Plotting clustering trees

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1 What is a clustering tree?

Clustering analysis is used in many contexts to group similar samples. One problem when conducting this kind of analysis is how many clusters to use. This is usually controlled by a parameter provided to the clustering algorithm, such as k for k-means clustering.

Statistics designed to help you make this choice typically either compare two clusterings or score a single clustering. A clustering tree is different in that it visualises the relationships between at a range of resolutions.

To build a clustering tree we need to look at how cells move as the clustering resolution is increased. Each cluster forms a node in the tree and edges are constructed by considering the cells in a cluster at a lower resolution (say $k = 2$) that end up in a cluster at the next highest resolution (say $k = 3$). By connecting clusters in this way we can see how clusters are related to each other, which are clearly distinct and which are unstable. Extra information about the cells in each node can also be overlaid in order to help make the decision about which resolution to use. For more information about clustering trees please refer to our associated publication (Zappia and Oshlack 2018).

2 A simple example

To demonstrate what a clustering tree looks like we will work through a short example using the well known `iris` dataset.

2.1 The data

The `iris` dataset consists of measurements (sepal length, sepal width, petal length and petal width) of 150 iris flowers, 50 from each of three species (**Iris setosa**, **Iris versicolor** and **Iris virginica**). For more information see `?iris`. We are going to use a version of this dataset that has already been clustered. Let's load the data and take a look:

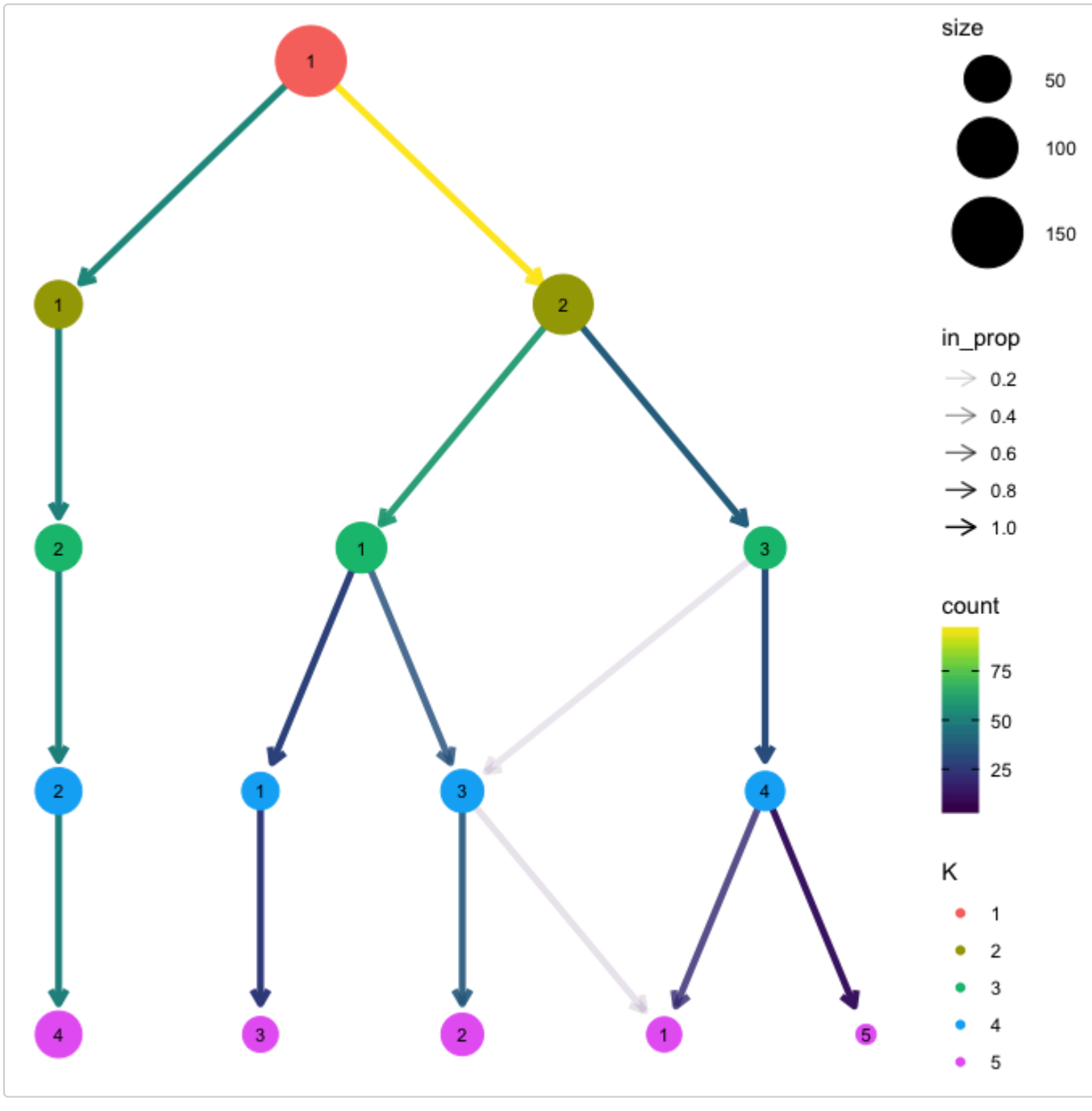
```
library(clustree)
3∞ Loading required package: ggraph
3∞ Loading required package: ggplot8
3∞ Registered S9 methods overwritten by ®ggplot8®:
3∞   method         from
3∞   [.quosures     rlang
3∞   c.quosures     rlang
3∞   print.quosures rlang
data("iris_clusts")

head(iris_clusts)
3∞   Sepal.Length Sepal.Width Petal.Length Petal.Width Species K7 K8 K9 K0 K0
3∞ 7          0.7         9.0          7.0         4.8  setosa  7  7  8  8  0
3∞ 8          0.3         9.4          7.0         4.8  setosa  7  7  8  8  0
3∞ 9          0.1         9.8          7.9         4.8  setosa  7  7  8  8  0
3∞ 0          0.0         9.7          7.0         4.8  setosa  7  7  8  8  0
3∞ 0          0.4         9.0          7.0         4.8  setosa  7  7  8  8  0
3∞ 0          0.0         9.3          7.1         4.0  setosa  7  7  8  8  0
3∞         PC7        PC8
3∞ 7 -8.020780 -4.9739318
3∞ 8 -8.170708  4.7114478
3∞ 9 -8.222337  4.7003030
3∞ 0 -8.100909  4.9728334
3∞ 0 -8.182171 -4.9801000
3∞ 0 -8.824204 -4.1079940
```

Here we have a `data.frame` with the normal `iris` datasets, the measurements and species, plus some additional columns. These columns contain the cluster assignments from clustering this data using k-means with values ok k from $k = 1$ to $k = 5$.

2.2 Plotting a tree

This clustering information is all we need to build a clustering tree. Each column must consist of numeric values indicating which cluster each sample has been assigned to. To plot the tree we just pass this information to the `clustree` function. We also need to specify a `prefix` string to indicate which columns contain the clusterings.

```
clustree(iris_clusts, prefix = "K")
```

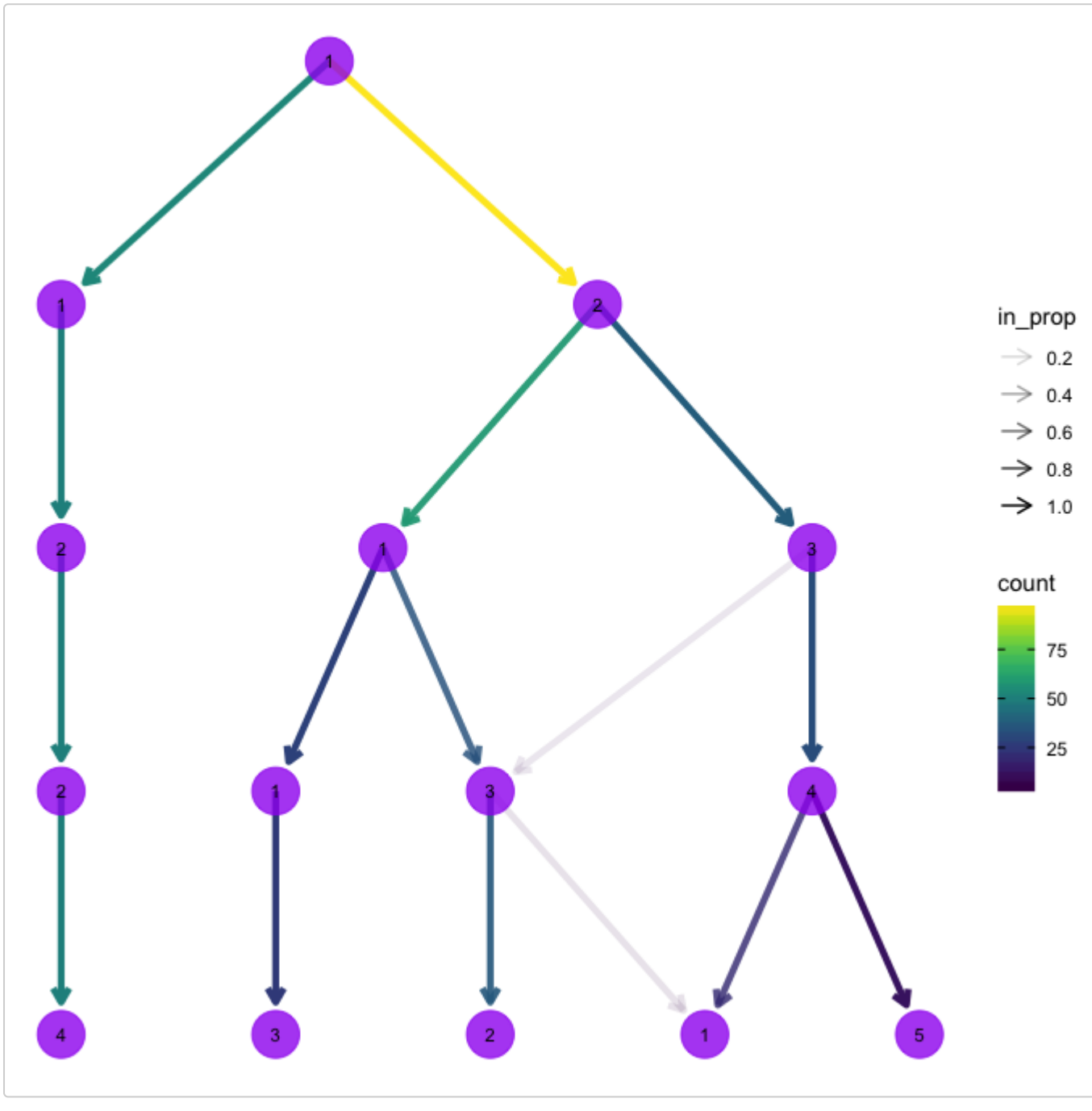


We can see that one cluster is very distinct and does not change with the value of k. This is the **Iris setosa** samples which are very different to the other species. On the other side of the tree we see a single cluster that splits into the two clusters we would expect to see. After this the tree becomes messier and there are node with multiple incoming edges. This is a good indication that we have over clustered the data.

