

Individual-microfilariae whole-genome-sequencing and sibling identification in *Onchocerca volvulus*: toward identification of treatment failure in clinical trials

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Introduction

- **Trial macrofilaricides** are in development for *Onchocerca volvulus*, the agent of river blindness
- **Adult female worms live in inaccessible nodules**: macrofilaricide efficacy is measured by surgery and histology
- A **diagnostic technique based on identifying parentage of microfilariae (Mf) from skin-snip biopsies** would remove the need for surgery
- We designed a method for estimating parentage and the number of breeders from Mf low-coverage whole-genome-sequencing data, and tested it on **11 Mf** of known parentage
- Further **optimization of laboratory protocols, increased sample size for validation, and information on *O. volvulus* spatial distribution** will enable the development of a viable method for use in clinical trials.

Methods - 1

1. Identifying siblings between pre-post Tx will identify Tx failure
2. Identifying individuals that share two parents infers full sibling (FS) relationships. The putative polyandry of *O. volvulus* raises the possibility of individuals sharing only one parent – Half siblings (HS)
3. Genotyping of maternally inherited mitochondria will allow inference of maternal HS relationships
4. We can use inferred FS and HS to infer the total number of parents contributing to a sample of Mf.
5. We can test this conceptual method on Mf of known parentage dissected from gravid adult female *O. volvulus*

Methods - 2

- **30 Mf from 3 gravid females from 2 patients** were dissected and whole-genome-sequenced
- Genotype likelihoods (GL) inferred from Mf with coverage **>3X**
- Filtered on depth, HWE, missingness
- Relatedness (R0, R1) calculated in **IBSRelate**
- Mitochondrial SNPs for median-spanning-network
- **For more detail please follow the link at the bottom**

