

# Single-cell RNA-sequencing: variance stabilizing transformations

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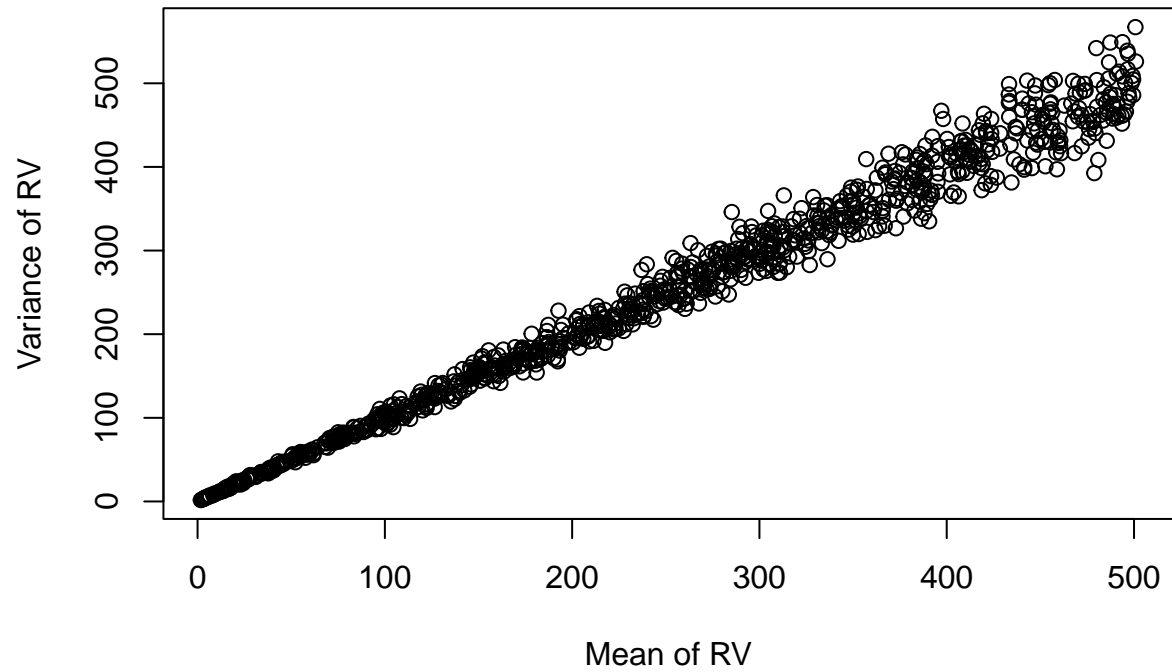
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## 1 Approximating the variance stabilizing transformation of a Poisson random variable

