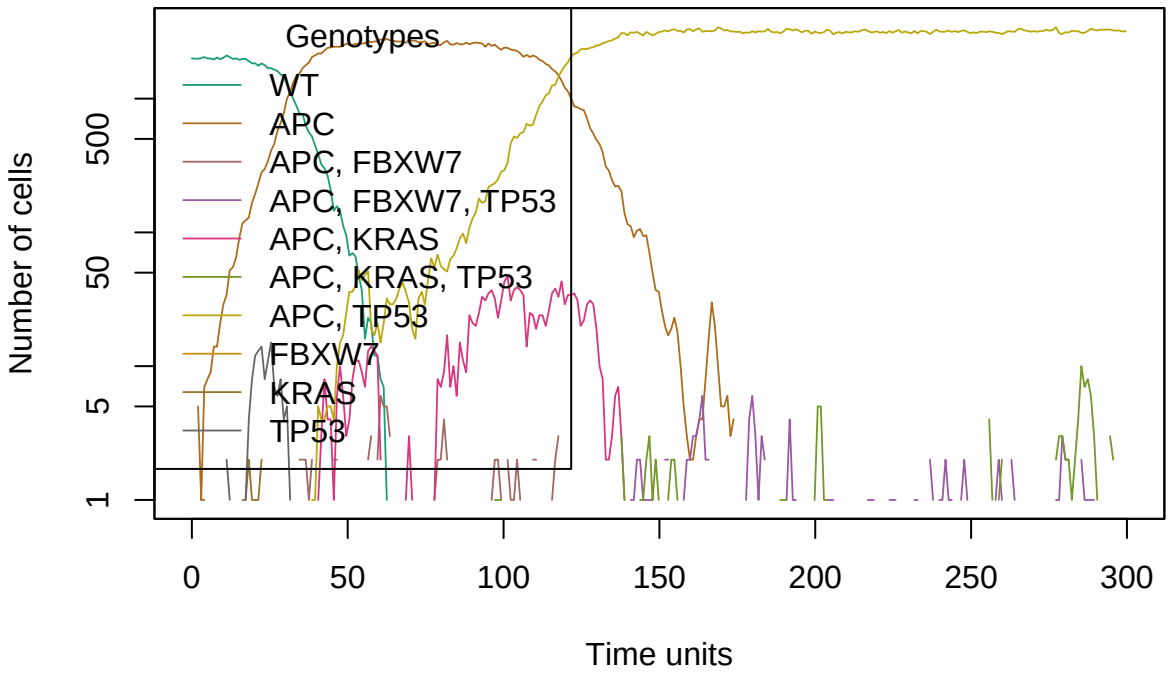

Figure 16: Simulation of cancer progression for the simplified model when onlyCancer = TRUE. Genotypes are shown as lines

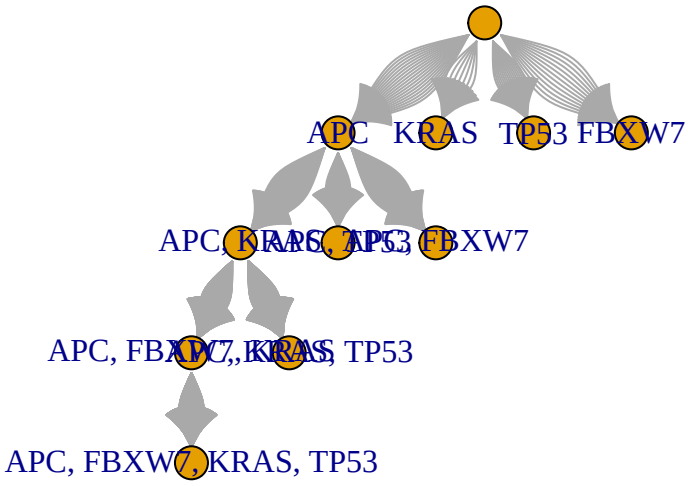

Figure 17: Genealogical relationships of clones

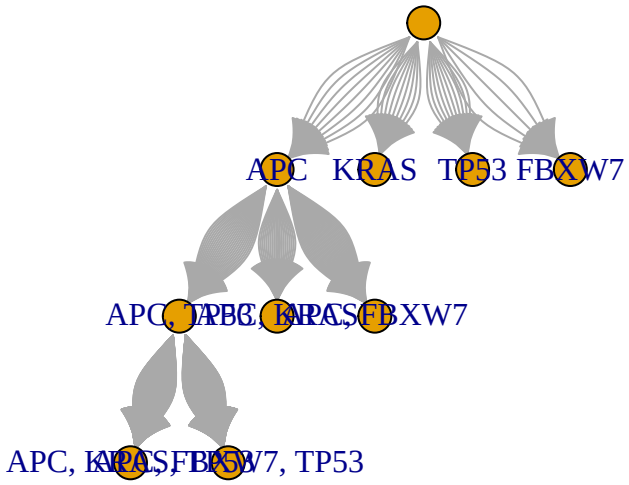

Figure 18: Genealogical relationships of clones when onlyCancer = TRUE

4.3 Synthetic Lethality

Synthetic lethality is a special type of epistasis. Therefore, we used the epistasis module inside allFitnessEffects to define an epistatic interaction between TP53 and APC (see Figure 19) in addition to the restriction imposed by the DAG (XOR relationships). The fitness values were assigned such that a scenario where synthetic lethality via pairwise interaction occurs.

The fitness landscape shows that the genotype for which the synthetic lethality was specified has a lower fitness value as expected, although it is not a local minimum. Similarly to fitness landscape in Figure 12, the local maxima are composed by the genotypes that satisfy both epistatic interactions and restrictions imposed, whereas local minima are composed of genotypes that contain genes with synthetic lethality and other genes that have top-down dependencies (see Figure 20).

```
## Simplified model
## Define poset restrictions, mapping of genes to modules, and driver genes
CRC_W3 <- allFitnessEffects(data.frame(parent = c(rep("Root", 2), "A", "B", "C"),
                                       child = c("A", "B", rep("C", 2), "D"),
                                       s = c(0.2, 0.1, rep(0.05, 2), 0.01),
                                       sh = -0.5,
                                       typeDep = c(rep("XMPN", 4), "MN")),
                            epistasis = c("-A : B" = 0.1,
                                          "-B : A" = 0.2,
                                          "A:B" = -0.5),
                           geneToModule = c("Root" = "Root",
                                            "A" = "APC",
                                            "B" = "TP53",
                                            "C" = "KRAS",
                                            "D" = "FBXW7"),
                           drvNames = c("APC", "TP53", "KRAS", "FBXW7"))

# DAG representation
plot(CRC_W3, expandModules = TRUE, autofit = TRUE, lwdf = 2)
```

```
## Map genotypes to fitness
CRC_F1 <- evalAllGenotypes(CRC_W3, order = FALSE, addwt = TRUE)

(CRC_F1)
```

```
##                   Genotype  Fitness
## 1                       WT 1.000000
## 2                      APC 1.440000
## 3                    FBXW7 0.500000
## 4                     KRAS 0.500000
## 5                     TP53 1.210000
## 6              APC, FBXW7 0.720000
## 7               APC, KRAS 1.512000
## 8               APC, TP53 0.660000
## 9             FBXW7, KRAS 0.505000
## 10            FBXW7, TP53 0.605000
## 11             KRAS, TP53 1.270500
## 12       APC, FBXW7, KRAS 1.527120
## 13       APC, FBXW7, TP53 0.330000
## 14        APC, KRAS, TP53 0.330000
```

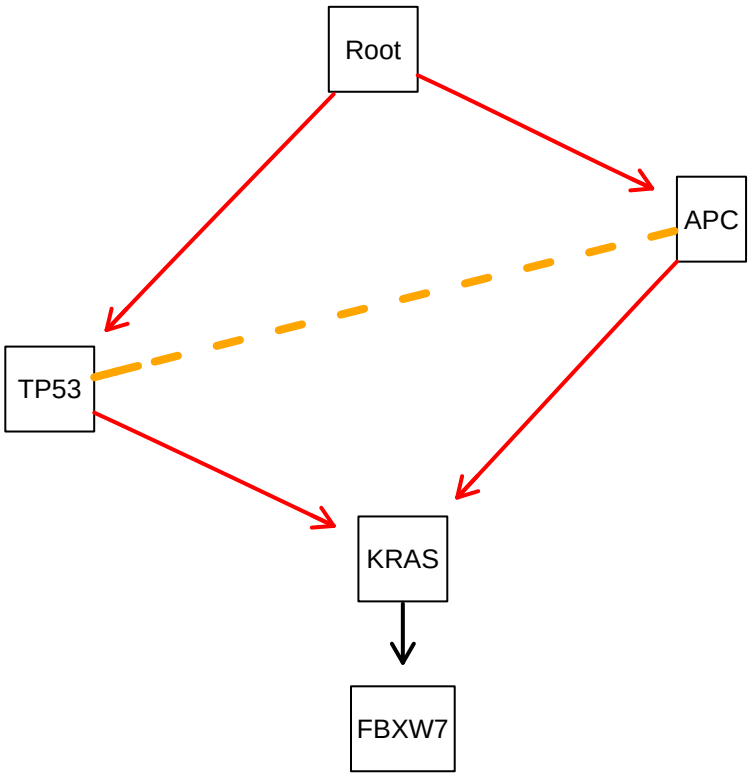

Figure 19: DAG with synthetic lethality

```
## 15      FBXW7, KRAS, TP53 1.283205
## 16 APC, FBXW7, KRAS, TP53 0.333300
```

```
## Plot of fitness landscape

plot(CRC_F1, use_ggrepel = TRUE)
```