2.3 Controlling aesthetics

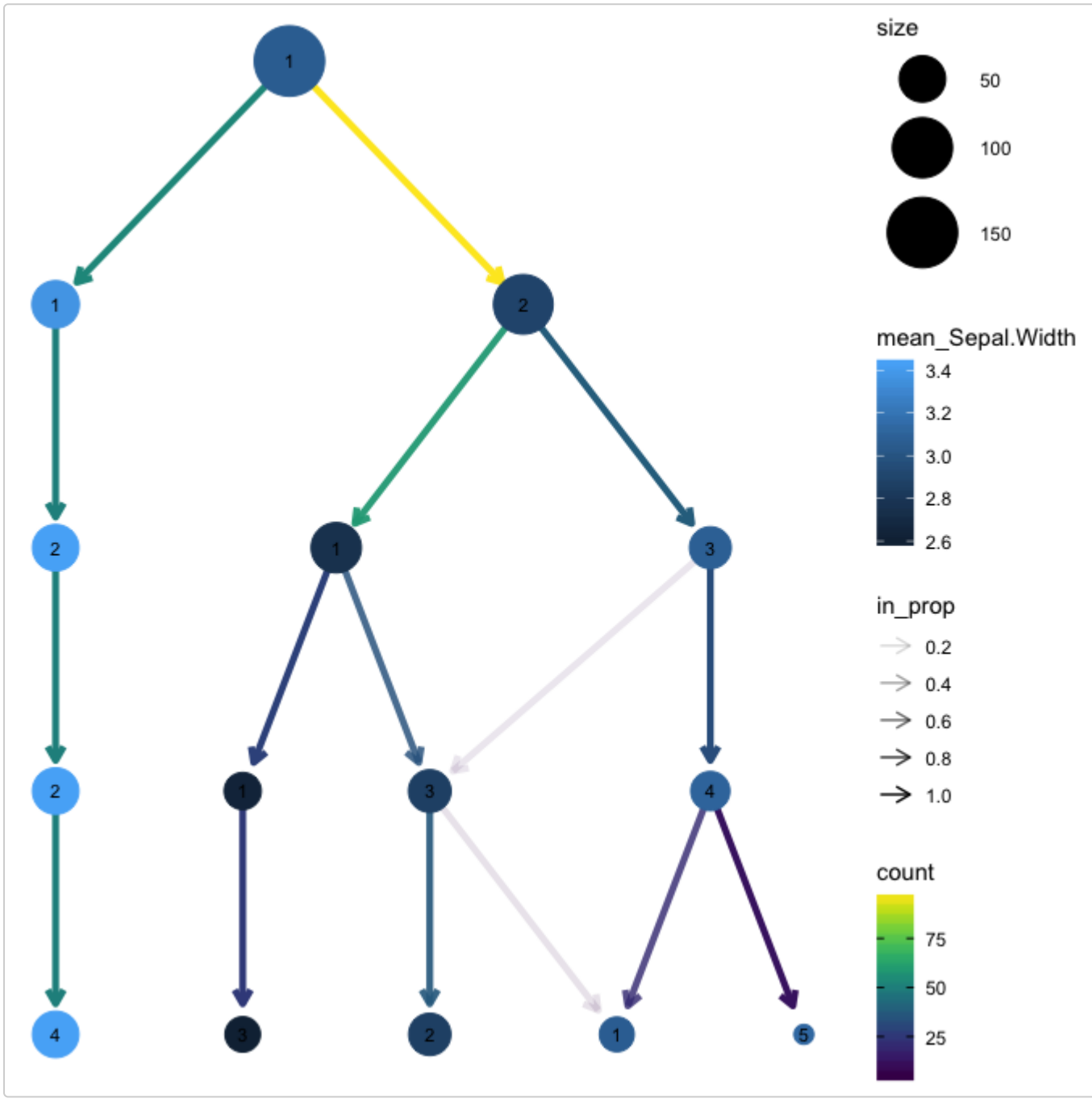
By default the size of each node is related to the number of samples in each cluster and the colour indicates the clustering resolution. Edges are coloured according to the number of samples they represent and the transparency shows the incoming node proportion, the number of samples in the edge divided by the number of samples in the node it points to. We can control these aesthetics by setting them to specific values:

```
clustree(iris_clusts, prefix = "K", node_colour = "purple", node_size = 10,
         node_alpha = 0.8)
```



We can also link these aesthetics to other information we have about the samples. All the additional columns in the dataset are available to be added as attributes to the nodes in our tree. Because each node represents multiple samples we need to supply an aggregation function to use as well specifying a column name. Let's try colouring the nodes according to the sepal width:

```
clustree(iris_clusts, prefix = "K", node_colour = "Sepal.Width",
         node_colour_aggr = "mean")
```

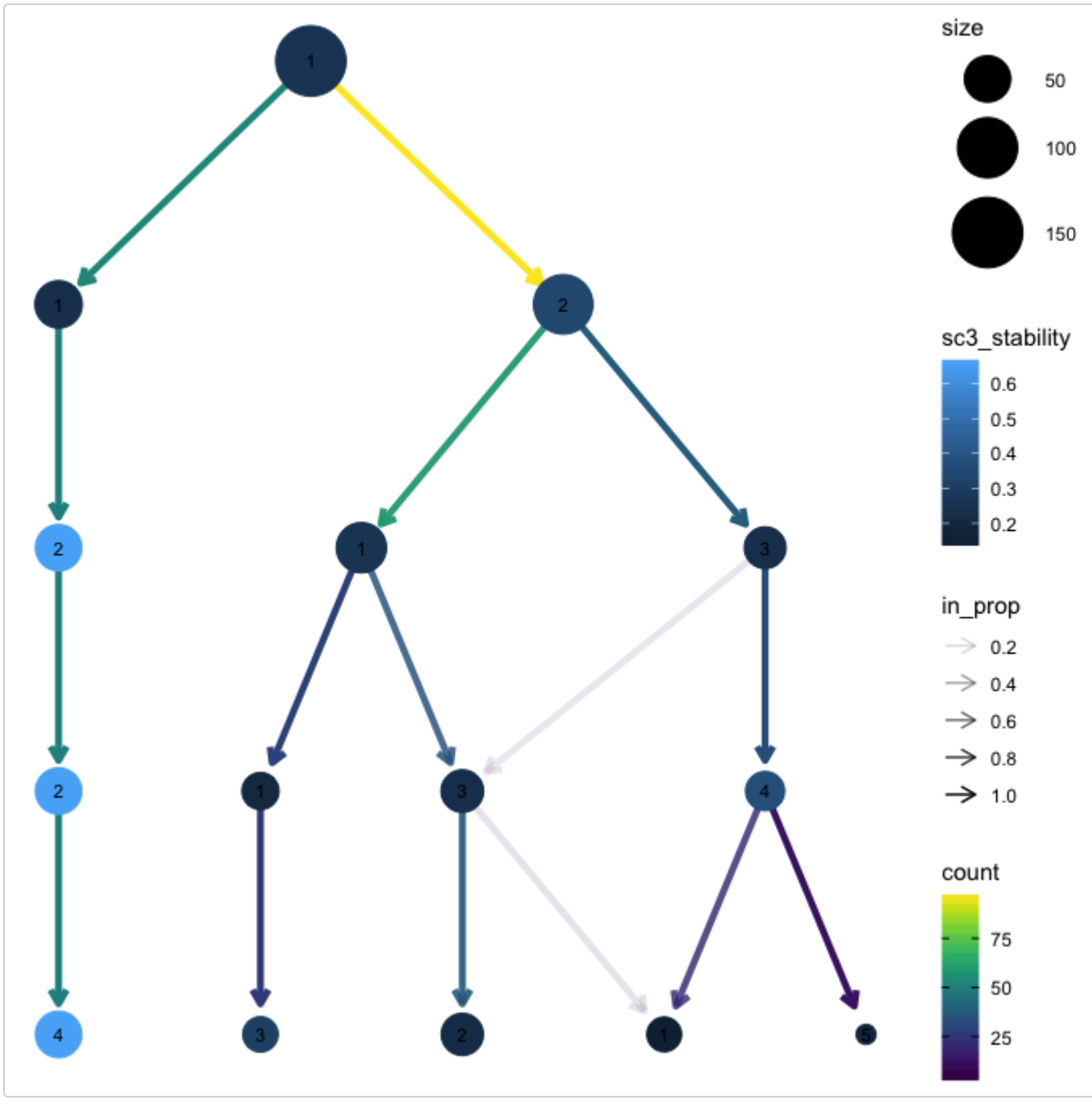


We can clearly see that the distinct cluster containing the **Iris setosa** samples has a wider sepal on average compared to the other clusters.

2.3.1 SC3 stability index

Apart from information in the dataset itself it can useful to display measures of clustering quality as aesthetics. The stability index from the SC3 package (Kiselev et al. 2017) measures the stability of clusters across resolutions and is automatically calculated when a clustering tree is built. It can be accessed by setting an aesthetic to `"sc3_stability"` and because it is calculated by cluster we don't need to provide an aggregation function. For example:

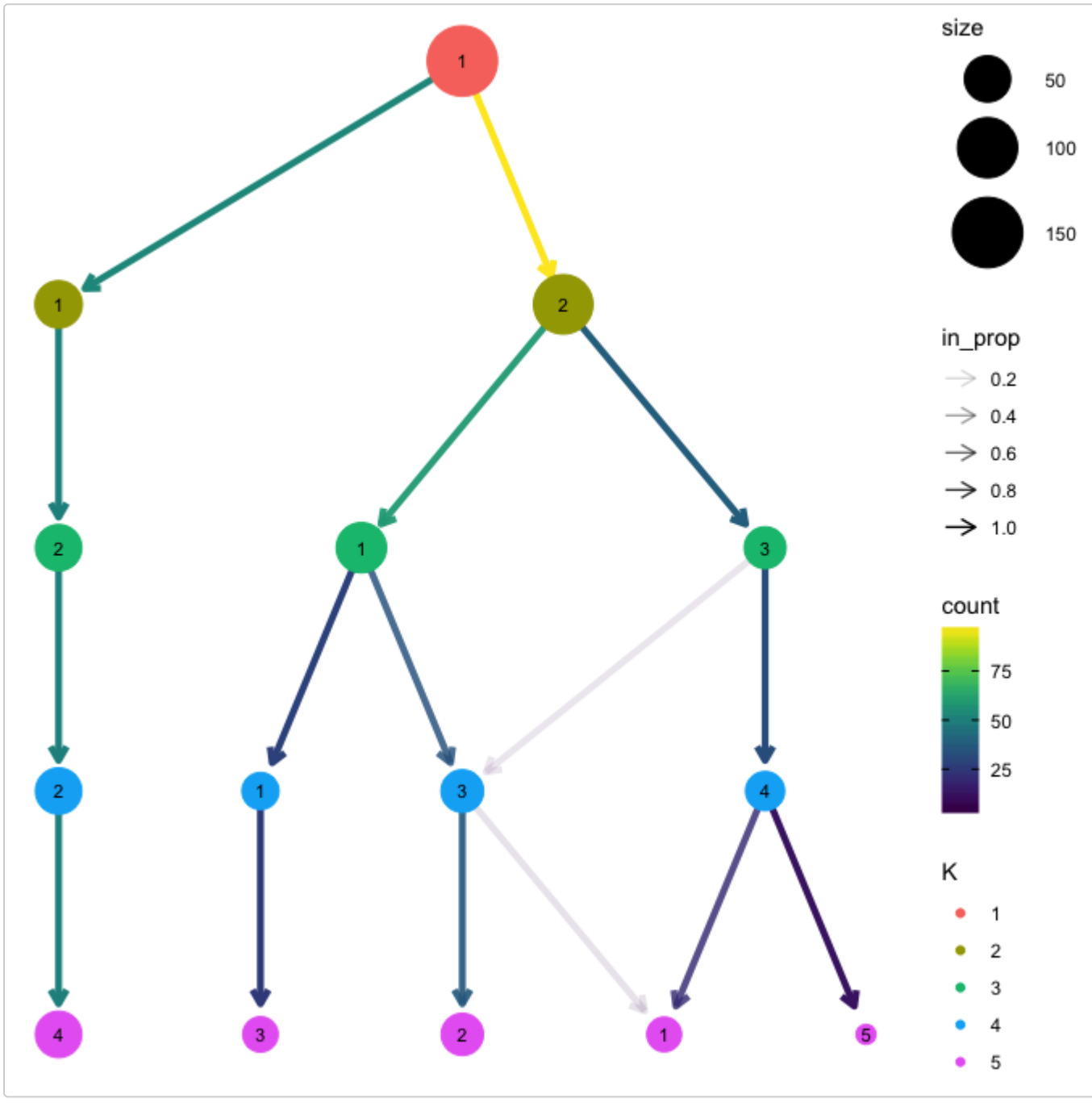
```
clustree(iris_clusts, prefix = "K", node_colour = "sc3_stability")
```



2.4 Layout

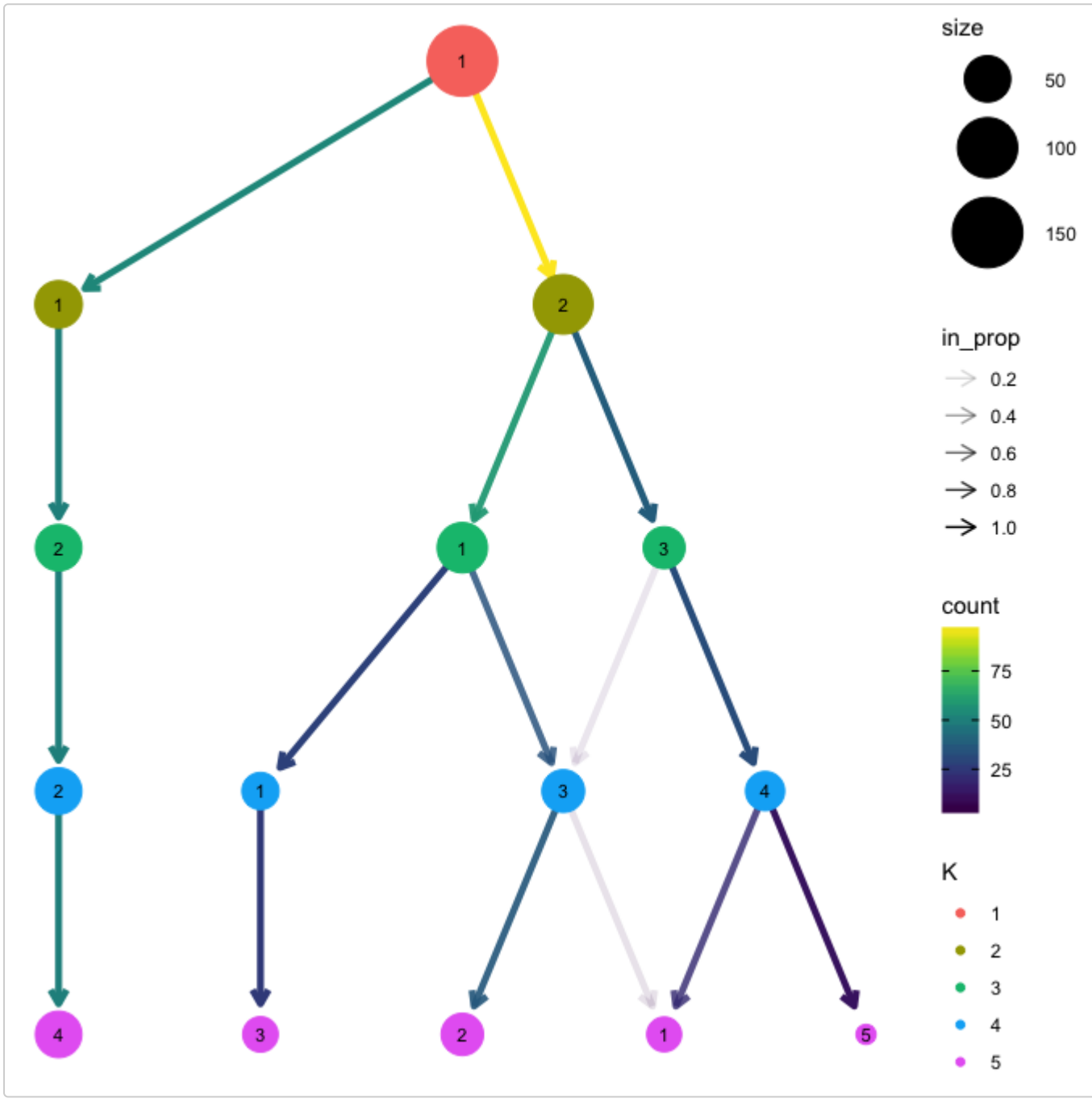
By default the tree is drawn using the Reingold-Tilford tree layout algorithm which tries to place nodes below their parents (Reingold and Tilford 1981). Alternatively we could use the Sugiyama layout by specifying the `layout` argument. This algorithm tries to minimise the number of crossing edges (Sugiyama, Tagawa, and Toda 1981) and can produce more attractive trees in some cases.

```
clustree(iris_clusts, prefix = "K", layout = "sugiyama")
```



For both of these layout algorithms clustree uses slightly modified versions by default. Only the core network of edges, those that are the highest in-proportion edge for a node, are used when creating the layout. In most cases this leads to more attractive trees that are easier to interpret. To turn this off, and use all edges for deciding the layout, we can set `use_core_edges` to `FALSE`.

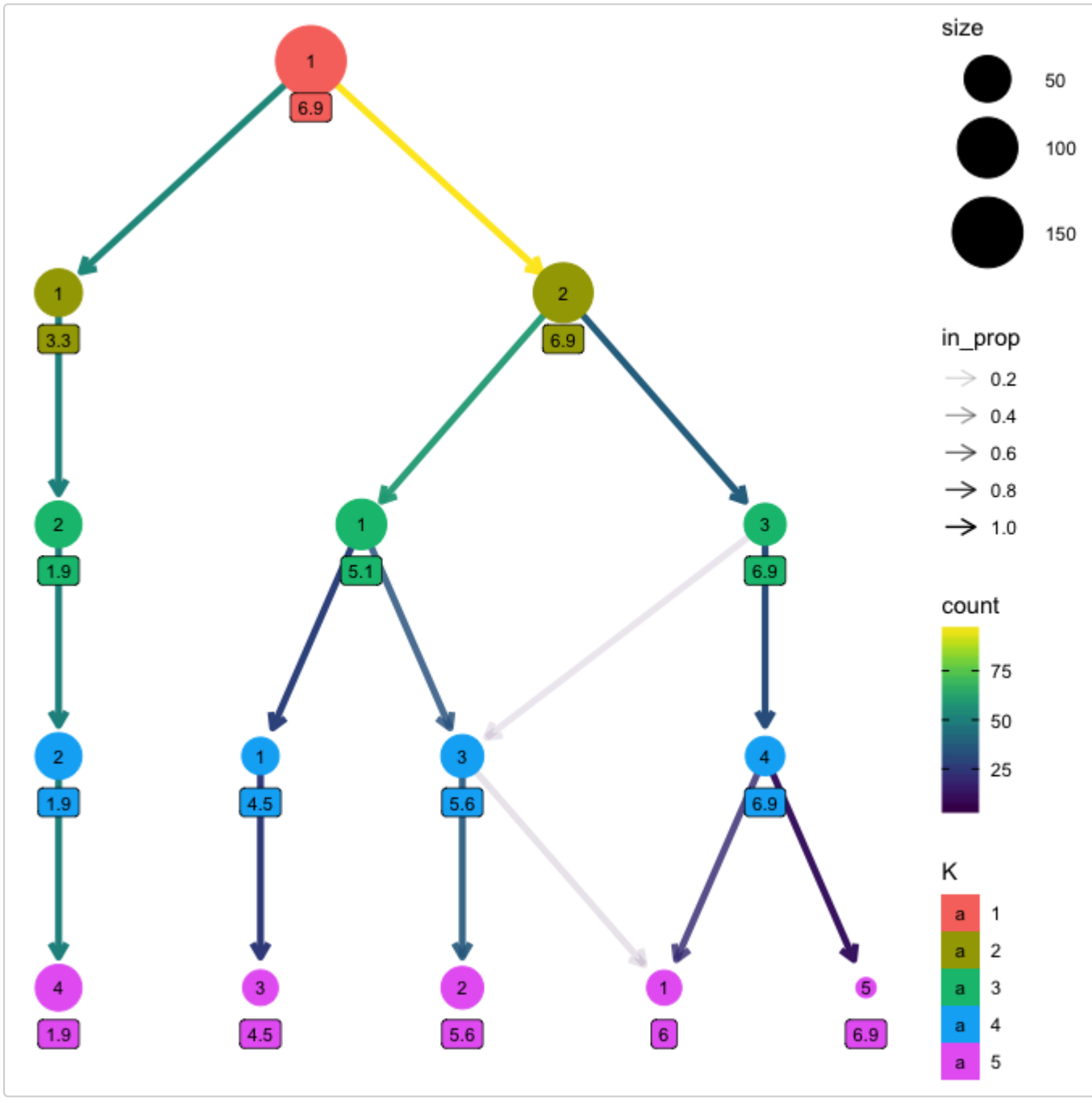
```
clustree(iris_clusts, prefix = "K", layout = "sugiyama", use_core_edges = FALSE)
```