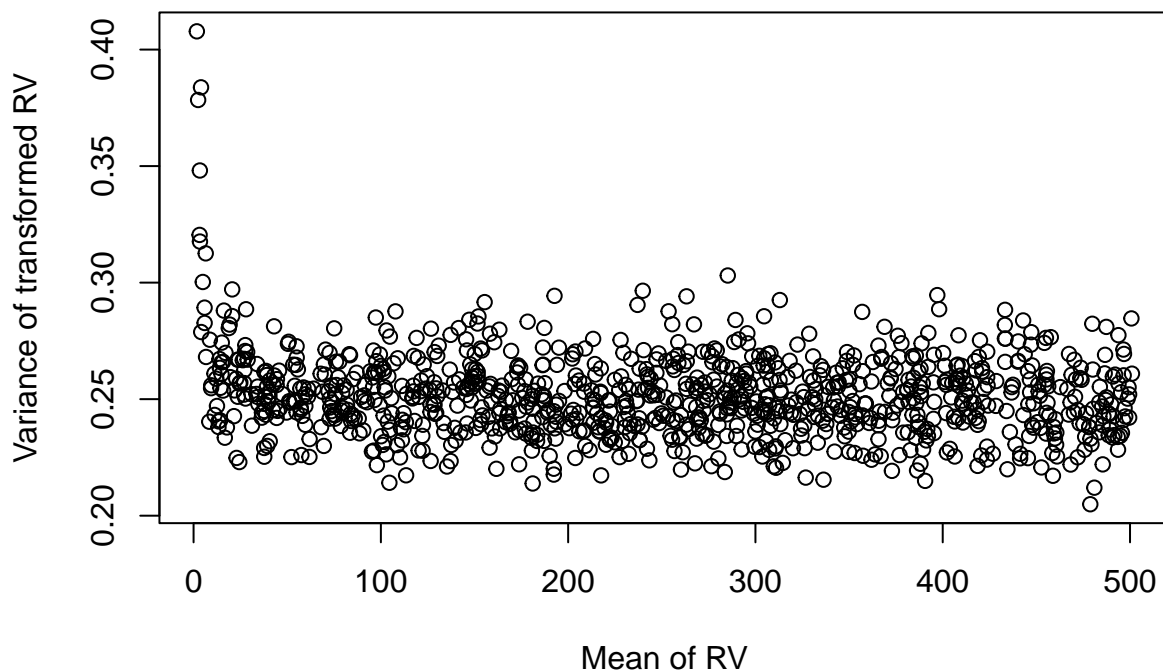
- A random variable  $Y \sim Poi(\mu)$  has  $Var(Y) = E(Y) = \mu$ .
- We are looking for a variance stabilizing transformation (VST)  $f(Y)$  such that  $Var(f(Y)) = c$ , with  $c$  any constant. In particular, we need  $Var(f(Y))$  to be independent of  $\mu$ .
- A first-order Taylor series gives us  $f(Y) \approx f(\mu) + (Y - \mu)f'(\mu)$ .
- Rearranging gives us  $\{f(Y) - f(\mu)\}^2 = (Y - \mu)^2 f'(\mu)^2$ .
- Which may be written as  $Var(f(Y)) = Var(Y)f'(\mu)^2$ .
- This shows us that **we want a transformation such that  $f'(\mu)^2 = 1/\mu$**  because then  $\forall \mu : Var(f(Y)) = 1$ ! Let's find out.
- The last bullet point can be written as  $f'(\mu) = \frac{1}{\sqrt{\mu}}$  and therefore  $f(\mu) = \int \frac{1}{\sqrt{\mu}} d\mu = 2\mu^{1/2}$ .
- Finally, this shows us that the transformation  $f(Y) = 2Y^{1/2}$  ensures  $Var(f(Y)) = 1$ . Similar, as is often written in the scientific literature, the transformation  $f(Y) = Y^{1/2}$  ensures  $Var(f(Y)) = 1/4$ .
- Note that these derivations all **rely on the first-order Taylor expansion to be a good approximation**. The VST will therefore work better for random variables with a high mean  $\mu$  as the distribution will be more discrete for random variables with a low mean. We show this using simulation below.

```
set.seed(871)
N <- 1e3
df <- data.frame(mean=rep(NA, N),
                  var=rep(NA, N),
                  transVar=rep(NA, N))
for(kk in 1:N){
  sampledMu <- runif(n=1, min=1, max=500)
  y <- rpois(n=500, lambda=sampledMu)
  df[kk,] <- c(mean(y), var(y), var(sqrt(y)))
}
```

```
plot(x=df$mean, y=df$var,  
     xlab = "Mean of RV", ylab="Variance of RV")
```



```
plot(x=df$mean, y=df$transVar,  
     xlab = "Mean of RV", ylab="Variance of transformed RV")
```



**Question.** Why is the first plot heteroscedastic?

Answer.

Remember that  $Var(\hat{\mu}) = \frac{\hat{\mu}}{n}$ . Since for all random variables in our simulation  $n$  is equal, in our case we have that  $Var(\hat{\mu}) = c\hat{\mu}$ . The variance on estimating the mean thus increases with the mean.

## 2 VST for scRNA-seq data based on a negative binomial distribution

### 2.1 Pearson residuals

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