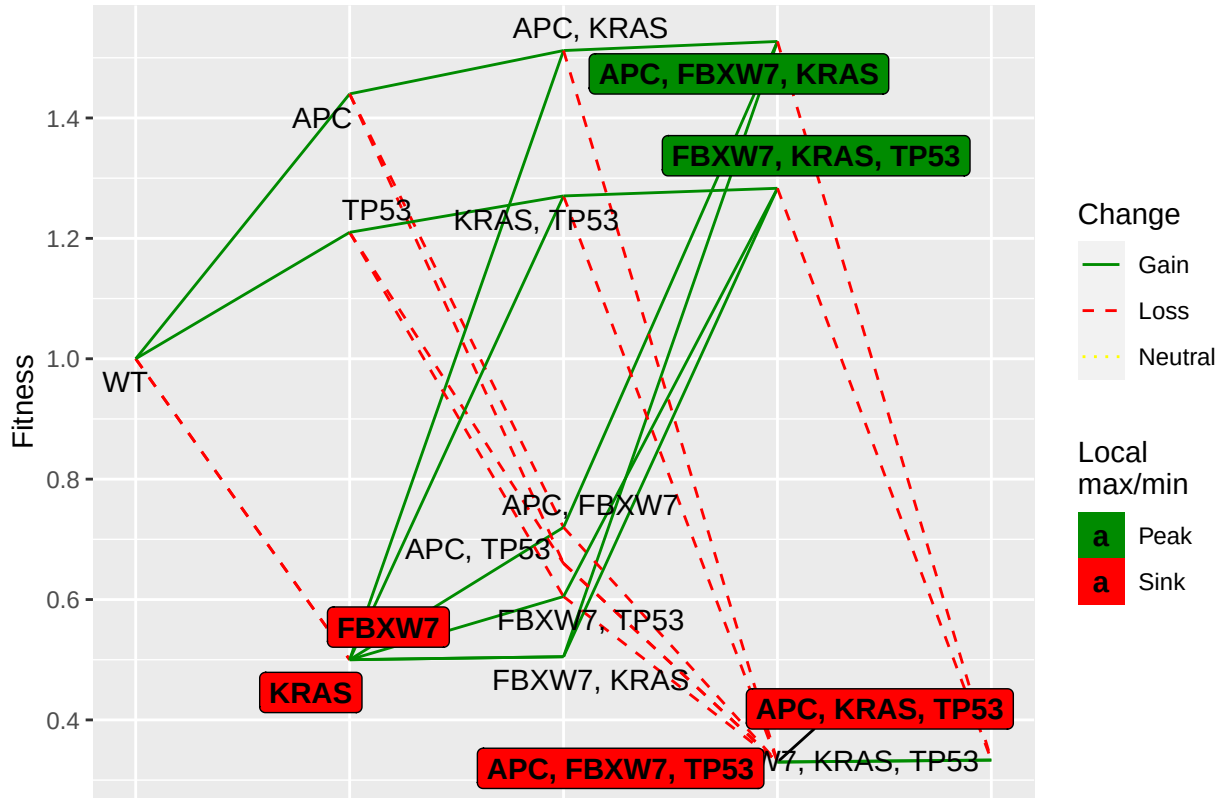

Figure 20: Fitness landscape inferred from simplified DAG with synthetic lethality

In order to simulate synthetic lethality via three-way interaction, we set fitness values that reflect a slightly deleterious effect (if two genes appear) or a highly deleterious effect (if three genes appear). Figure 21 shows the DAG derived for the three-way interaction between APC, TP53, and KRAS. The inferred fitness landscape shows that the global minimum is composed of the genotype that carries the synthetic lethality, whereas local maxima are composed of genotypes that follow the restrictions imposed in the DAG. Also, note that the global maximum is APC. This is not surprising given that APC is an earlier mutation and has the highest fitness values compared to other genes/genotypes (see Figure 22).

```
## Simplified model
## Define poset restrictions, mapping of genes to modules, and driver genes
CRC_W4 <- allFitnessEffects(data.frame(parent = c(rep("Root", 2), "A", "B", "C"),
                                       child = c("A", "B", rep("C", 2), "D"),
                                       s = c(0.2, 0.1, rep(0.05, 2), 0.01),
                                       sh = -0.5,
                                       typeDep = c(rep("XMPN", 4), "MN")),
                            epistasis = c("A : -B : -C" = 0.2,
```

```
                                        "-A : B : -C" = 0.1,
                                        "-A : -B : C" = 0.05,
                                        "A : B : -C" = 0.01,
                                        "-A : B : C" = 0.02,
                                        "-B : A : C" = 0.02,
                                        "A : B : C" = -0.5),
                          geneToModule = c("Root" = "Root",
                                           "A" = "APC",
                                           "B" = "TP53",
                                           "C" = "KRAS",
                                           "D" = "FBXW7"),
                          drvNames = c("APC", "TP53", "KRAS", "FBXW7"))

# DAG representation
plot(CRC_W4, expandModules = TRUE, autofit = TRUE, lwdf = 2)
```

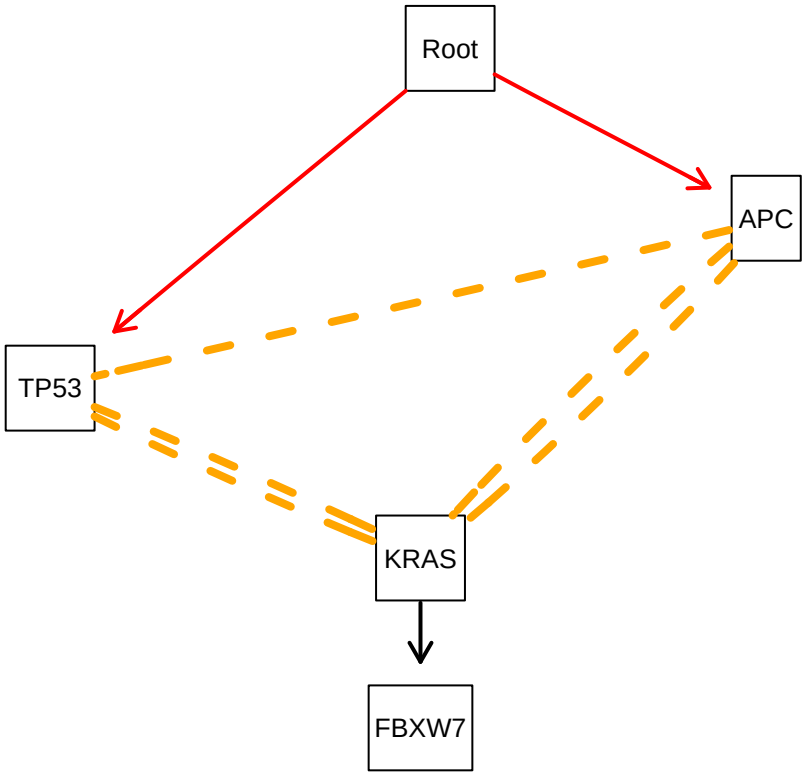

Figure 21: DAG with synthetic lethality (three-way interaction)

```
## Map genotypes to fitness
CRC_F2 <- evalAllGenotypes(CRC_W4, order = FALSE, addwt = TRUE)

(CRC_F2)
```

```
##                  Genotype  Fitness
```

```
## 1                     WT 1.000000
## 2                    APC 1.440000
## 3                 FBXW7 0.500000
## 4                   KRAS 0.525000
## 5                   TP53 1.210000
## 6             APC, FBXW7 0.720000
## 7              APC, KRAS 1.285200
## 8              APC, TP53 1.333200
## 9           FBXW7, KRAS 0.530250
## 10          FBXW7, TP53 0.605000
## 11           KRAS, TP53 1.178100
## 12      APC, FBXW7, KRAS 1.298052
## 13      APC, FBXW7, TP53 0.666600
## 14       APC, KRAS, TP53 0.330000
## 15     FBXW7, KRAS, TP53 1.189881
## 16 APC, FBXW7, KRAS, TP53 0.333300
```

```
## Plot of fitness landscape

plot(CRC_F2, use_ggrepel = TRUE)
```

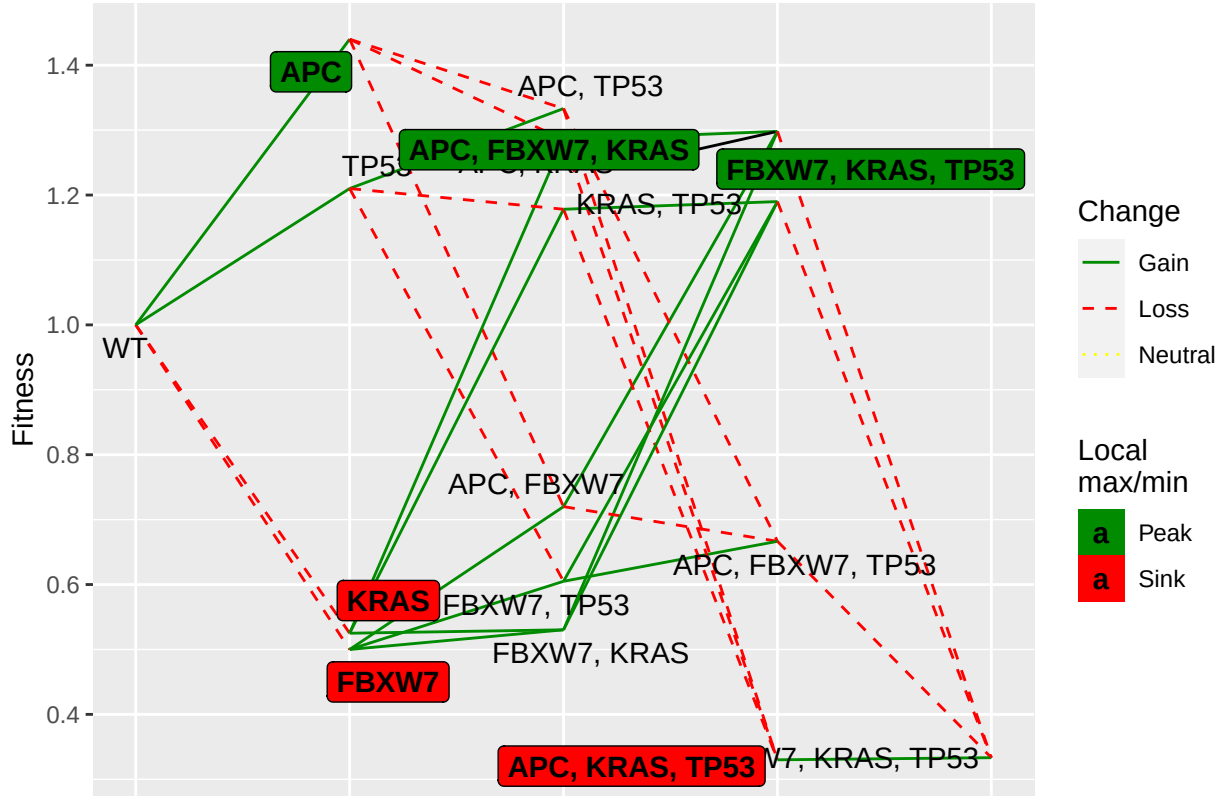

Figure 22: Fitness landscape inferred from simplified DAG with synthetic lethality (three-way interaction)