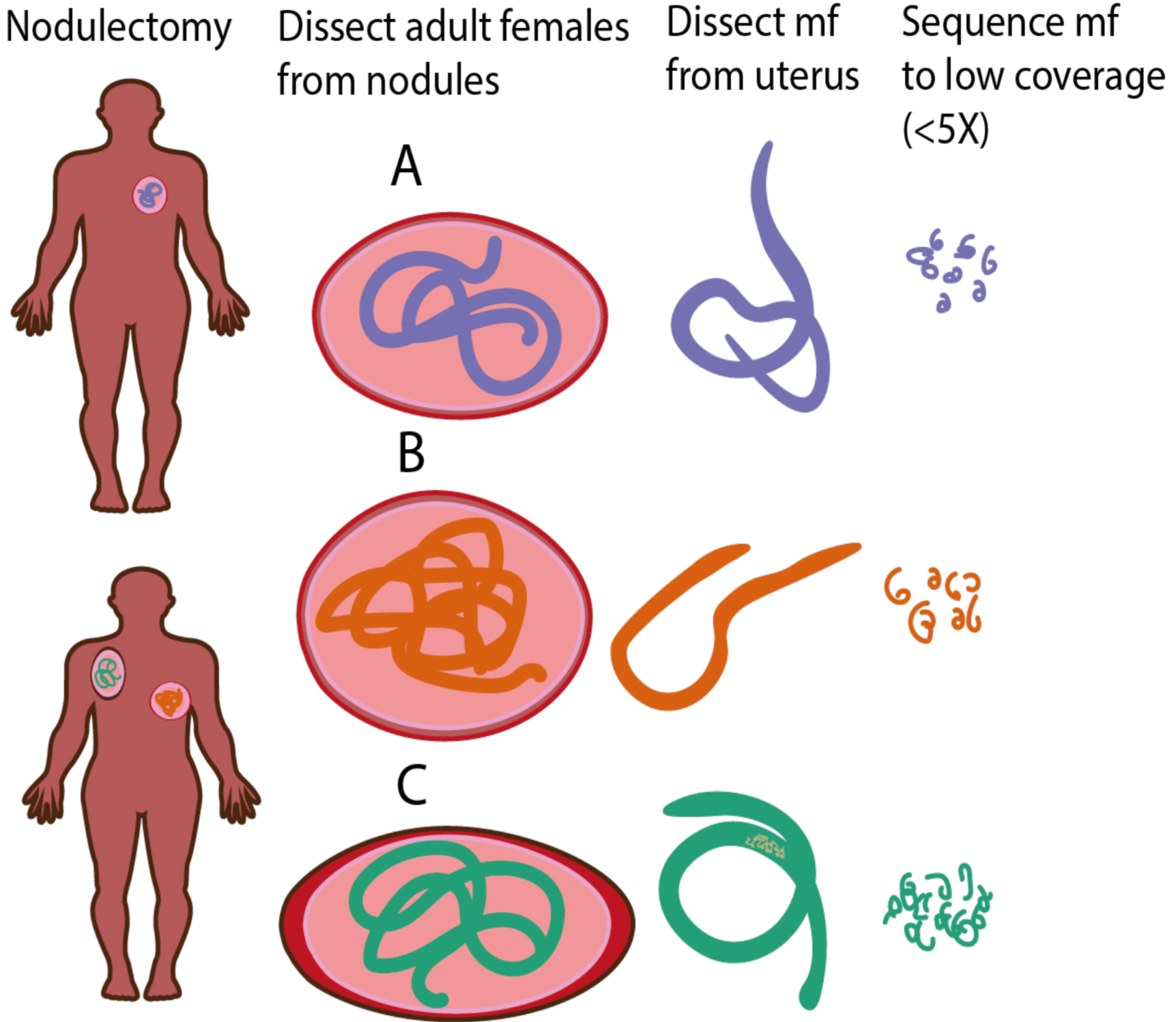


Figure 1: Experimental schema: 3 adult females from 2 patients had intrauterine Mf dissected and sequenced.

Results - 1

- 10 of 30 Mf had a coverage > 3X and were included
- Mitochondrial groups were defined by <3 SNPs between samples (**Fig 3**)
- FS defined by R0 < 0.5 and R1 > 0.5 (**Fig 2**)
- HS assignments noisy: not included (**Fig 2**)