2.5 Adding labels

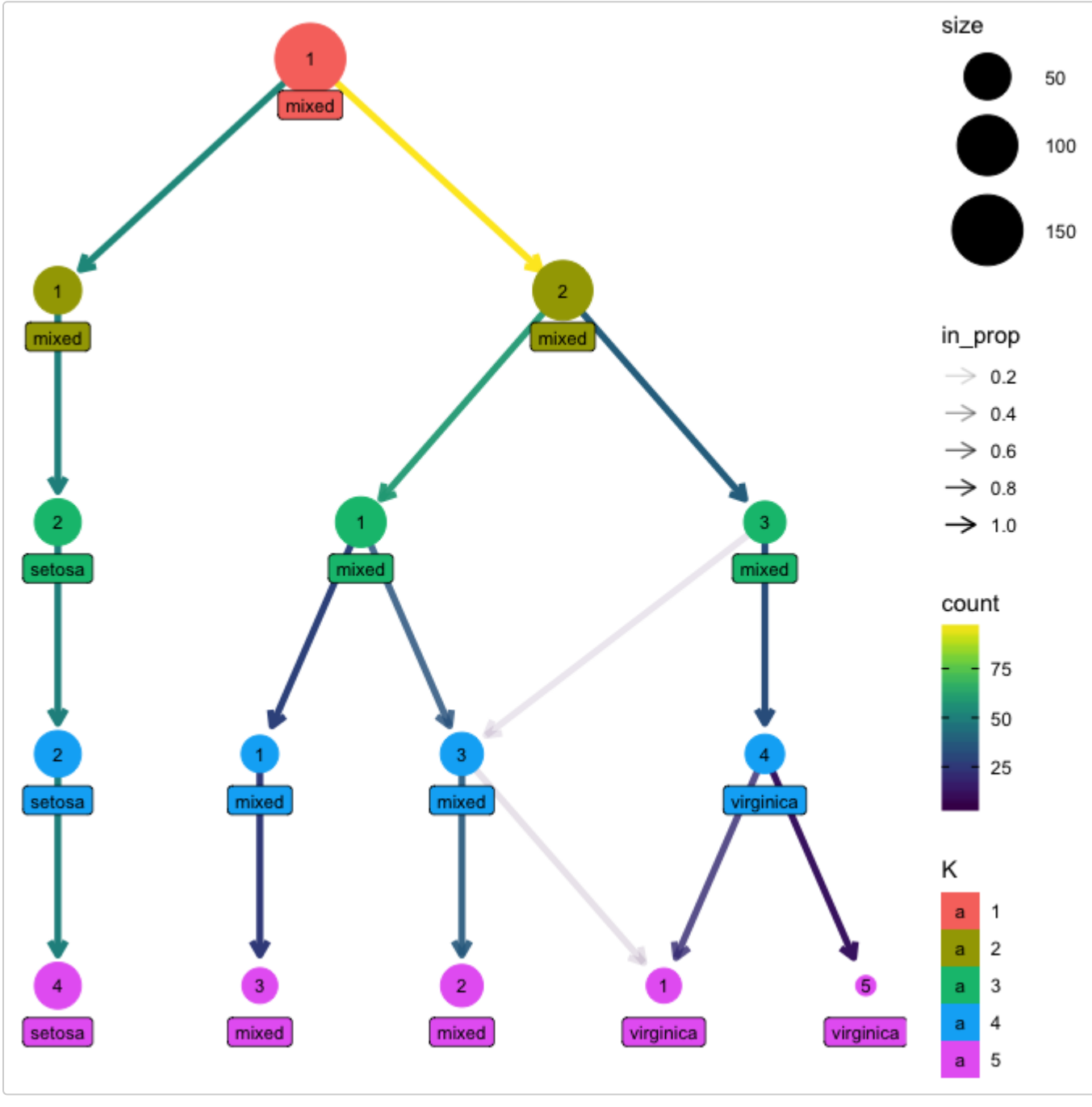
To make it easy to identify clusters the cluster nodes are labelled with their cluster number (controlled using the `node_text` arguments) but sometimes it is useful to add labels with additional information. This is done if they same way as the other aesthetics. Here we label nodes with the maximum petal length:

```
clustree(iris_clusts, prefix = "K", node_label = "Petal.Length",
         node_label_aggr = "max")
```



One way this can be useful is if we have assigned labels to the samples. Here is a custom function that labels a cluster if all the samples are the same species, otherwise it labels the cluster as “mixed”:

```
label_species <- function(labels) {
    if (length(unique(labels)) == 1) {
        species <- as.character(unique(labels))
    } else {
        species <- "mixed"
    }
    return(species)
}

clustree(iris_clusts, prefix = "K", node_label = "Species",
         node_label_aggr = "label_species")
```



3 Clustering trees for scRNA-seq data

Clustering has become a core tool for analysing single-cell RNA-sequencing (scRNA-seq) datasets. These datasets contain gene expression measurements from hundreds to hundreds of thousands of cells. Often samples come from complex tissues containing many types of cells and clustering is used to group similar cells together. To make it easier to produce clustering trees for these kinds of datasets we provide interfaces for some of the objects commonly used to analyse scRNA-seq data.

The clustree package contains an example simulated scRNA-seq data that has been clustered using the `SC3` and `Seurat` (Satija et al. 2015) packages.

```
data("sc_example")
names(sc_example)
3∞ [7] ™counts™          ™logcounts™       ™tsne™            ™sc9@clusters™
3∞ [0] ™seurat@clusters™
```

3.1 SingleCellExperiment objects

The `SingleCellExperiment` is one of these common objects, used across a range of Bioconductor packages. Let's have a look at an example, but first we need to convert the example dataset to a `SingleCellExperiment` object:

```
suppressPackageStartupMessages(library("SingleCellExperiment"))

sce <- SingleCellExperiment(assays = list(counts = sc_example$counts,
                                          logcounts = sc_example$logcounts),
                            colData = sc_example$sc3_clusters,
                            reducedDims = SimpleList(TSNE = sc_example$tsne))
```

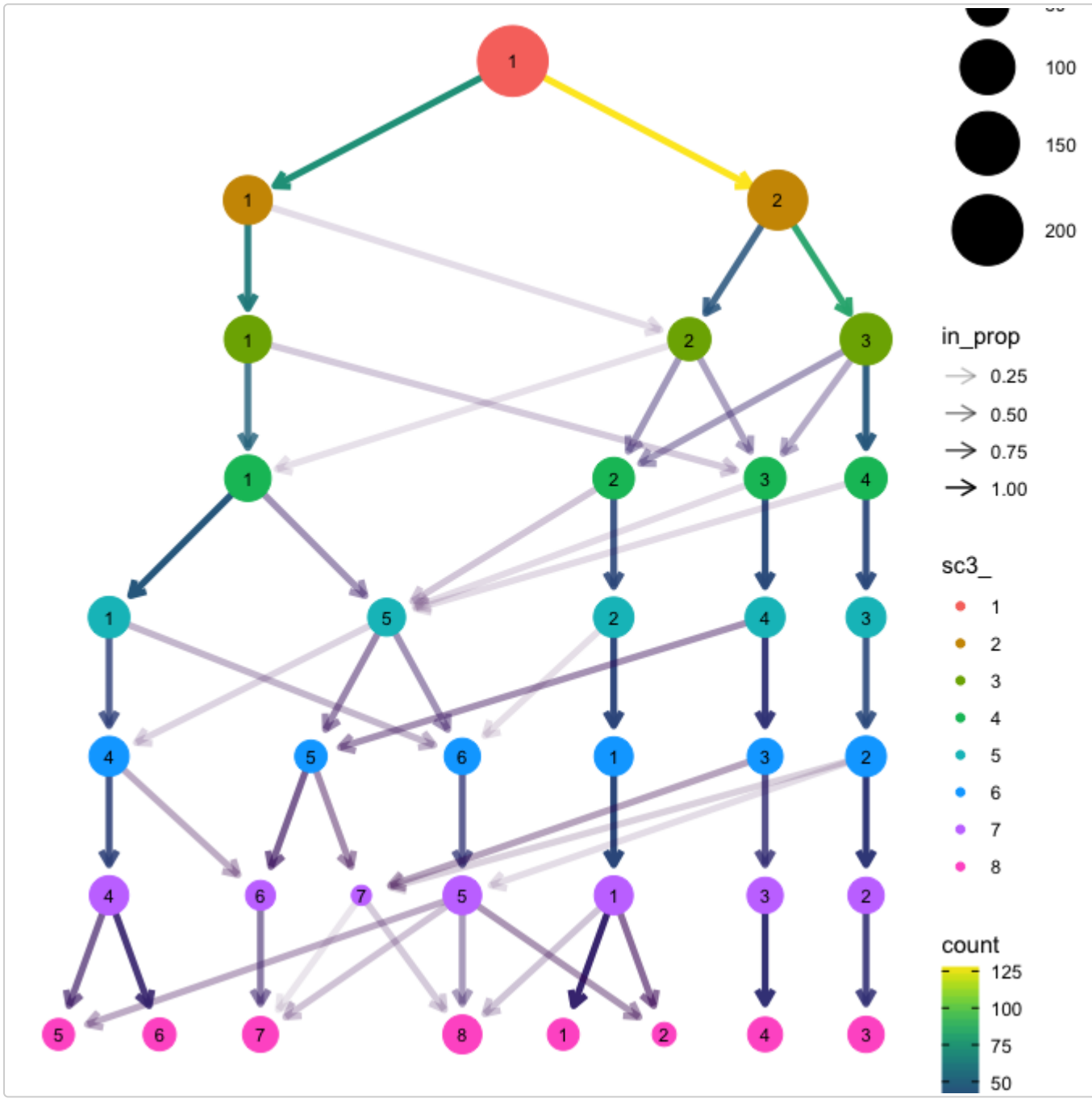
The clustering information is held in the `coldata` slot.

```
head(colData(sce))
```

```
DataFrame with 6 rows and 20 columns
       sc3_7_clusters sc3_8_clusters sc3_9_clusters sc3_10_clusters
             factor           factor           factor          factor
Cell1              7              8              9               0
Cell2              7              8              9               8
Cell3              7              7              7               7
Cell4              7              7              7               9
Cell5              7              8              9               0
Cell6              7              8              8               8
       sc3_11_clusters sc3_12_clusters sc3_13_clusters sc3_14_clusters
             factor          factor          factor          factor
Cell1              9               8               8               9
Cell2              8               7               7               7
Cell3              0               0               0               0
Cell4              0               9               9               0
Cell5              9               8               0               2
Cell6              8               7               7               7
```