4.4 Synthetic Viability

Synthetic viability is specified for genotype APC, TP53 (see Figure 23). Here the genotypes composed only by APC or TP53 are deleterious. Figure 24 shows the fitness landscape for synthetic viability via pairwise interaction. Note that the global maximum is composed by the genotype that contains all genes. On the other hand, local minima are composed of genotypes that contain one gene that has a deleterious effect. Note that despite the lower fitness value of genotype FBXW7, KRAS, it conforms a local maximum, although the restrictions imposed in the DAG are not completely satisfied. Moreover, in this fitness landscape, the global maximum may not be reached because the mutational paths required lead to a region composed of multiple valleys. It is important to mention that order of effects could provide a more realistic fitness landscape. For example, a possible path that leads to the global maxima requires a mutation in KRAS before a mutation in FBXW7.

```
## Simplified model
## SM because synthetic viability requires both parent nodes.
## Define poset restrictions, mapping of genes to modules, and driver genes
CRC_W5 <- allFitnessEffects(data.frame(parent = c(rep("Root", 2), "A", "B", "C"),
                                       child = c("A", "B", rep("C", 2), "D"),
                                       s = c(0.2, 0.1, rep(0.05, 2), 0.01),
                                       sh = -0.5,
                                       typeDep = c(rep("MN", 5))),
                            epistasis = c("-A : B" = -0.2,
                                          "-B : A" = -0.3,
                                          "A:B" = 0.5),
                           geneToModule = c("Root" = "Root",
                                            "A" = "APC",
                                            "B" = "TP53",
                                            "C" = "KRAS",
                                            "D" = "FBXW7"),
                           drvNames = c("APC", "TP53", "KRAS", "FBXW7"))

# DAG representation
plot(CRC_W5, expandModules = TRUE, autofit = TRUE, lwdf = 2)
```

```
## Map genotypes to fitness
CRC_F3 <- evalAllGenotypes(CRC_W5, order = FALSE, addwt = TRUE)
(CRC_F3)
```

```
##                  Genotype Fitness
## 1                      WT 1.00000
## 2                     APC 0.84000
## 3                  FBXW7 0.50000
## 4                    KRAS 0.50000
## 5                    TP53 0.88000
## 6             APC, FBXW7 0.42000
## 7              APC, KRAS 0.42000
## 8              APC, TP53 1.98000
## 9            FBXW7, KRAS 0.50500
## 10           FBXW7, TP53 0.44000
## 11            KRAS, TP53 0.44000
## 12      APC, FBXW7, KRAS 0.42420
## 13      APC, FBXW7, TP53 0.99000
## 14       APC, KRAS, TP53 2.07900
```

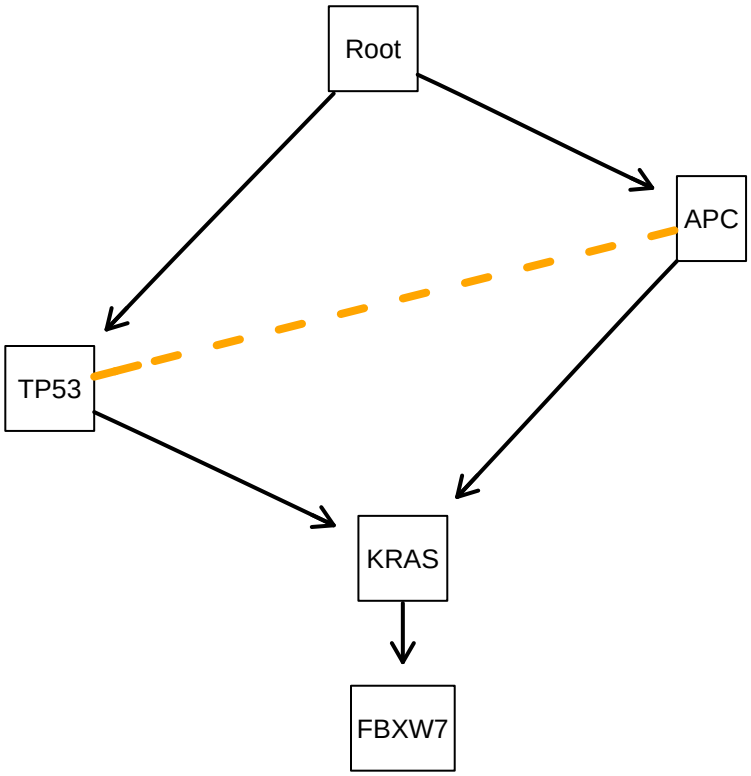

Figure 23: DAG with synthetic viability (pairwise interaction)

```
## 15      FBXW7, KRAS, TP53 0.44440
## 16 APC, FBXW7, KRAS, TP53 2.09979
```

```
## Plot of fitness landscape

plot(CRC_F3, use_ggrepel = TRUE)
```

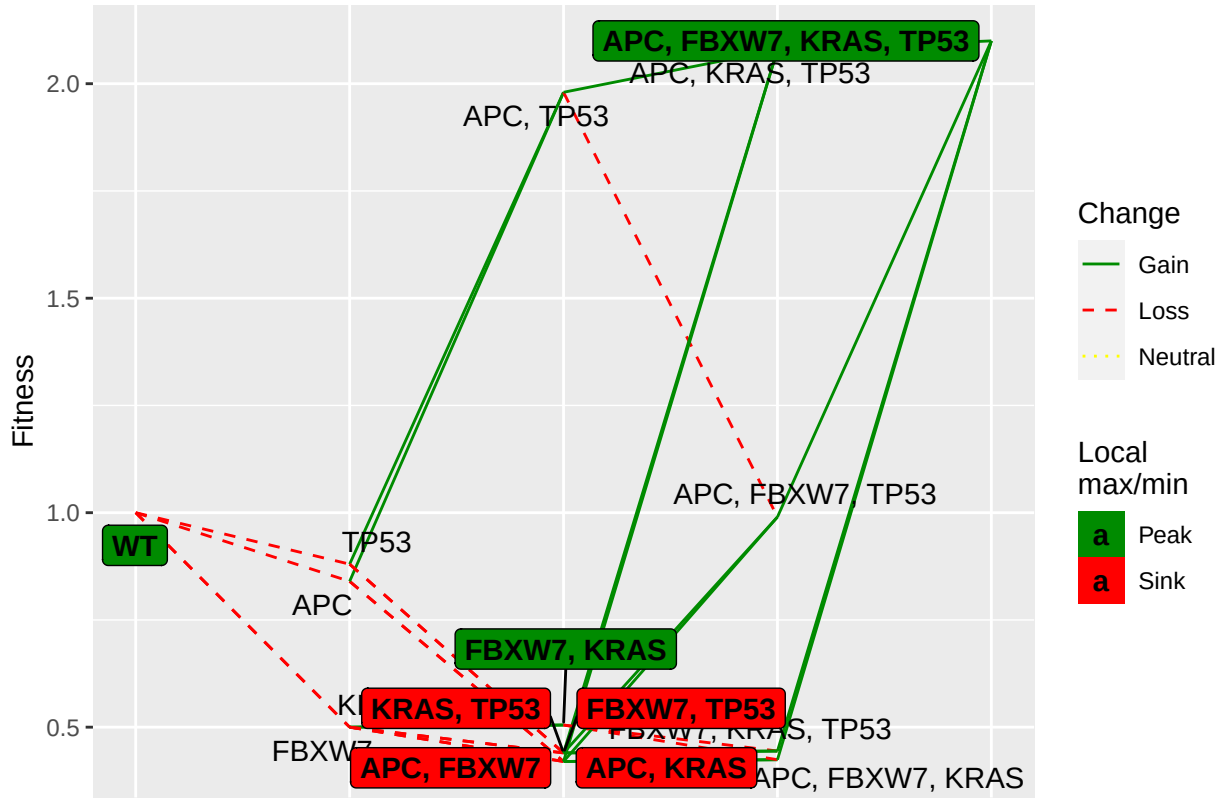

Figure 24: Fitness landscape inferred from simplified DAG with synthetic viability

Figure 25 shows synthetic viability with a three-way interaction between APC, TP53, and KRAS. For this, we specified highly deleterious effects if APC, TP53, or KRAS appear independently, whereas slightly deleterious effects were set if two of those genes appear in a genotype. The fitness landscape for this scenario (see Figure 26) shows that the order of restrictions and epistatic interactions used lead to the global maximum composed by the genotype APC, TP53, KRAS, FBXW7. This result supports the idea that DAGs are better suited to represent sign epistasis (5). Nevertheless, as mentioned above, including the order of effects can give more realistic fitness values associated with genotypes.

In this work, we have represented synthetic lethality via pairwise and three-way interactions. However, this can be achieved if the DAG is composed by individual genes instead of modules because modules does not allow to define epistatic relationships between genes of the same module. This is important because genes of the same module can participate in the same pathway, as discussed in (1,2).

```
## Simplified model
## SM because synthetic viability requires both parent nodes.
## Define poset restrictions, mapping of genes to modules, and driver genes
```

```
CRC_W6 <- allFitnessEffects(data.frame(parent = c(rep("Root", 2), "A", "B", "C"),
                                       child = c("A", "B", rep("C", 2), "D"),
                                       s = c(0.2, 0.1, rep(0.05, 2), 0.01),
                                       sh = -0.5,
                                       typeDep = c(rep("MN", 5))),
                            epistasis = c("A : -B : -C" = -0.2,
                                          "-A : B : -C" = -0.2,
                                          "-A : -B : C" = -0.3,
                                          "A : B : -C" = -0.05,
                                          "-A : B : C" = -0.01,
                                          "A : -B : C" = -0.01,
                                          "A : B : C" = 0.5),
                           geneToModule = c("Root" = "Root",
                                            "A" = "APC",
                                            "B" = "TP53",
                                            "C" = "KRAS",
                                            "D" = "FBXW7"),
                           drvNames = c("APC", "TP53", "KRAS", "FBXW7"))

# DAG representation
plot(CRC_W6, expandModules = TRUE, autofit = TRUE, lwdf = 2)
```