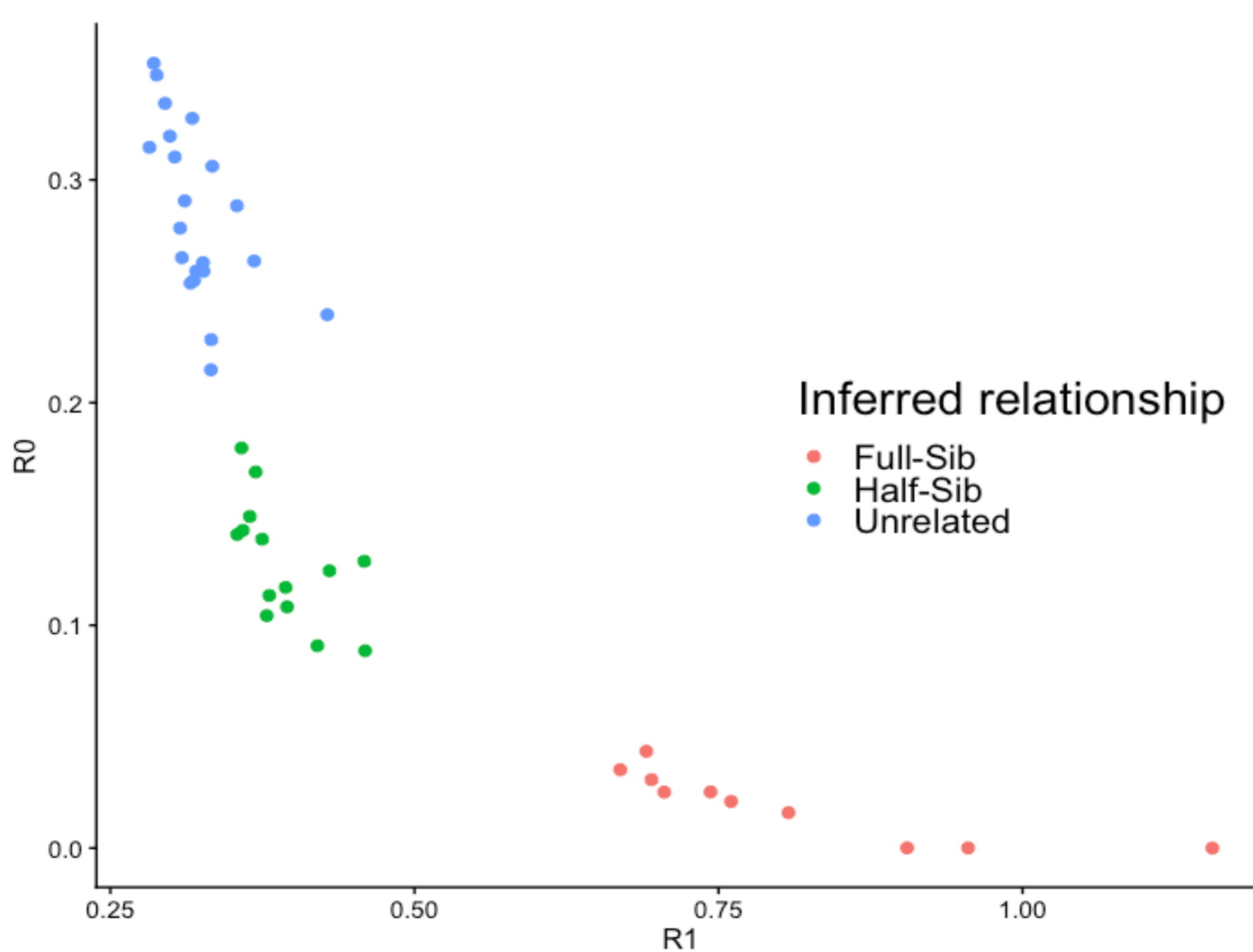


Figure 2: Plot of R0/R1 for pairs of intrauterine Mf. Point colour indicates inferred relationship based on thresholds in *Waples et al, 2013*.

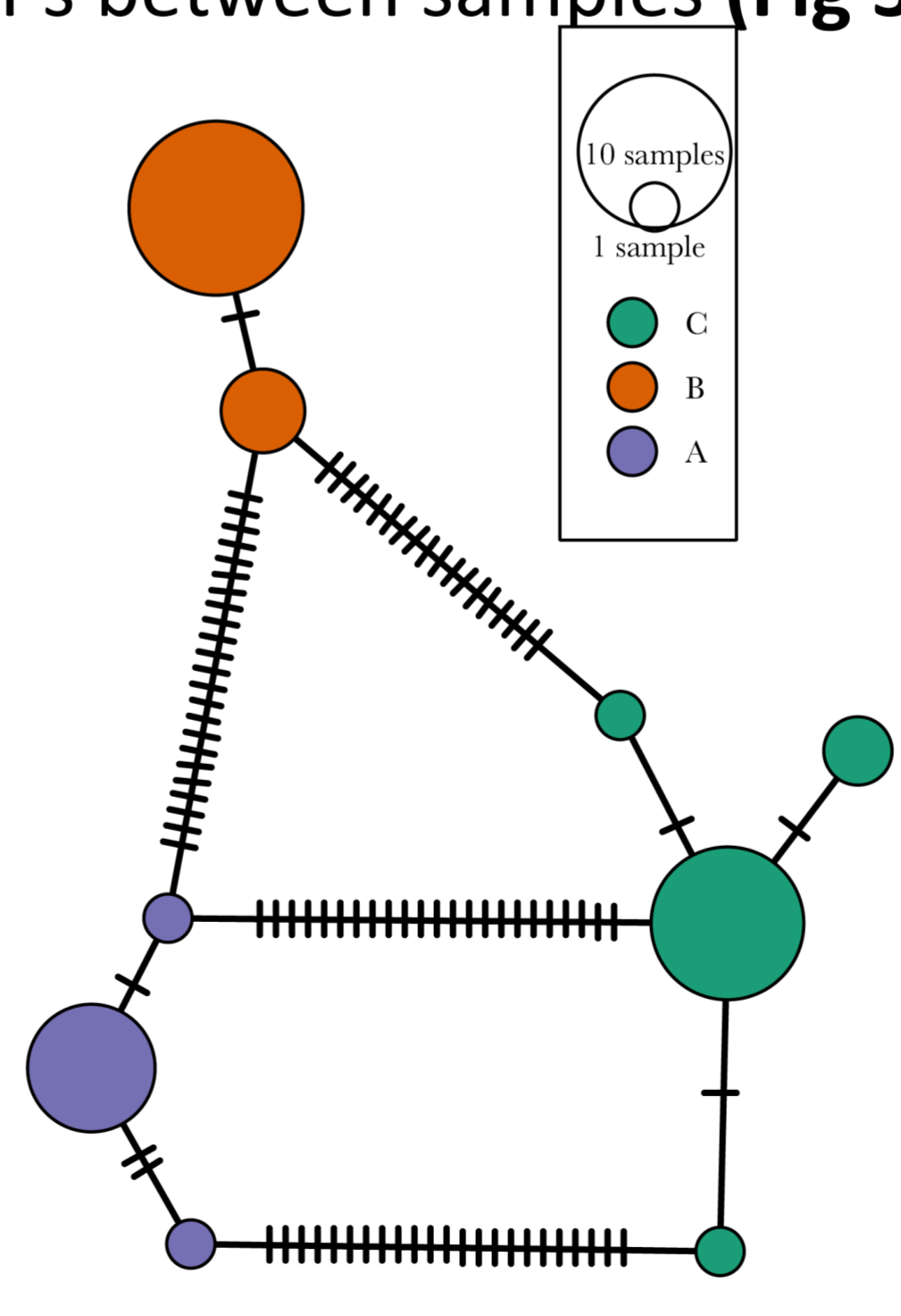


Figure 3: Median-spanning network of mitochondrial genomes from intrauterine Mf. Colour indicates female ID (see Fig 1), hatches indicate pairwise SNP distance.