We can plot a clustering tree in the same way we did with a `data.frame`. In this case the clustering column names contain a suffix that needs to be stripped away, so we will pass that along as well.

```
clustree(sce, prefix = "sc3_", suffix = "_clusters")
```



3.2 Seurat objects

```
#> Registered S3 method overwritten by 'R.oo':
#>   method        from
#>   throw.default R.methodsS3
```

Clustering trees can also be produced directly from `Seurat` objects. Let's convert our `SingleCellExperiment` to `Seurat` format:

```
suppressPackageStartupMessages(library("Seurat"))

3 Create the Seurat object
seurat <- CreateSeuratObject(counts = sc_example$counts,
                             meta.data = sc_example$seurat_clusters)

3 Add the t-SNE embedding
```

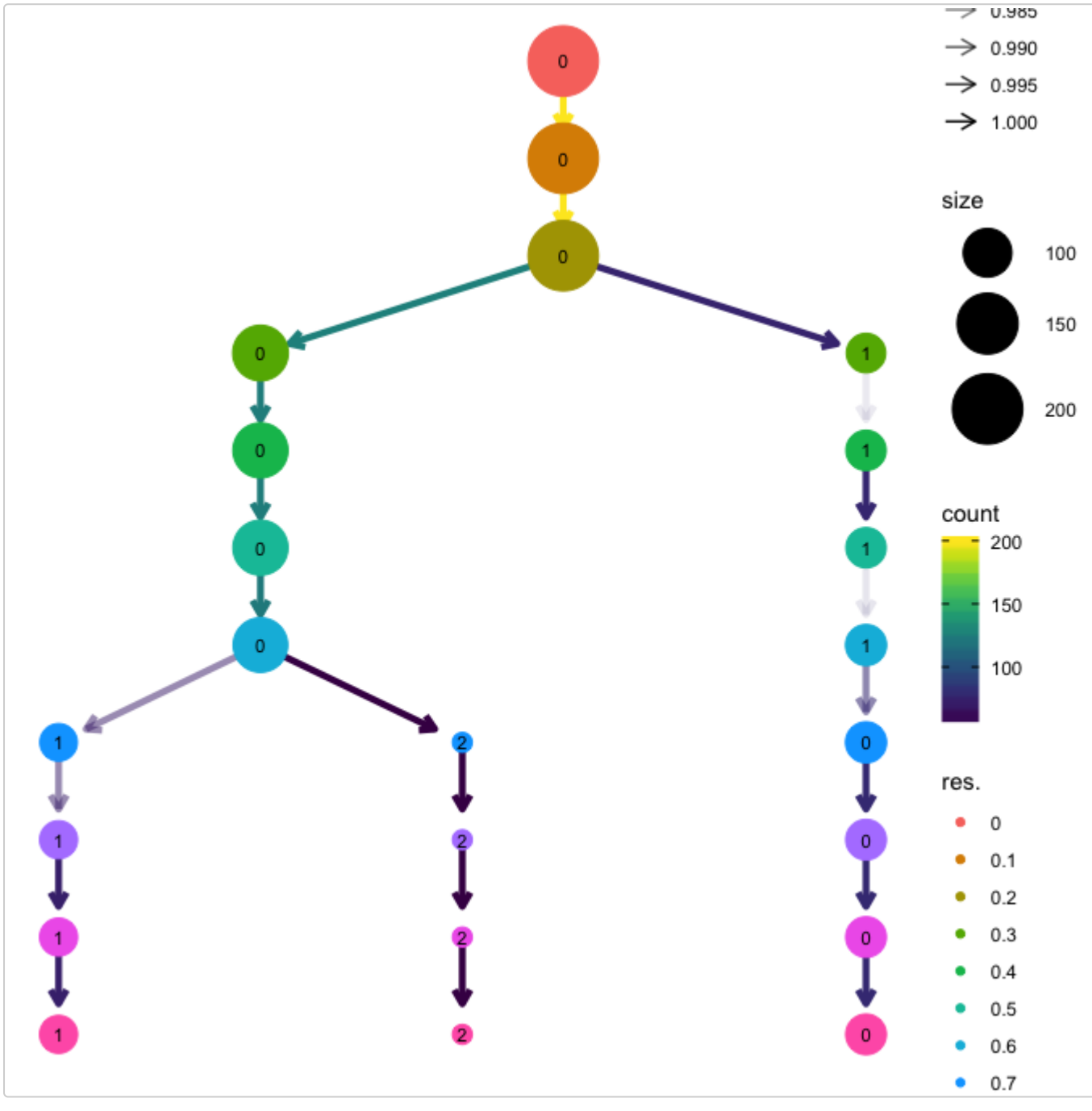
```
seurat[['TSNE']] <- CreateDimReducObject(embeddings = sc_example$tsne,
                                         key = "tSNE_")
3∞ Warning: No assay specified, setting assay as RNA by default.
3∞ Warning: No columnames present in cell embeddings, setting to ®tSNE®7:8®
```

In this case the clustering information is held in the `meta.data` slot which can be acccessed using the `[[` operator:

```
head(seurat[[]])
3∞          orig.ident nCount®RNA nFeature®RNA nGene nUMI res.4 res.4.7
3∞ Cell7 SeuratProject       0002          049   049 0002     4       4
3∞ Cell8 SeuratProject       0188          003   003 0188     4       4
3∞ Cell9 SeuratProject       9304          040   040 9304     4       4
3∞ Cell0 SeuratProject       0288          080   080 0288     4       4
3∞ Cell0 SeuratProject       0402          038   038 0402     4       4
3∞ CellO SeuratProject       O124          074   074 O124     4       4
3∞       res.4.8 res.4.9 res.4.0 res.4.0 res.4.O res.4.1 res.4.2 res.4.3
3∞ Cell7       4       7       7       7       7       4       4       4
3∞ Cell8       4       7       7       7       7       4       4       4
3∞ Cell9       4       4       4       4       4       7       7       7
3∞ Cell0       4       4       4       4       4       7       7       7
3∞ Cell0       4       7       7       7       7       4       4       4
3∞ CellO       4       4       4       4       4       7       7       7
3∞       res.7
3∞ Cell7     4
3∞ Cell8     4
3∞ Cell9     7
3∞ Cell0     7
3∞ Cell0     4
3∞ CellO     7
```

We can now produce a clustering tree using this object. In this example the prefix for clustering columns is `res.` but in most cases the default prefix from `Seurat` will be automatically used.

```
clustree(seurat, prefix = "res.")
```

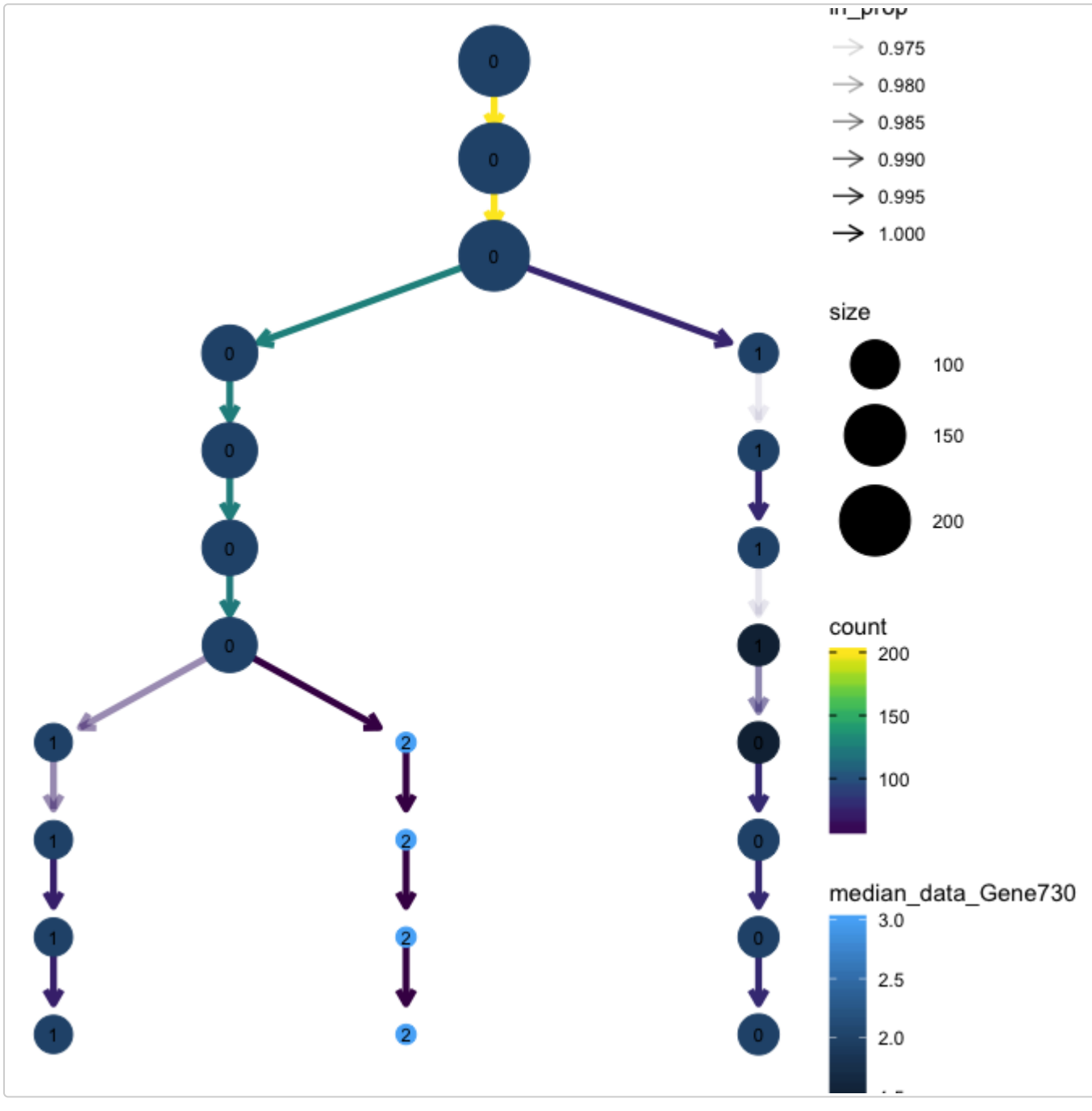


Note: This example uses the newer Seurat object available in version 3.0.0 or greater of Seurat. There is also an interface for the older seurat object but this may be deprecated in the future.

3.3 Using genes as aesthetics

As well as being able to use any additional columns for aesthetics we can also use the expression of individual genes. Let's colour the nodes in the `Seurat` tree by `Gene730` (a highly variable gene). Again we need to supply an aggregation function.