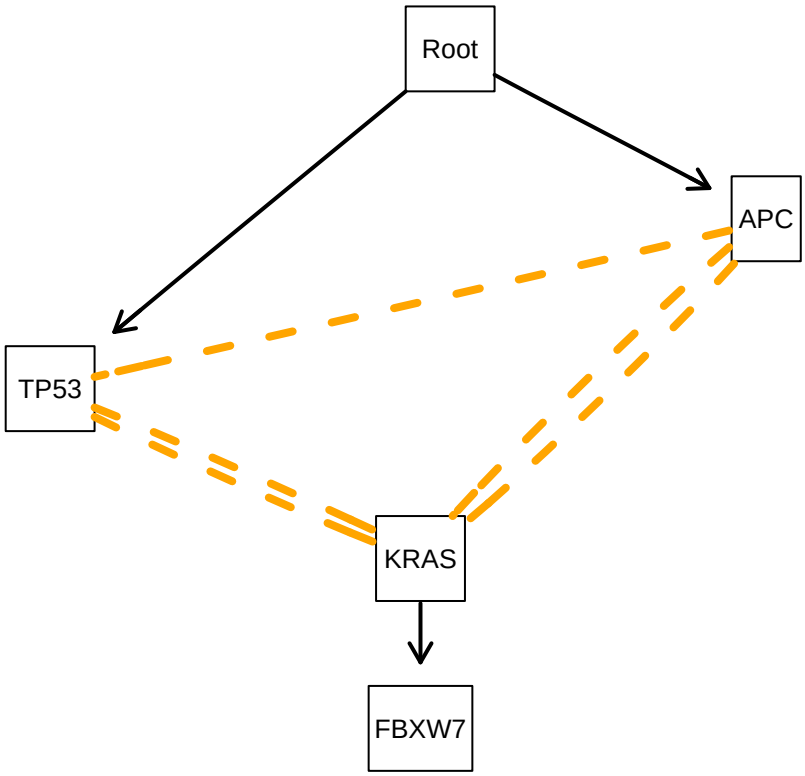

Figure 25: DAG with synthetic viability (three-way interaction)

```
## Map genotypes to fitness
CRC_F4 <- evalAllGenotypes(CRC_W6, order = FALSE, addwt = TRUE)
(CRC_F4)
```

```
##                   Genotype  Fitness
## 1                       WT 1.000000
## 2                      APC 0.960000
## 3                   FBXW7 0.500000
## 4                     KRAS 0.350000
## 5                     TP53 0.880000
## 6               APC, FBXW7 0.480000
## 7                APC, KRAS 0.594000
## 8                APC, TP53 1.254000
## 9              FBXW7, KRAS 0.353500
## 10             FBXW7, TP53 0.440000
## 11              KRAS, TP53 0.544500
## 12       APC, FBXW7, KRAS 0.599940
## 13       APC, FBXW7, TP53 0.627000
## 14        APC, KRAS, TP53 2.079000
## 15      FBXW7, KRAS, TP53 0.549945
## 16 APC, FBXW7, KRAS, TP53 2.099790
```

```
## Plot of fitness landscape

plot(CRC_F4, use_ggrepel = TRUE)
```

5 A Probabilistic Model of Mutually Exclusive Linearly Ordered Driver Pathways

Mohaghegh Neyshabouri et al. (11) proposed a probabilistic model of mutually exclusive linearly ordered driver pathways and analyze one large dataset of colorectal adenocarcinoma (COADREAD) from the IntOGen-mutations database. Their model assumes driver genes are over-represented among those mutated across a large tumor collection and, thus, they can be identified in terms of frequency. Also, those genes participating in the same pathway are mutated in a mutually exclusive manner because more than one mutation in a pathway does not give any selective advantage to the clone.

Like with previous generative models, we mapped the COADREAD generative model to an actual evolutionary model using different **OncoSimulR** functionalities. This time, we extended what authors modeled using the frequency-dependent fitness specification to illustrate how differently fitness landscapes evolve even though they are built from exact CPMs when we consider this additional evolutionary event.

Figure 7.C from (11) shows the CPM inferred from the COADREAD dataset, consisting of seven modules with between 1 to 4 genes each. The model clearly reconstructs the well-known initiator events in colorectal cancer, including mutations in APC, TP53, and KRAS (19). Using the DAG of restrictions as a starting point[1], the evolutionary model was created specifying the same genotype fitness for all modules as authors do not state any differences in fitness for when the restrictions in the DAG are satisfied (s). However, based on the confidence parameter used by the authors to assess the reliability of modeled restrictions, different fitness are set when the DAG of restrictions are not satisfied (sh) (Table 2). Since this method reconstructs linear models (*i.e.* oncogenic trees), there is no need to specify any particular type of dependency between

[1]Several genes were removed from the original set in order to get a clear fitness landscape

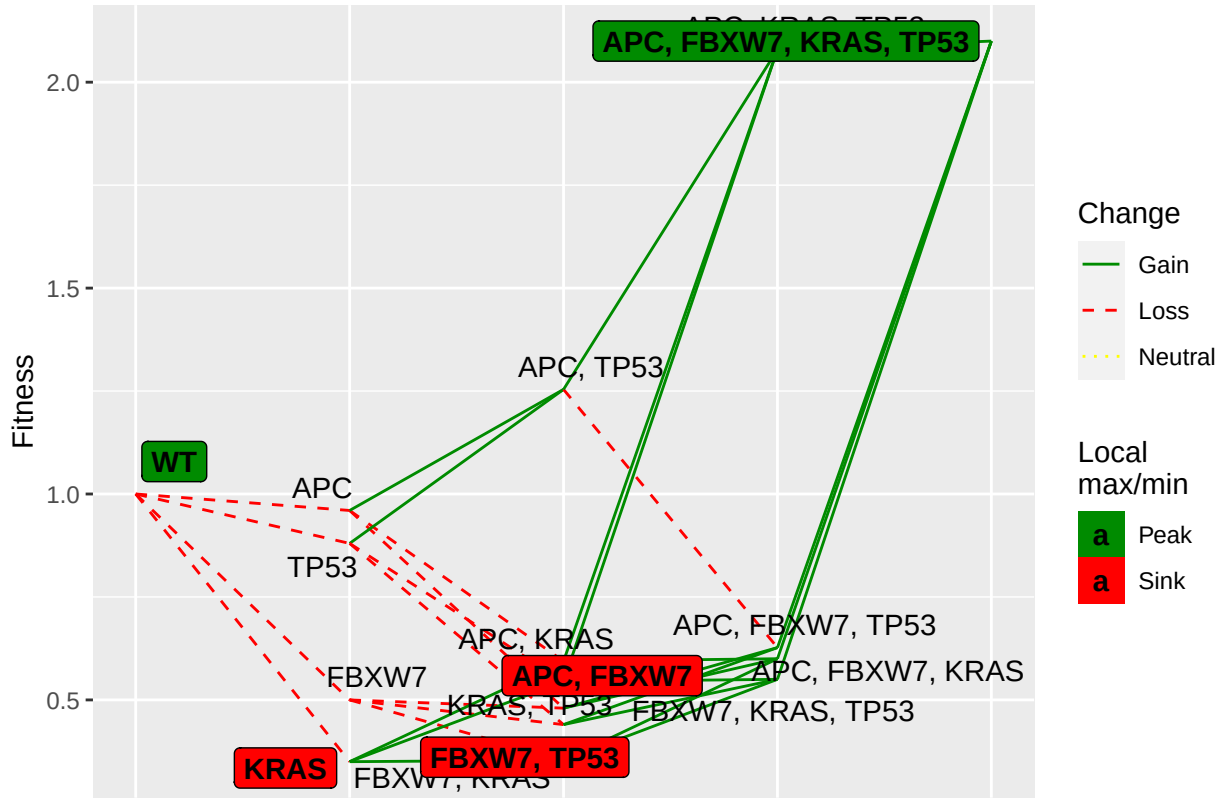

Figure 26: Fitness landscape inferred from simplified DAG with synthetic viability (three-way interaction)

modules (typeDep), so we set it to monotonic (MN) as it is a mandatory argument for allFitnessEffects function. Figure 27 shows the DAG of restrictions created with `allFitnessEffects`, recapitulating the poset inferred in (11).

Table 2: Confidence parameter for each module transition

Module	Confidence parameter (%)
APC	100
TP53	100
KRAS	100
PIK3CA, NRAS, LRP1B	100
FBXW7, TCF7L2, FAT4, ARID1A	87.7
ATM, SMAD2, ERBB3, MTOR, CTNNB1	86.9
SOX9, SMAD4	66.7

```
## Restriction table, including DAG of restrictions
## specifications and associated fitness
COADREAD_rT <- data.frame(
              parent = c("Root", "A", "B", "C", "D", "E", "F"), # Parent nodes
              child = c("A", "B", "C", "D", "E", "F", "G"), # Child nodes
                            s = 0.5,
                            sh = c(rep(-1, 4), rep(-.5, 2), -.2),
                            typeDep = "MN")


## Create fitness specifications from DAG of restrictions considering modules
COADREAD_fitness <- allFitnessEffects(
              COADREAD_rT,
              geneToModule = c(
                 "Root" = "Root",
                 "A" = "APC",
                 "B" = "TP53",
                 "C" = "KRAS",
                 "D" = "PIK3CA, NRAS",
                 "E" = "FBXW7, ARID1A",
                 "F" = "ATM, SMAD2",
                 "G" = "SOX9, SMAD4")) # Modules


## DAG of restrictions representation
plot(COADREAD_fitness, expandModules = TRUE, autofit = TRUE)
```

```
## Evaluation of all possible genotypes fitness
## under the previous fitness specifications
COADREAD_FL <- evalAllGenotypes(COADREAD_fitness, max = 131072)
```

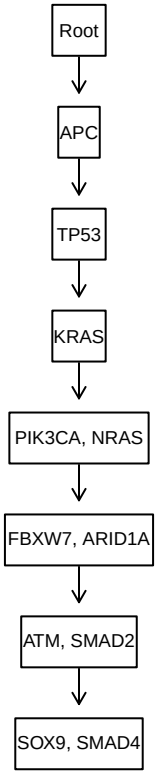

Figure 27: DAG of restrictions for the COADREAD dataset

```
## Fitness landscape representation
plotFitnessLandscape(COADREAD_FL)
```