Results - 2

- 4 FS groups (**Fig 4**)
- In **Figure 5**, FS groups connected by lines (a.k.a edges) are members of the same mitochondrial group (**Fig 2**)
- **2 nodes connected by a line represent 3 parents (distinct males, sharing a female) (Figure 5)**
- According to (**Fig 5**), the number of unique parents in a sample can be calculated by **2*no. Nodes – no. edges**
- **Therefore we identify 3 females and 4 males as parents to this sample of 10 Mf**

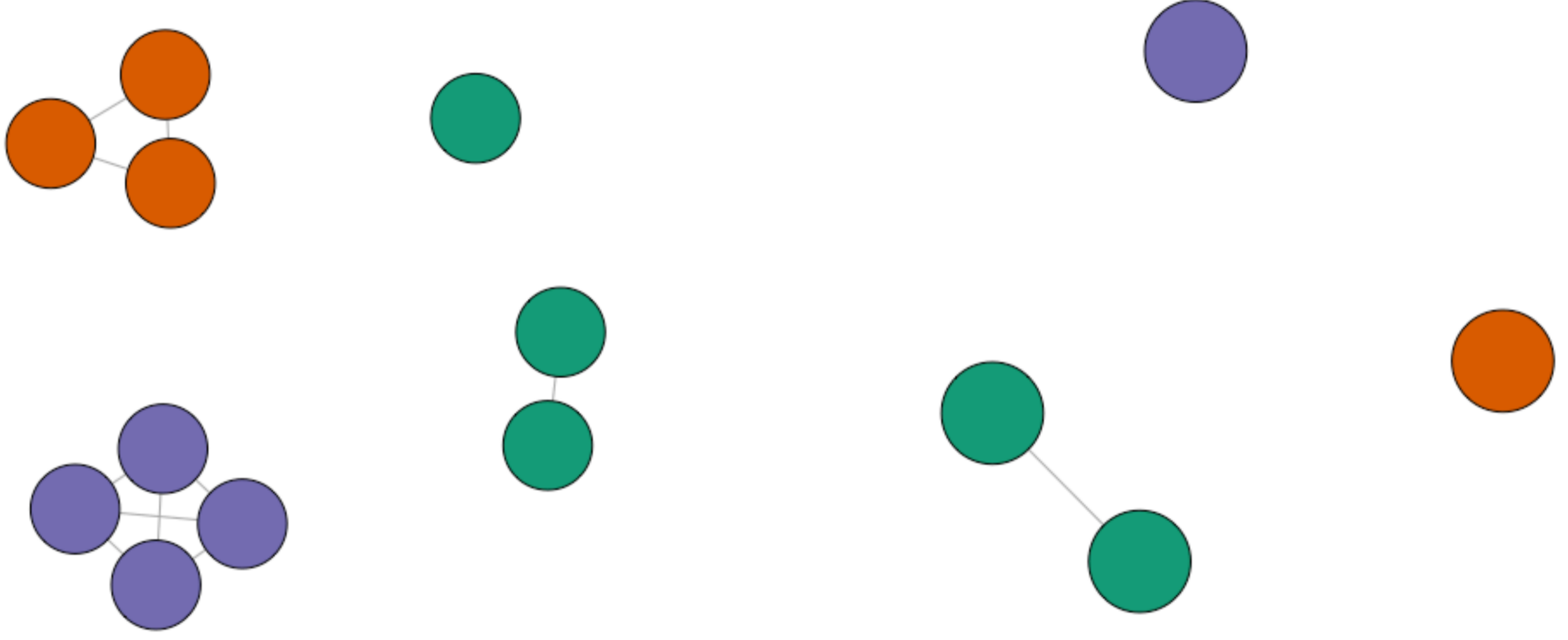


Figure 4: Relationships between intrauterine Mf (nodes). Node colour = female from which dissected (**Fig 1**). Edges indicate FS relationships. Figure 5: FS groups (nodes) with mitochondrial group sharing indicated by edges. Node colour = see **Fig 1**.

Conclusions

- With relatedness inference, the **Nb (male and female) can be calculated**
- Presence of **shared females (maternal HS or shared mitochondrial group) will identify treatment success or failure**
- Need more samples to:
 - Define uncertainty around relatedness inference
 - Define uncertainty around mitochondrial group designation
- Nb used in models (in development) incorporating sporadic fecundity and differing simulated adult worm burdens for **predicting likelihood of capturing total adult worm burden from a sample of Mf**.