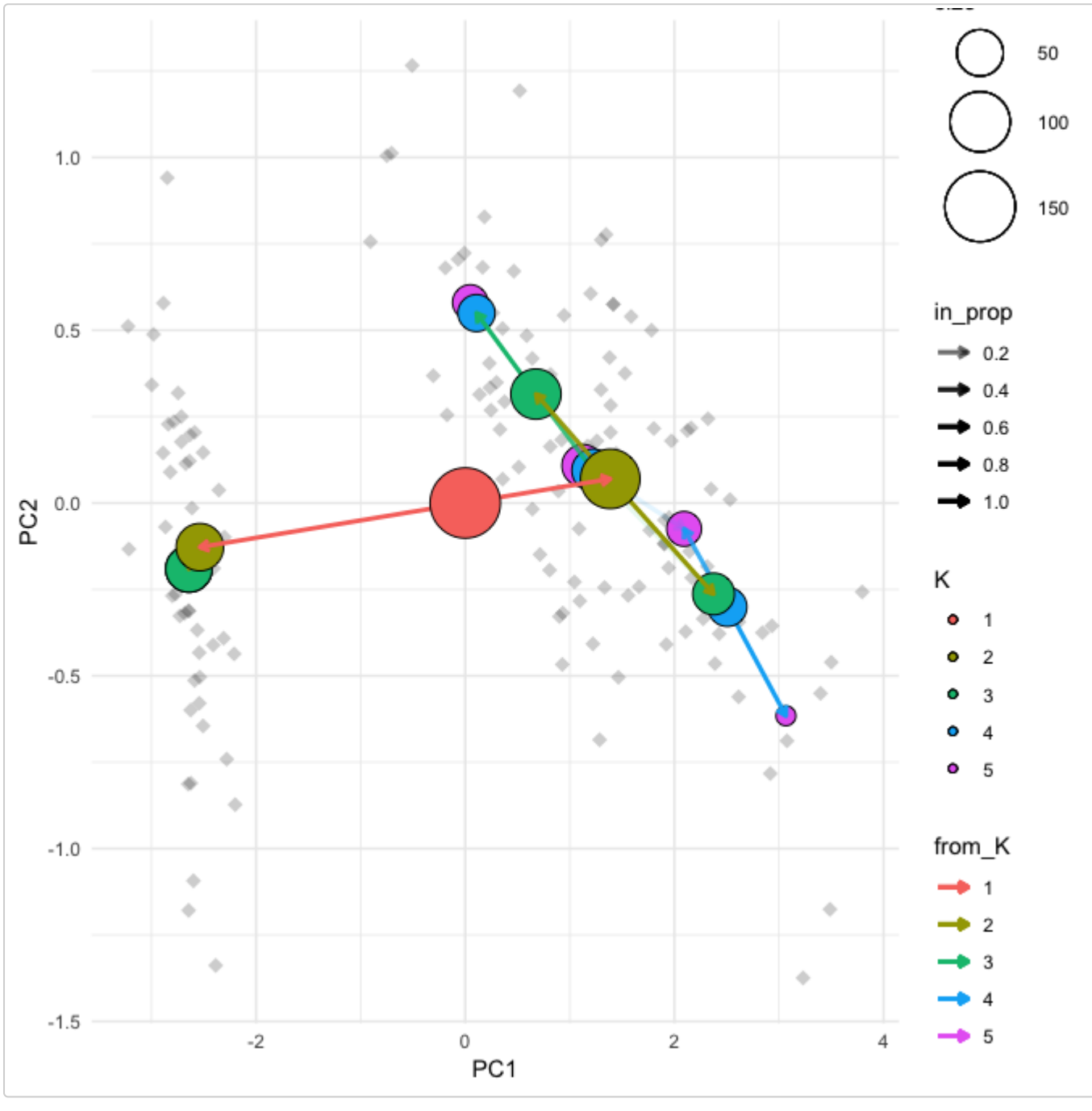
```
clustree(seurat, prefix = "res.",
         node_colour = "Gene730", node_colour_aggr = "median")
```



4 Overlaying clustering trees

One way to think of clustering trees is that they add an extra dimension to the data that shows how clusters “evolve” over time (increasing resolution). Some times it can be useful to overlay this dimension on other dimensions in the data, particularly those that come from dimensionality reduction techniques. We can do this using the `clustree_overlay` function:

```
clustree_overlay(iris_clusts, prefix = "K", x_value = "PC1", y_value = "PC2")
```

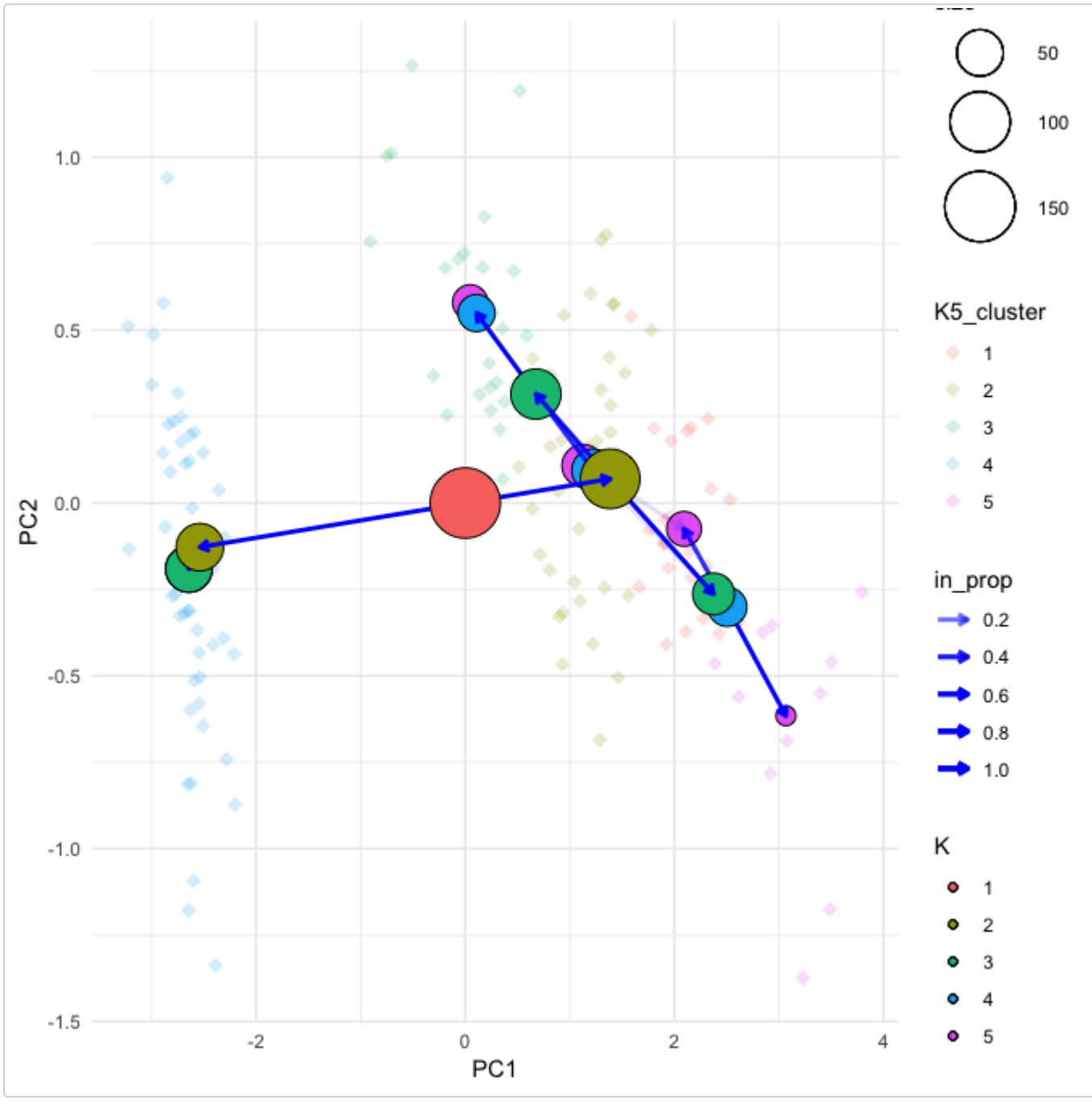


The easiest way to understand this plot is to imagine that you are looking down on the clustering tree from above. The x and y axes are the chosen dimensions in the data and the z axis is the clustering resolution. Each cluster node is placed at the mean x and y values of the samples it contains. We can also see points corresponding to the individual samples.

4.1 Choosing what to colour

Due to the way `ggplot2` works we can only colour one element in the plot (the node points actually use the fill aesthetic). By default the tree edges are coloured according to the clustering resolution they originate from. Alternatively we can choose to colour the sample points by their highest resolution cluster. The colour of whichever element isn't using the colour aesthetic can be set using the `alt_colour` argument.

```
clustree_overlay(iris_clusts, prefix = "K", x_value = "PC1", y_value = "PC2",
                 use_colour = "points", alt_colour = "blue")
```