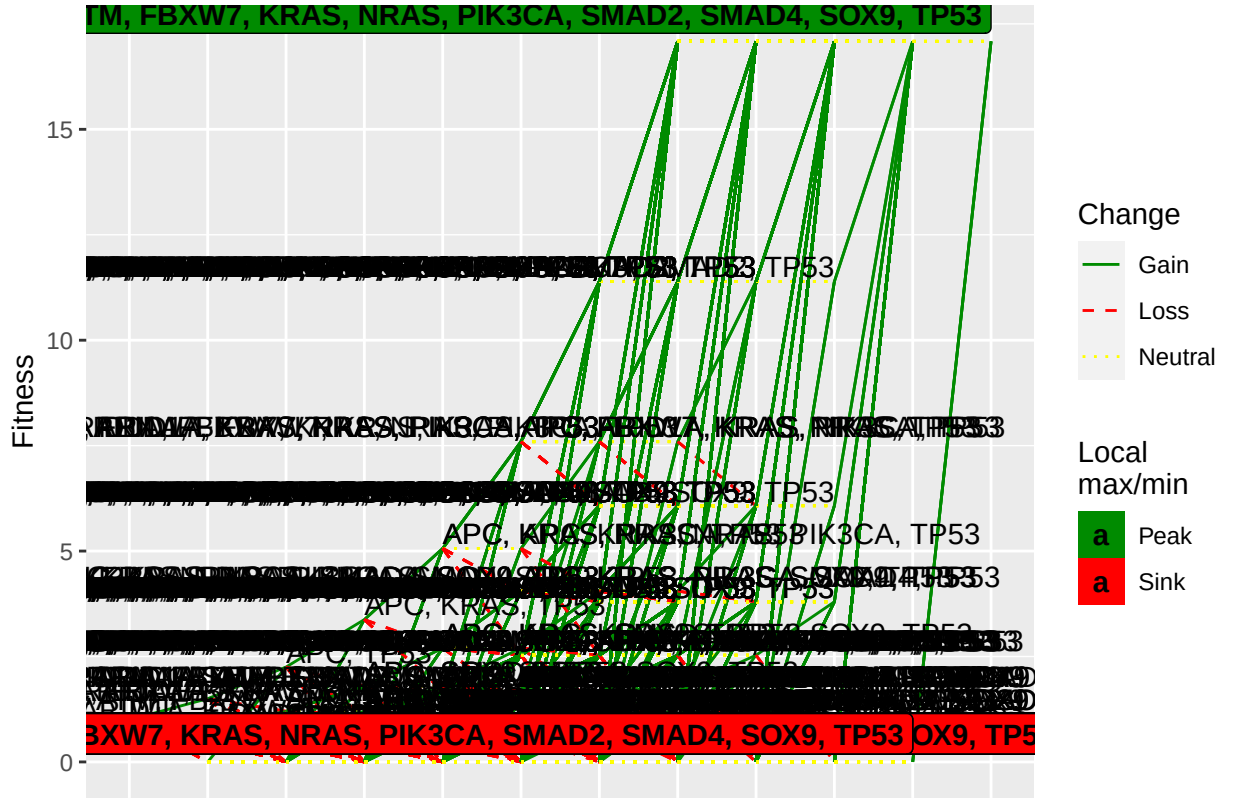

Figure 28: Fitness landscape corresponding with the DAG of restrictions for the COADREAD dataset

Figure 28 plots all possible genotypes in a very busy fitness landscape. Although it is not possible to visualize genotypes clearly, we can see an exponential trend towards local maxima, corresponding to the clones carrying seven driver-genes genotypes. Stands out how very different genotypes behave the same due to the mutual exclusivity effect, which is probably simplifying much more complex interactions among genes, especially the effect in fitness different genes from the same module could have if we did not account for the mutual exclusivity phenomenon. In the next section, we discuss genotypes distribution with a simplified model.

5.1 Simplified Cancer Progression Model

As we did with previous models, for illustrating purposes we designed a simplified version of the CPM by (11) to assess its reliability when considering other evolutionary scenarios. This time, we take the first four modules of the complete DAG (maintaining same fitness parameters), being the fourth the only one carrying two genes and, thus, affected by mutual exclusivity circumstance (Figure 29).

The fitness landscape shown in Figure 30 includes some of the possible genotypes. Clearer than in Figure 28, we see fitness increases exponentially following the restrictions established in the DAG (smooth landscape). Local minima (red squares) correspond to genotypes violating these constraints, and gain changes (green lines) are consistent with fulfilled restrictions. However, one of the local maxima genotypes (green squares)

corresponds to a genotype violating mutual exclusivity (PIK3CA and NRAS appear together). This is explained because the module functionality in OncoSimulR sets fitness to zero when genes from the same module mutate at a time as there is a null effect since both genes participate from the same pathway. Because there is not a decrease in the fitness of the genotype (i.e. no deleterious effect), it can still be a local maximum. In theory, the three best-fitted genotypes can inhabit the global maxima. Yet, it is unlikely that those three genotypes can appear and fixate simultaneously in the population.

```
## Restriction table, including five-modules DAG of
## restrictions specifications and associated fitness
COADREAD_rT_5d <- data.frame(parent = c("Root", "A", "B", "C"), # Parent nodes
                             child = c("A", "B", "C", "D"), # Child nodes
                             s = 0.5,
                             sh = c(rep(-1, 4)),
                             typeDep = "MN")

## Create fitness specifications from simplified DAG of restrictions
COADREAD_fitness_5d <- allFitnessEffects(COADREAD_rT_5d,
                                         geneToModule = c( "Root" = "Root",
                                                           "A" = "APC",
                                                           "B" = "TP53",
                                                           "C" = "KRAS",
                                                           "D" = "PIK3CA, NRAS"),
                                         drvNames = c("APC", "TP53", "KRAS",
                                                      "PIK3CA", "NRAS"))

## Simplified DAG of restrictions representation
plot(COADREAD_fitness_5d, expandModules = TRUE, autofit = TRUE)
```

```
## Evaluation of all possible genotypes fitness under the previous fitness specifications
(COADREAD_FL_5d <- evalAllGenotypes(COADREAD_fitness_5d))
```

```
##                         Genotype Fitness
## 1                            APC  1.5000
## 2                           KRAS  0.0000
## 3                           NRAS  0.0000
## 4                         PIK3CA  0.0000
## 5                           TP53  0.0000
## 6                       APC, KRAS  0.0000
## 7                       APC, NRAS  0.0000
## 8                     APC, PIK3CA  0.0000
## 9                       APC, TP53  2.2500
## 10                     KRAS, NRAS  0.0000
## 11                   KRAS, PIK3CA  0.0000
## 12                     KRAS, TP53  0.0000
## 13                   NRAS, PIK3CA  0.0000
## 14                     NRAS, TP53  0.0000
## 15                   PIK3CA, TP53  0.0000
## 16               APC, KRAS, NRAS  0.0000
## 17             APC, KRAS, PIK3CA  0.0000
## 18               APC, KRAS, TP53  3.3750
## 19             APC, NRAS, PIK3CA  0.0000
## 20               APC, NRAS, TP53  0.0000
## 21             APC, PIK3CA, TP53  0.0000
```

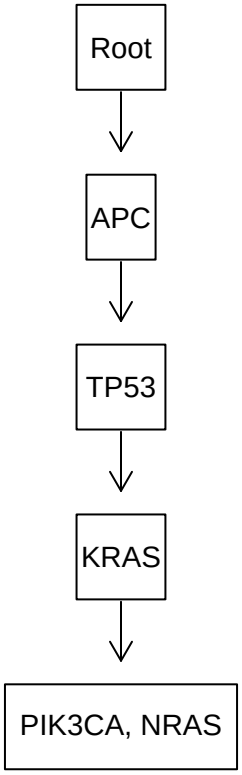

Figure 29: Simplified DAG of restrictions for the COADREAD dataset

```
## 22             KRAS, NRAS, PIK3CA  0.0000
## 23               KRAS, NRAS, TP53  0.0000
## 24             KRAS, PIK3CA, TP53  0.0000
## 25             NRAS, PIK3CA, TP53  0.0000
## 26       APC, KRAS, NRAS, PIK3CA  0.0000
## 27         APC, KRAS, NRAS, TP53  5.0625
## 28       APC, KRAS, PIK3CA, TP53  5.0625
## 29       APC, NRAS, PIK3CA, TP53  0.0000
## 30      KRAS, NRAS, PIK3CA, TP53  0.0000
## 31 APC, KRAS, NRAS, PIK3CA, TP53  5.0625
```

```
## Fitness landscape representation
plotFitnessLandscape(COADREAD_FL_5d, use_ggrepel = TRUE)
```

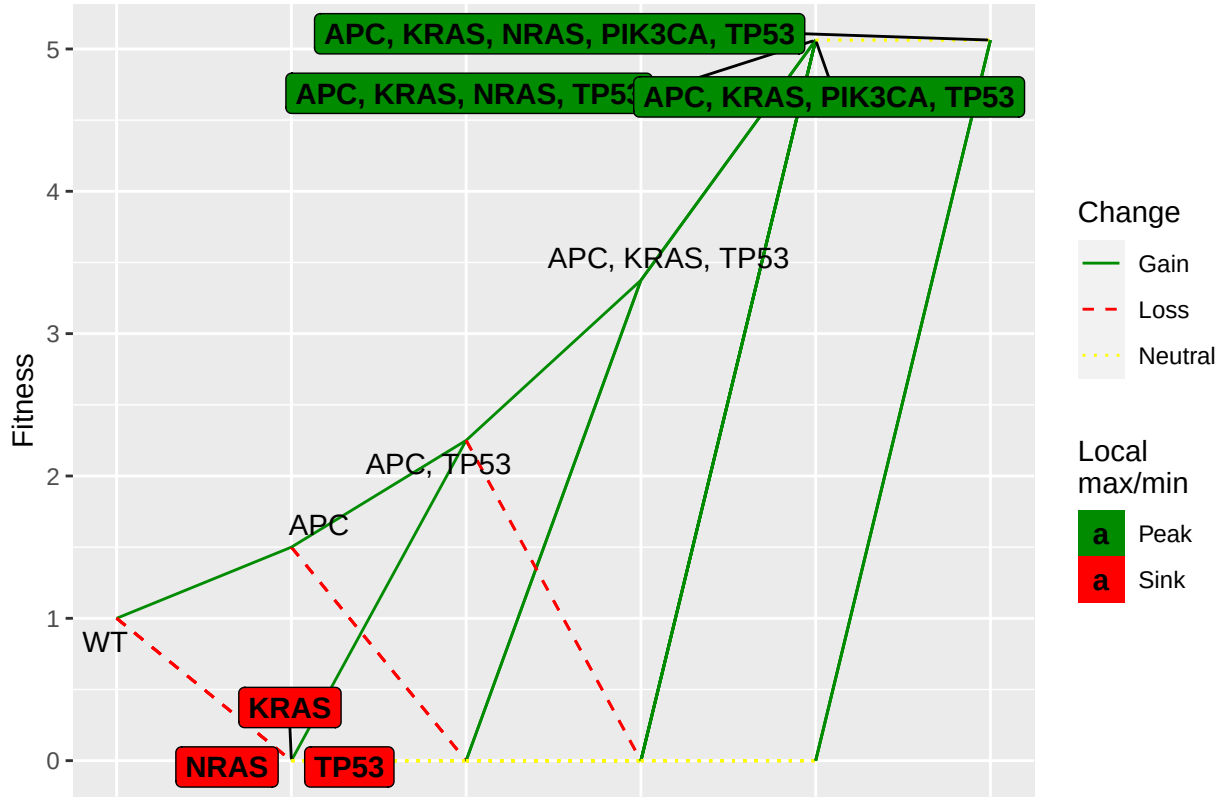

Figure 30: Fitness landscape corresponding with the simplified DAG of restrictions for the COADREAD dataset

The previous assumption can be better addressed by simulating tumor progression under the proposed evolutionary model. Accordingly, we would expect clones to evolve following the optimal pathway of the fitness landscape that satisfied the DAG of restrictions. We use the oncoSimulPop function to simulate tumor progression in ten different individuals and test whether the genotype APC, TP53, KRAS, PIK3CA, NRAS would ever fixate even at a very small frequency. Parameters are adjusted appropriately so that simulation stops when APC, TP53, KRAS, PIK3CA, NRAS genotype is reached at a frequency of 0.1 at least ("onlyCancer = TRUE, fixation = c(c("_, APC, TP53, KRAS, PIK3CA, NRAS"), fixation_tolerance = 0.9)").

