④   By valid I suppose we mean unbiased.

① $\theta_{FL} = m_1$

$$E\theta_{FL} = \bar{E}m_1 = \frac{\theta}{1} = \theta \implies \text{unbiased}$$

② $\theta_{W_i} = \frac{1}{\sum\limits_{i=1}^{n-1}\frac{1}{i}}\sum\limits_{i=1}^{n-1}m_i$

$$E\theta_W = \frac{1}{\sum\frac{1}{i}}\cdot\sum\frac{\theta}{i} = \theta \implies \text{unbiased}$$

③ $\theta_L = \frac{1}{n-1}\sum\limits_{i=1}^{n-1}im_i$

$$E\theta_L = \frac{1}{n-1}\sum i\cdot\frac{\theta}{i} = \theta \implies \text{unbiased}$$

④ $\theta_\pi = \frac{2}{n(n-1)}\sum\limits_{i=1}^{n-1}i(n-i)m_i$

$$E\theta_\pi = \frac{2}{n(n-1)}\underbrace{\sum\limits_{i=1}^{n-1}i(n-i)\frac{\theta}{i}}_{\frac{n(n-1)}{2}} = \theta \implies \text{unbiased}$$

⑤ $\theta_H = \frac{2}{n(n-1)}\sum\limits_{i=1}^{n-1}i^2 m_i$

$$E\theta_H = \frac{2}{n(n-1)}\underbrace{\sum\limits_{i=1}^{n-1}i\cdot\frac{\theta}{i}}_{\binom{n}{2}} = \theta \implies \text{unbiased}$$

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⑤   I suppose that we have set of reads with (nearly) uniform coverage across genome and the same sequencing depth for all individuals. + ~~reads are the~~

We can build the "SNP matrix" but for reads. (sort of) ...
The Tajima estimate could be calculated between reads in such a way: $\theta_T = \frac{1}{\binom{n}{2}}\sum\limits_{ij}T_{ij}\frac{l_g}{l_r}$

where l_g is the len of genome
and l_r is the len   #reads   hamming distance between
of reads   reads

$\theta_\pi = \frac{2}{n(n-1)}\sum\limits_{i=1}^{n-1}i(n-i)m_i$ where n - # of reads/coverage depth per individual
and m_i - #mutations in ith indiv / coverage depth period