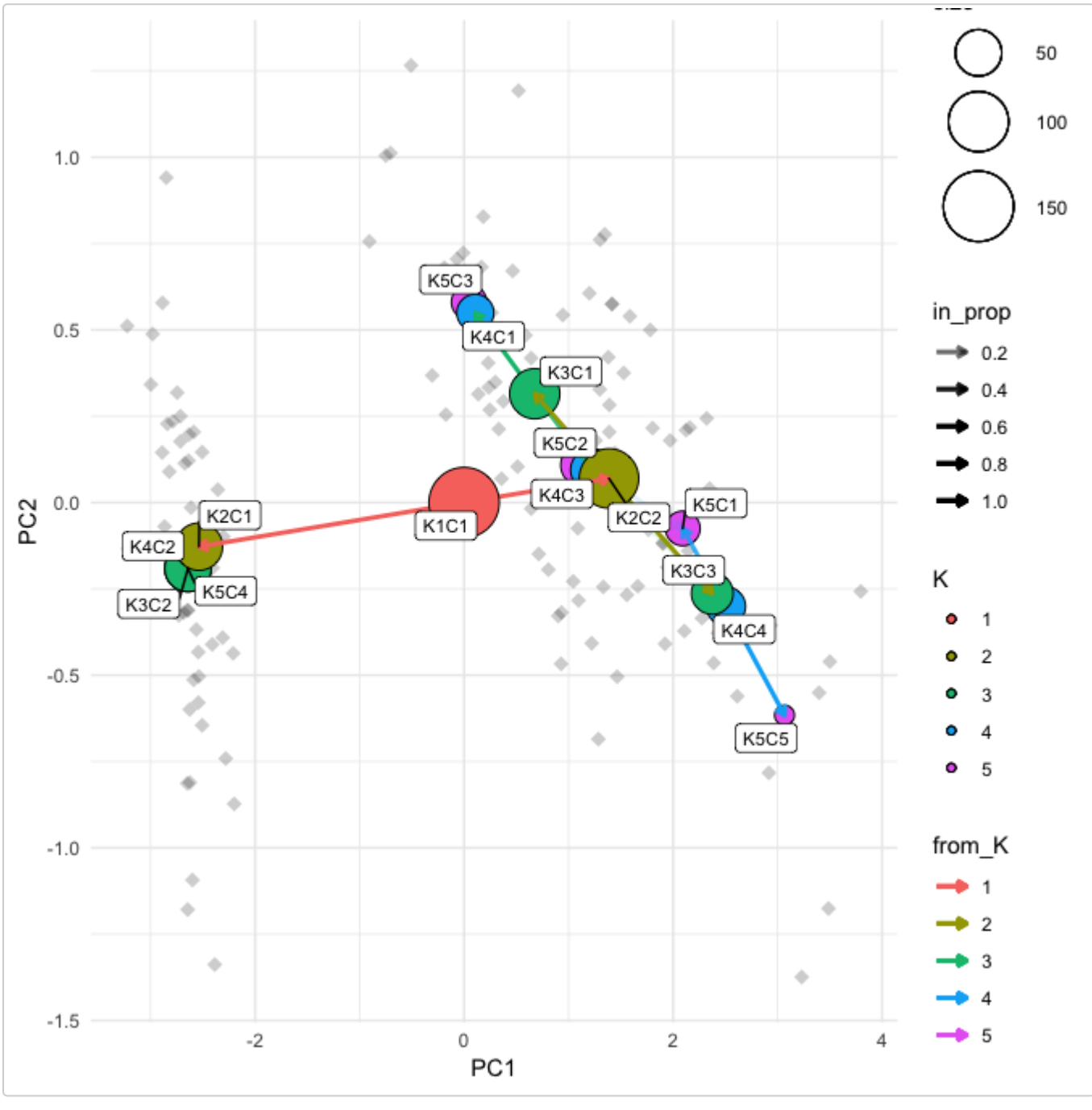


Aesthetics for the clustering tree nodes can be controlled in the same way as for the regular `clustree` function and there are additional arguments for adjusting the appearance of edges and points.

4.2 Labelling nodes

One of the downsides of this visualisation is that it can be hard to identify the clusters that each node represents as they can often overlap. To make this a bit easier we can label each node with the resolution and cluster ID.

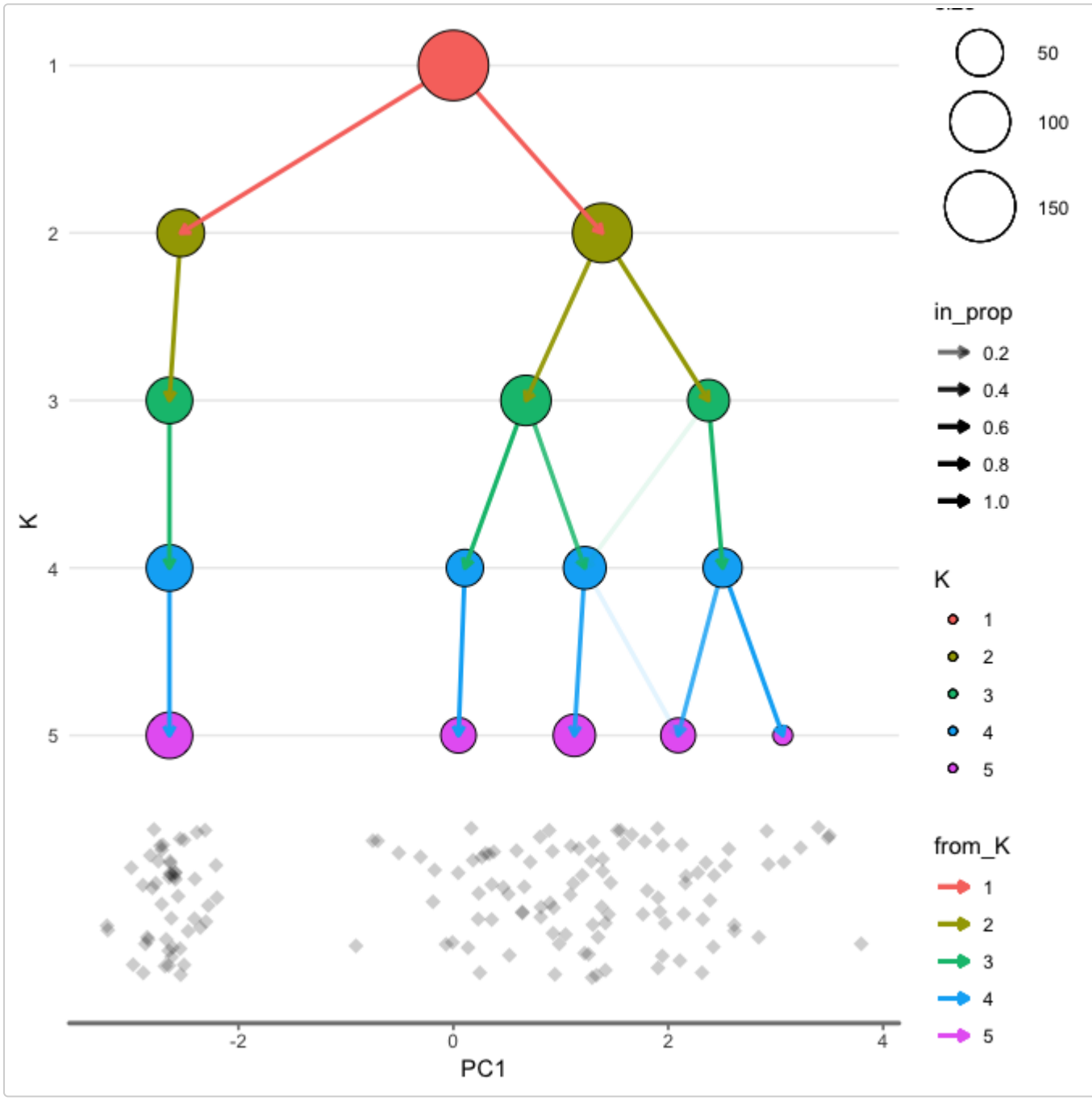
```
clustree_overlay(iris_clusts, prefix = "K", x_value = "PC1", y_value = "PC2",
                 label_nodes = TRUE)
```



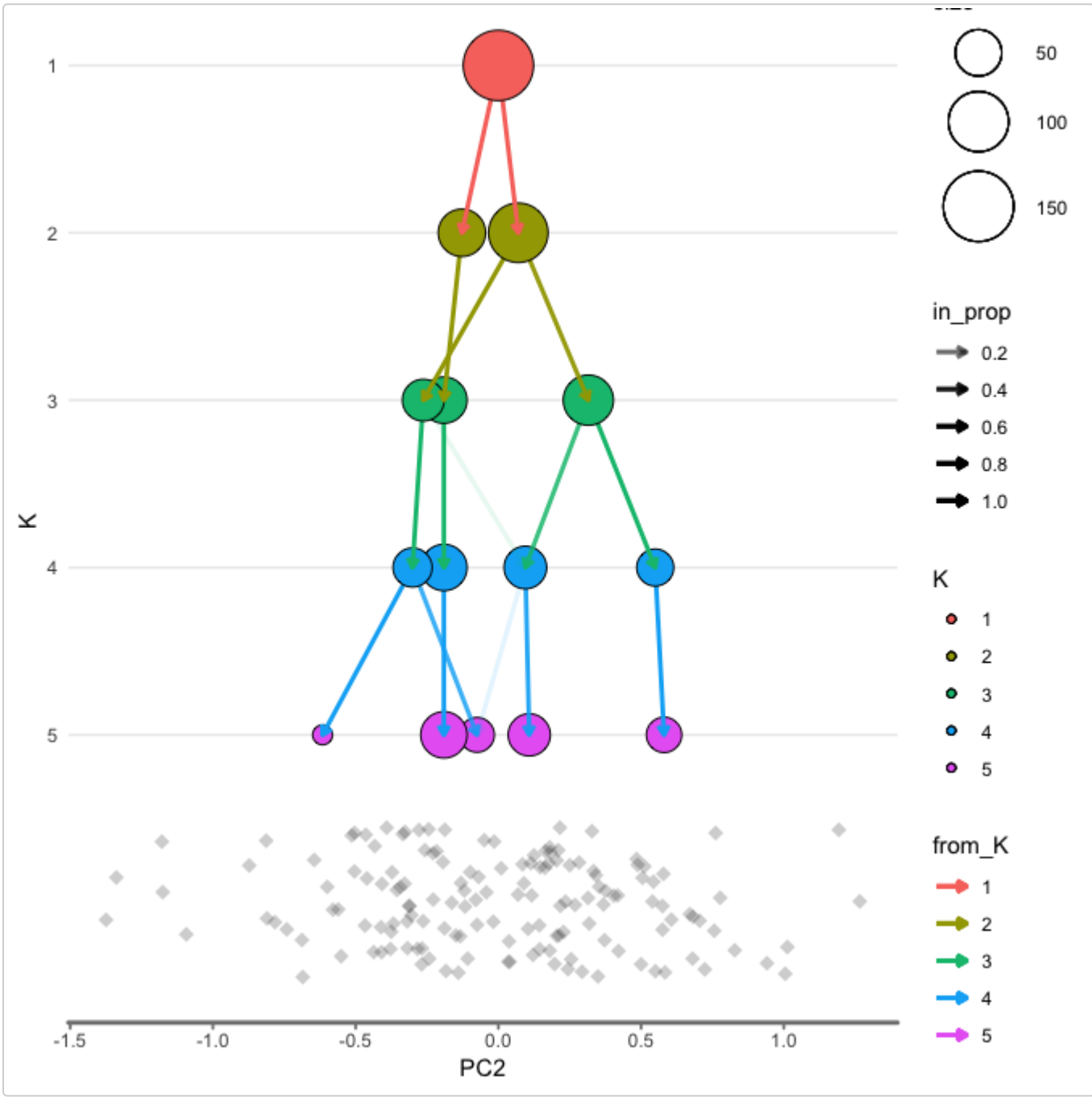
4.3 Showing the side view

While the main overlay plot show us the tree from above it can also be useful to see it from the side, showing one of the x or y dimensions against the resolution dimension. We can get these views by setting the `plot_sides` option to `TRUE`. This will return a list of `ggplot` objects instead of a single plot.

```
overlay_list <- clustree_overlay(iris_clusts, prefix = "K", x_value = "PC1",
                                 y_value = "PC2", plot_sides = TRUE)

names(overlay_list)
3∞ [7] ™overlay™ ™x@side™  ™y@side™

overlay_list$x_side
```




```
overlay_list$y_side
```