Figure 31 shows a representative stacked plot of the genotype abundance over time. Surprisingly, APC, TP53, KRAS, PIK3CA, NRAS genotype appeared in the ten simulations significantly (data not shown), able to coexist with clones of the same fitness. Often, it arises from APC, TP53, KRAS, PIK3CA or APC, TP53, KRAS, NRAS clones, which appear together even less frequently, probably because both descend from the same ancestor and have much higher fitness, leading to its extinction once one of the two emerges. Figure 32 supports this idea as it clearly plots the behavior of each clone separately, displaying abrupt extinctions the moment the following clone with higher fitness appears. Retrieving the phylogeny of the clones (representative graph of one simulation in Figure 33), we confirm there is a tendency of the clones to mutate until the complete genotype APC, TP53, KRAS, PIK3CA, NRAS, either following the linear mutual exclusivity path or from a more diverse landscape.

```
set.seed(125)

## Simulate cancer progression in 10 individuals until APC,
## TP53, KRAS, PIK3CA, NRAS genotype fixates
COADREAD_Simul_5d <- oncoSimulPop(
                10, COADREAD_fitness_5d,
                model = "McFL", ## Model used
                mu = 1e-4, ## Mutation rate
                sampleEvery = 0.02, ## How often the whole population is sampled
                keepEvery = 1,
                initSize = 200, ## Initial population size
                finalTime = 2000,
                keepPhylog = TRUE, ## Allow to see parent-child relationships
                onlyCancer = TRUE,
                detectionSize = NA,
                fixation = c(c("_, APC, TP53, KRAS, PIK3CA, NRAS"),
                             fixation_tolerance = 0.7),
                detectionDrivers = NA,
                detectionProb = NA,
                max.num.tries = 500,
                max.wall.time = 20,
                errorHitMaxTries = TRUE)
```

```
## You are running Windows. Setting mc.cores = 1
```

```
## Plot of simulation for genotypes
plot(COADREAD_Simul_5d[[3]],
show = "genotypes",
type = "stacked",
ylim = c(0, 35000))
```

```
plot(COADREAD_Simul_5d[[5]],
     show = "genotypes",
     type = "line",
     ylim = c(1, 100000000))
```

```
## Parent-child relationship derived from simulation
plotClonePhylog(COADREAD_Simul_5d[[3]],
                N = 0, ## Specify clones that exist
                keepEvents = TRUE ## Arrows showing how many times each clones appeared
)
```

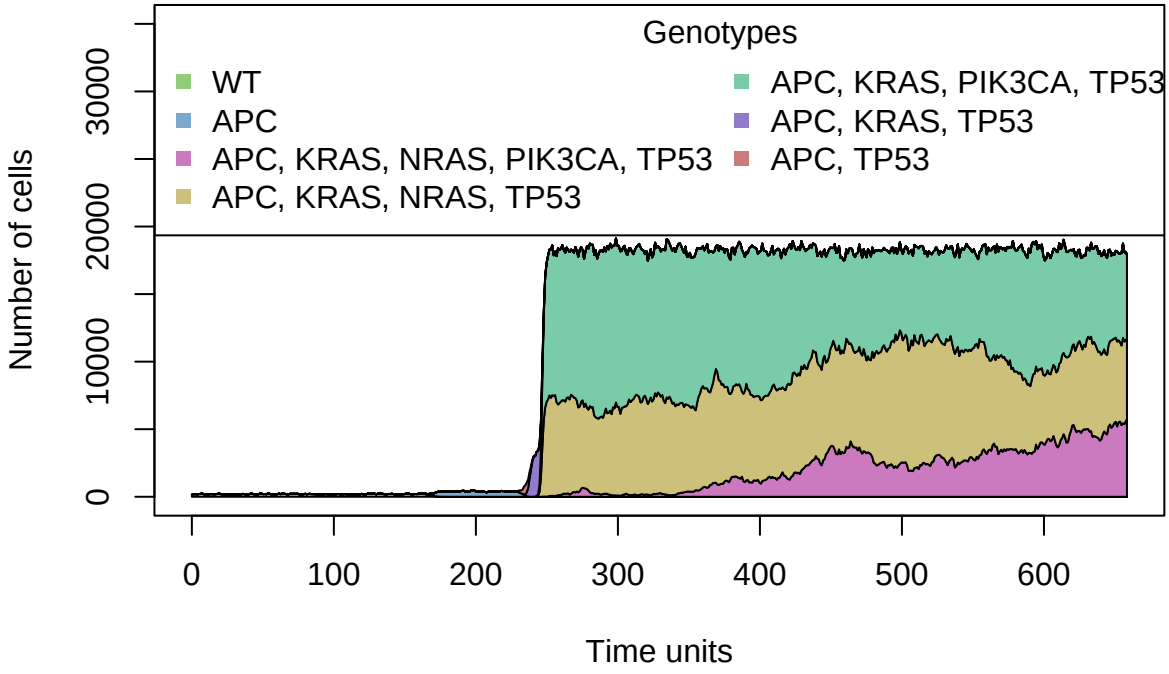

Figure 31: One of the 10 simulations of cancer progression using the four-modules fitness landscape until APC, TP53, KRAS, PIK3CA, NRAS genotype arises (stacked plot)

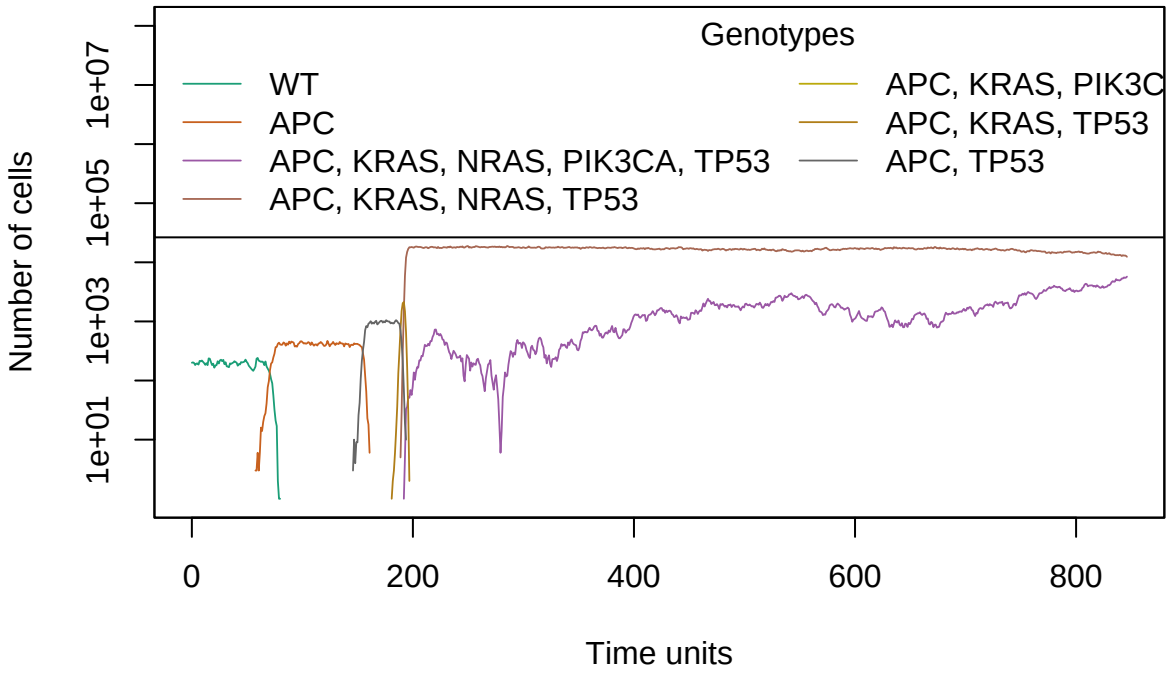

Figure 32: One of the 10 simulations of cancer progression using the four-modules fitness landscape until APC, TP53, KRAS, PIK3CA, NRAS genotype arises (line plot)

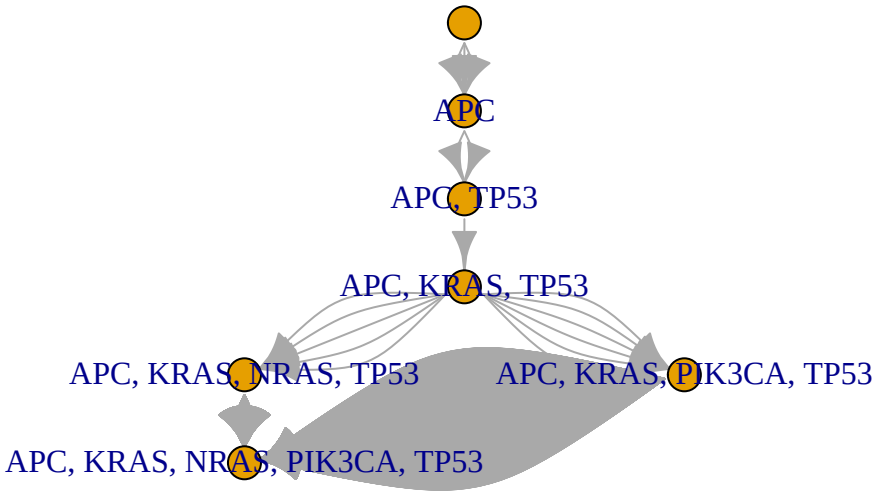

Figure 33: Parent-child relationship derived from one of the 10 simulations

5.2 Frequency-dependent Fitness

As previously introduced, clones that coexist in a tumor can influence the fitness of each other in a frequency-dependent manner when a mutation produces a phenotype able to modulate the tumor microenvironment. OncoSimulR incorporates the frecuencyDependentFitness specification to allow modeling interactions among clones during tumor progression. In the simulations of the simplified model, we significantly observed APC, TP53, KRAS, PIK3CA, NRAS genotype in the final stage of the simulation, an unexpected event considering PIK3CA and NRAS are mutually exclusive. This might denote there is an additional evolutionary event directing the very infrequent coexistence of these two genes in tumor samples (11). Here, we propose APC, TP53, KRAS, PIK3CA, NRAS genotype fitness could depend on the frequency of APC, TP53, KRAS, PIK3CA, and APC, TP53, KRAS, NRAS genotypes in the context of a competitive relationship among clones for nutrients. APC, TP53, KRAS, PIK3CA, NRAS clones would be more energetically demanding and thus, coexistence with other clones would be detrimental (considering the three clones have the same fitness).

To use the frecuencyDependentFitness functionality, it is necessary to set to TRUE de frequencyDependentFitness parameter in the allFitnessEffects function, as well as providing a "mapping of genotypes to fitness" data frame. Fitness values are taken from the fitness specifications previously used (subsection 5.1). To evaluate genotypes with the evalAllGenotypes function is mandatory the parameter spPopSizes to build a fitness landscape in accordance with the size of the different clones. Here, we define a scenario in which all the clones except for the four-genes and five-genes clones are almost extinct. In this context, we can see in the fitness landscape (Figure 34) an expected fitness decrease for the APC, TP53, KRAS, PIK3CA, NRAS genotype, which is not a local maximum anymore.

```
## Mapping of genotypes to frequency-dependent fitness
# Not explicitly mapped genotypes are assigned a fitness of cero
COADREAD_gen_freqdep <- data.frame(
                Genotype = c("WT", "APC","APC, TP53",
                             "APC, TP53, KRAS",
                             "APC, TP53, KRAS, PIK3CA",
                             "APC, TP53, KRAS, NRAS",
                             "APC, TP53, KRAS, PIK3CA, NRAS"),
                             Fitness = c("1", "1.5",
                                         "2.25", "3.375", "5.0625", "5.0625",
            "5.0625 - ((f_APC_TP53_KRAS_PIK3CA + f_APC_TP53_KRAS_NRAS))/2"),
                             stringsAsFactors = FALSE)

## Fitness specifications
COADREAD_fitness_freqdep <- allFitnessEffects(genotFitness = COADREAD_gen_freqdep,
                                              frequencyDependentFitness = TRUE,
                                              frequencyType = "rel")

## Evaluate all genotypes considering population sizes of the clones
(COADREAD_FL_freqdep <- evalAllGenotypes(COADREAD_fitness_freqdep,
                                 spPopSizes = c("WT" = 5, "APC" = 5, "APC, TP53" = 5,
                                                "APC, TP53, KRAS" = 10,
                                                "APC, TP53, KRAS, PIK3CA" = 50,
                                                "APC, TP53, KRAS, NRAS" = 50,
                                                "APC, TP53, KRAS, PIK3CA, NRAS" = 50)))
```

```
##                          Genotype  Fitness
## 1                              WT 1.000000
## 2                             APC 1.500000
## 3                            KRAS 0.000000
```

```
## 4                          NRAS 0.000000
## 5                         PIK3CA 0.000000
## 6                           TP53 0.000000
## 7                       APC, KRAS 0.000000
## 8                       APC, NRAS 0.000000
## 9                     APC, PIK3CA 0.000000
## 10                      APC, TP53 2.250000
## 11                     KRAS, NRAS 0.000000
## 12                   KRAS, PIK3CA 0.000000
## 13                     KRAS, TP53 0.000000
## 14                   NRAS, PIK3CA 0.000000
## 15                     NRAS, TP53 0.000000
## 16                   PIK3CA, TP53 0.000000
## 17                APC, KRAS, NRAS 0.000000
## 18              APC, KRAS, PIK3CA 0.000000
## 19                APC, KRAS, TP53 3.375000
## 20              APC, NRAS, PIK3CA 0.000000
## 21                APC, NRAS, TP53 0.000000
## 22              APC, PIK3CA, TP53 0.000000
## 23             KRAS, NRAS, PIK3CA 0.000000
## 24               KRAS, NRAS, TP53 0.000000
## 25             KRAS, PIK3CA, TP53 0.000000
## 26             NRAS, PIK3CA, TP53 0.000000
## 27       APC, KRAS, NRAS, PIK3CA 0.000000
## 28         APC, KRAS, NRAS, TP53 5.062500
## 29       APC, KRAS, PIK3CA, TP53 5.062500
## 30       APC, NRAS, PIK3CA, TP53 0.000000
## 31      KRAS, NRAS, PIK3CA, TP53 0.000000
## 32 APC, KRAS, NRAS, PIK3CA, TP53 4.776786
```

```
## Fitness landscape representation
plotFitnessLandscape(COADREAD_FL_freqdep)
```

Next, we try to run the same simulation we did before with these new fitness specifications. After modifying the fixation_tolerance parameter so that we could detect the APC, TP53, KRAS, PIK3CA, NRAS genotype rapidly, it never arises. This can be explained by its slightly lower fitness compared to its ancestors that have a very high frequency at the moment PIK3CA or NRAS mutate to generate the fivefold-mutated genotype, leading APC, TP53, KRAS, PIK3CA, NRAS clones near to extinction. Running an additional short simulation, in which we just set onlyCancer as FALSE (so as for the simulation to run until finalTime), in Figure 35 we do not see the presence of the fivefold-mutated clone, yet Figure 36 shows an oscillating pattern of growth for this clone. Also, the phylogeny recorded for each simulation shows the appearance of APC, TP53, KRAS, PIK3CA, NRAS genotype (Figure 37. Either way, its frequency is considerably low that it cannot trigger the onlyCancer condition to end the first simulation. Although we cannot assure whether the phenomenon of frequency-dependent fitness could be influencing the lack of coexistence of PIK3CA and NRAS in real colorectal cancer samples (11), these findings support there are probably additional evolutionary events leading to genotype frequency besides mutual exclusivity.

```
## Simulate cancer progression in 10 individuals until APC, TP53, KRAS,
## PIK3CA, NRAS genotype fixates

set.seed(125)
COADREAD_Simul_freqdep <- oncoSimulIndiv(
               COADREAD_fitness_freqdep,
               model = "McFL", ## Model used
```

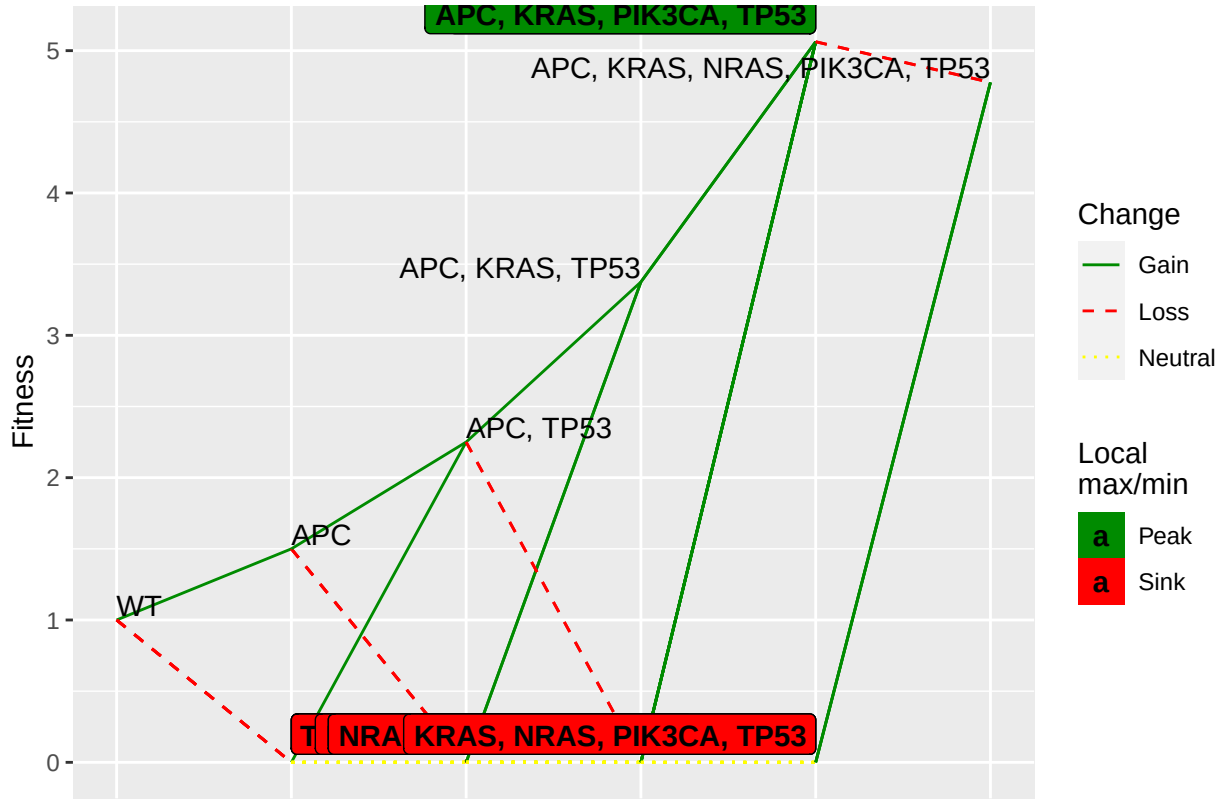

Figure 34: Fitness landscape corresponding with the simplified DAG for the COADREAD dataset accounting for frequency-dependent fitness

```
                mu = 1e-4, ## Mutation rate
                sampleEvery = 0.02, ## How often the whole population is sampled
                keepEvery = 1,
                initSize = 200, ## Initial population size
                finalTime = 2000,
                keepPhylog = TRUE, ## Allow to see parent-child relationships
                onlyCancer = TRUE,
                detectionSize = NA,
                fixation = c(c("_, APC, TP53, KRAS, PIK3CA, NRAS"),
                             fixation_tolerance = 0.99),
                detectionDrivers = NA,
                detectionProb = NA)
```

```
##
##  Hitted wall time. Exiting.
##  Hitting wall time is regarded as an error.
```

```
## Simulate cancer progression in 10 individuals for a final time of 300 time units

set.seed(125)
COADREAD_Simul_freqdep <- oncoSimulPop(10,
                COADREAD_fitness_freqdep,
                model = "McFL", ## Model used
                mu = 1e-4, ## Mutation rate
                sampleEvery = 0.02, ## How often the whole population is sampled
                keepEvery = 1,
                initSize = 200, ## Initial population size
                finalTime = 300,
                keepPhylog = TRUE, ## Allow to see parent-child relationships
                onlyCancer = FALSE,
                detectionSize = NA,
                fixation = NA,
                detectionDrivers = NA,
                detectionProb = NA)
```

```
## You are running Windows. Setting mc.cores = 1
```

```
## Plot of simulation for genotypes
plot(COADREAD_Simul_freqdep[[9]],
show = "genotypes",
type = "stacked",
ylim = c(0, 35000))
```

```
plot(COADREAD_Simul_freqdep[[9]],
     show = "genotypes",
     type = "line",
     ylim = c(1, 100000000))
```

```
## Parent-child relationship derived from simulation
plotClonePhylog(COADREAD_Simul_freqdep[[9]],
```