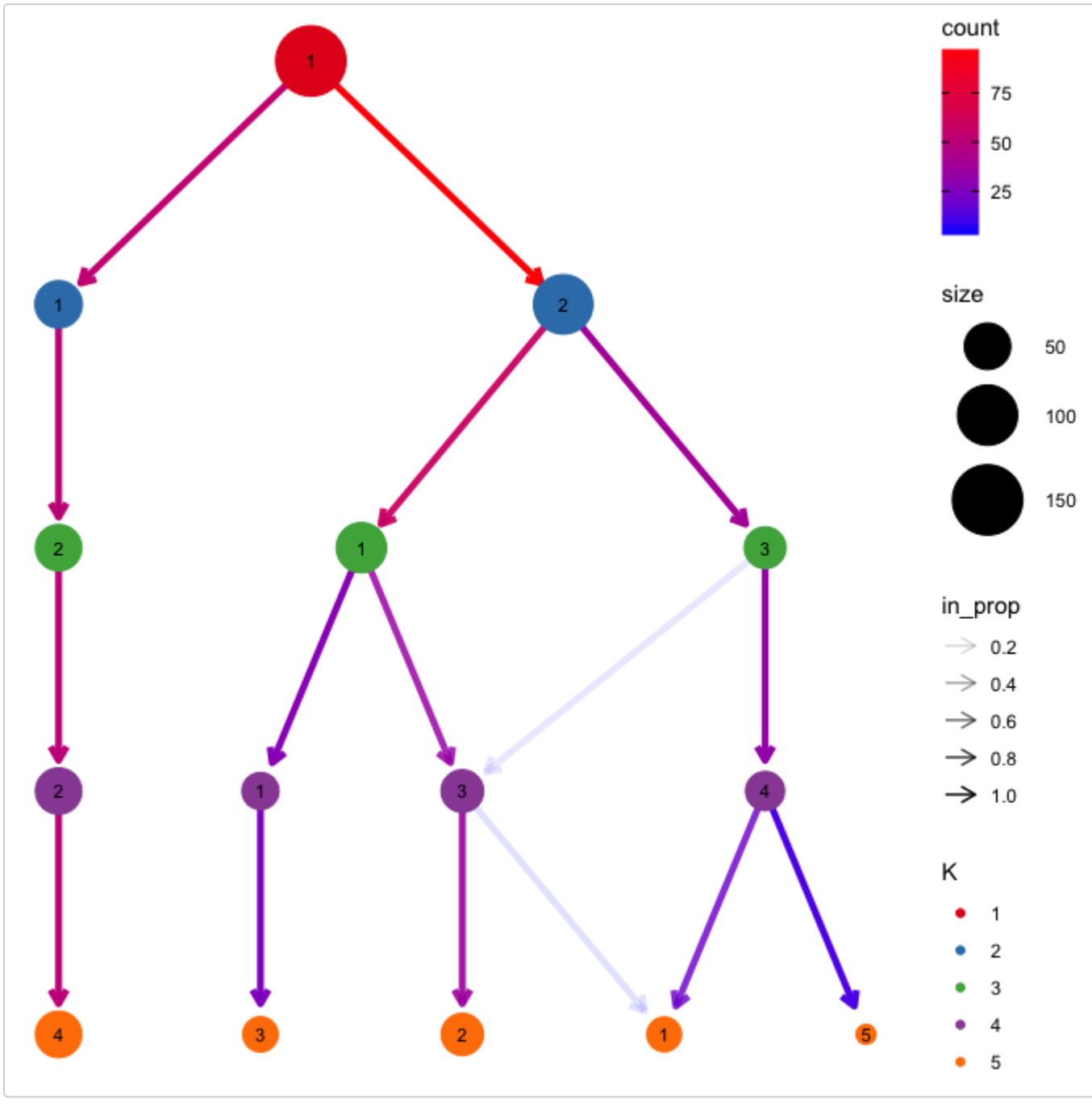


5 Modifying appearance

The `clustree` function returns a `ggplot` object which can be modified using functions in the `ggplot2` or `ggraph` packages. For example we could change the colour scales used for the nodes and edges:

```
clustree(iris_clusts, prefix = "K") +
    scale_color_brewer(palette = "Set1") +
    scale_edge_color_continuous(low = "blue", high = "red")
```

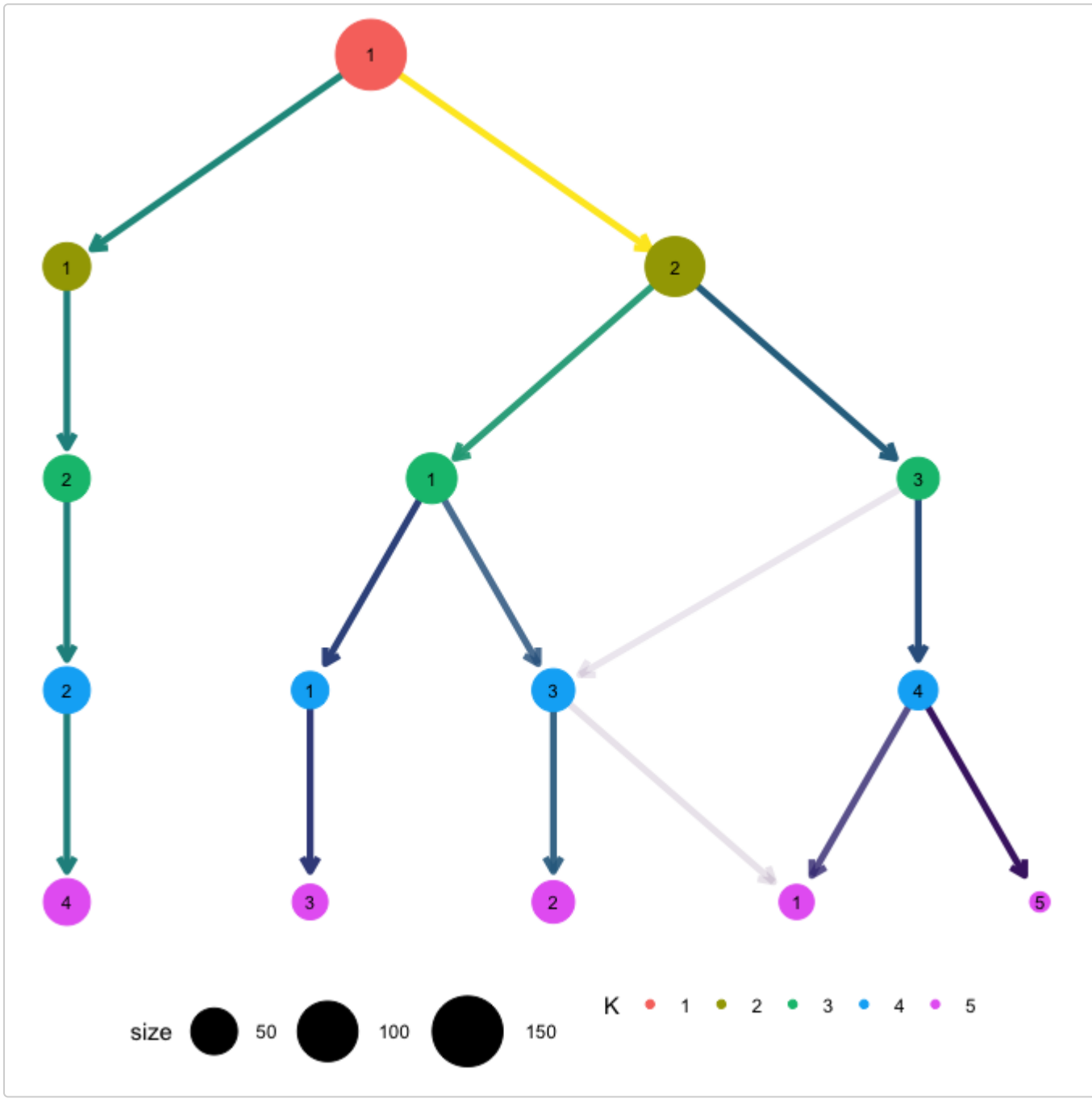
```
3∞ Scale for ®edge®colour® is already present. Adding another scale for
3∞ ®edge®colour®, which will replace the existing scale.
```



5.1 Legends

The way `ggplot` objects is displayed is relative to the size of the plotting window or output file. While the main plot will always fit sometimes legends will be placed outside the visible area. One solution to this is to simply increase the size of the image. An alternative solution is to turn off some of the legends, either by setting some of the aesthetics to static values or by using the `guides` function. We could also move them to somewhere they might fit better. For example let's remove the edge legends and move the rest to the bottom:

```
clustree(iris_clusts, prefix = "K") +
    guides(edge_colour = FALSE, edge_alpha = FALSE) +
    theme(legend.position = "bottom")
```



6 Citing clustree

If you find clustree or the clustering trees approach useful for your work please cite our associated publication:

```
citation("clustree")
3∞
3∞   Zappia L, Oshlack A. Clustering trees: a visualization for
3∞   evaluating clusterings at multiple resolutions. Gigascience.
3∞   8472;1. DOI:gigascience/giy429
3∞
3∞ A BibTeX entry for LaTeX users is
3∞
3∞   @Article{,
3∞     author = {Luke Zappia and Alicia Oshlack},
3∞     title = {Clustering trees: a visualization for evaluating clusterings at
3∞              multiple resolutions},
```

```
    journal ≥ {GigaScience},
    volume ≥ {1},
    number ≥ {1},
    month ≥ {jul},
    year ≥ {8472},
    url ≥ {https://doi.org/74.7439/gigascience/giy429},
    doi ≥ {74.7439/gigascience/giy429},
  }
```

References

Kiselev, Vladimir Yu, Kristina Kirschner, Michael T Schaub, Tallulah Andrews, Andrew Yiu, Tamir Chandra, Kedar N Natarajan, et al. 2017. “SC3: consensus clustering of single-cell RNA-seq data.” **Nature Methods** 14 (5):483–86. https://doi.org/10.1038/nmeth.4236.

Reingold, E M, and J S Tilford. 1981. “Tidier Drawings of Trees.” **IEEE Transactions on Software Engineering** SE-7 (2):223–28. https://doi.org/10.1109/TSE.1981.234519.

Satija, Rahul, Jeffrey A Farrell, David Gennert, Alexander F Schier, and Aviv Regev. 2015. “Spatial reconstruction of single-cell gene expression data.” **Nature Biotechnology** 33 (5). Nature Publishing Group:495–502. https://doi.org/10.1038/nbt.3192.

Sugiyama, K, S Tagawa, and M Toda. 1981. “Methods for Visual Understanding of Hierarchical System Structures.” **IEEE Transactions on Systems, Man, and Cybernetics** 11 (2):109–25. https://doi.org/10.1109/TSMC.1981.4308636.

Zappia, Luke, and Alicia Oshlack. 2018. “Clustering trees: a visualization for evaluating clusterings at multiple resolutions.” **GigaScience** 7 (7). https://doi.org/10.1093/gigascience/giy083.