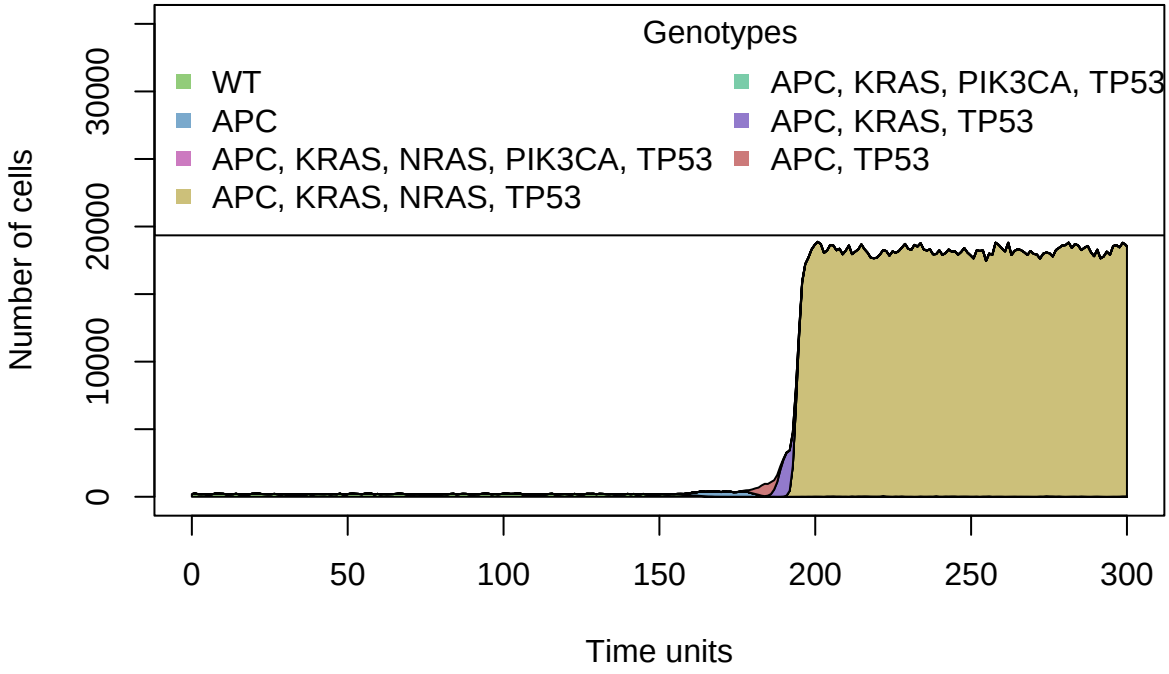

Figure 35: One of the 10 simulations of cancer progression using the frequency-dependent fitness model for the COADREAD dataset (stacked plot)

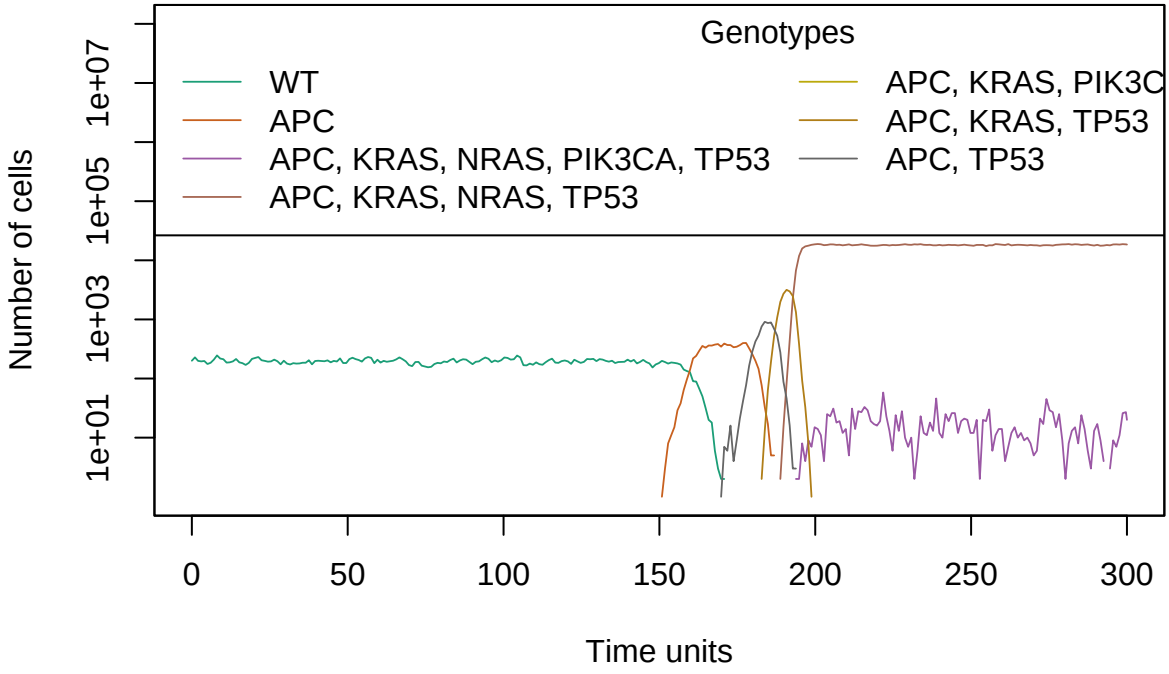

Figure 36: One of the 10 simulations of cancer progression using the frequency-dependent fitness model for the COADREAD dataset (line plot)

```
                N = 0, ## Specify clones that exist
                keepEvents = TRUE ## Arrows showing how many times each clones appeared
)
```

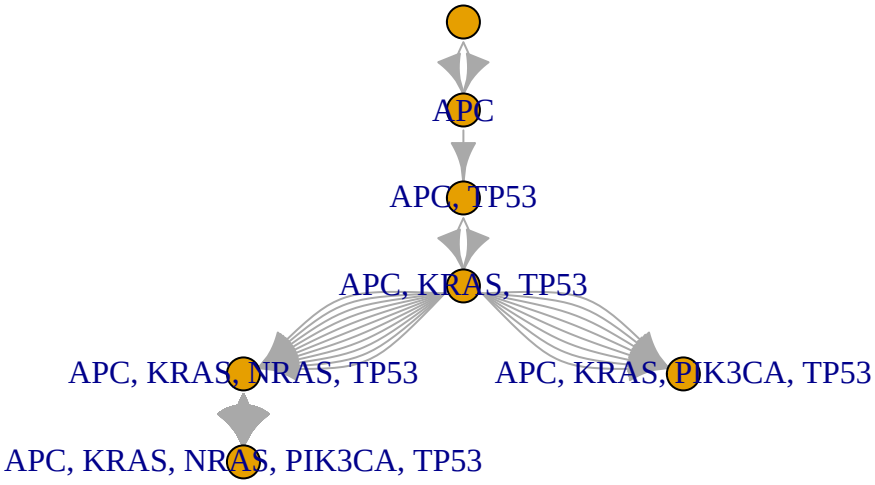

Figure 37: Parent-child relationship derived from one of the 10 simulations

6 Discussion and Conclusions

Cancer progression models (CPMs) are constructed upon genotype frequency data obtained from cross-sectional tumor samples from different individuals. They provide information on mutational restrictions in the form of Directed Acyclic Graphs (DAGs), specifying strict cancer progression paths. Accordingly, CPMs do not allow deviations from DAGs of restrictions, meaning any genotype not included in the model cannot exist (13). However, cancer progression occurs in an evolutionary scenario that makes the process much more flexible, as well as conditioned by other phenomena. Thus, it is paramount to approach cancer progression from an evolutionary perspective, accounting for fitness specification of genotypes that can arise in a specific temporary space. Fitness landscapes include all possible paths of tumor progression and enable to study of different outcomes depending on the selected evolutionary model (5,9).

In this work, we mapped various CPMs (1,10,11) to different evolutionary models and analyzed the diverse fitness landscapes produced by the restrictions used. Authors in (1,10,11) apply a generative approach to derive constraints and mutual exclusivity between genes. Maintaining the mutual exclusivity assumptions, we produced the corresponding fitness landscapes under different fitness specifications, including deviation from monotonicity, order effects, reciprocal sign epistasis, and frequency-dependent fitness. Authors ambiguously

define what is the relationship between mutually exclusive genes (i.e. null or lethal epistatic effects). We tested how this consideration strongly affects the resulting fitness landscape, with synthetic lethality being the relationship that best fits the mutual exclusivity restrictions state in the CPMs. Conversely, a null effect on fitness does not avoid genotypes violating the mutual exclusivity restriction to reach the highest fitness peaks in the models and eventually arise in the simulations.

Order of effects considers the order in which mutations are accumulated when mapping the fitness of genotypes. The same genotype can be associated with different fitness values depending if the mutations acquired before violate or not restrictions defined in the model. The favored genotypes were the ones consistent with specified gene dependencies. In this context, the fitness landscape will show the same genotype at different heights, depending on the order in which mutations are acquired. Frequency-dependent fitness also shapes the fitness landscape so that mutually exclusive genes are less likely to appear together due to a lower fitness in the presence of other clones. All this evidence supports the idea that cancer progression is better explained through an evolutionary perspective, as evolution significantly modulates the genotype space that clones can explore. Current CPMs, based on probabilistic methodologies, cannot capture these phenomena and, thus, conclusions derived from them should be questioned.

OncoSimulR (13) is a very complete and convenient clone-based genetic simulator to evaluate cancer progression under evolutionary models. Apart from enabling fitness specification considering order and restriction of mutations, epistasis, and frequency-dependent for running cancer simulations, it displays all possible paths of tumor progression in fitness landscapes, so it is possible to examine differences among evolutionary models and their subsequent cancer simulations. However, fitness landscapes are limited to a small set of genes that allow easy visualization and interpretation of all possible paths, decreasing the capacity of this software. In addition, it does not display DAG nor fitness landscape when order effects are used to define fitness effects. Likewise, synthetic lethality can be better represented if the DAG is composed of individual genes instead of modules because modules do not allow to define epistatic relationships between genes of the same module, which is important for genes that participate in the same pathway. Also, large and realistic gene sets cannot be used in DAGs because the number of possible genotypes increases at 2^n, where n is the number of genes, which hampers the mapping of genotypes to fitness.

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