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Estimating infectivity rates and attack windows for two viruses

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ABSTRACT

Cells exist in an environment in which they are simultaneously exposed to a number of viral challenges. In some cases, infection by one virus may preclude infection by other viruses. Under the assumption of independent times until infection by two viruses, a procedure is presented to estimate the infectivity rates along with the time window during which a cell might be susceptible to infection by multiple viruses. A test for equal infectivity rates is proposed and interval estimates of parameters are derived. Additional hypothesis tests of potential interest are also presented. The operating characteristics of these tests and the estimation procedure are explored in simulation studies.

1. Introduction

Normal cells exist in an environment that contains a number of potential viruses. Cells can be infected by multiple viruses, but the interaction of viruses within a cell is difficult to study. These different viruses can infect cells at different rates, reflecting differing virulence. Analyzing multiple infection of cells by different viruses has been an interesting scientific problem for a long time. In [3], an early discussion of the probability of multiple virus infection is introduced and the Poisson distribution is used to model multiple infection. The Poisson distribution has been widely used to model the number of organisms (or cells) killed (or infected) by viruses. The "one-hit" model introduced in [2,4], and the "multiple-hit" cases discussed in both [1,3] have been used to discuss the probability of infection given contact with a virus or multiple viruses.

Although the condition of observing coexistence of competing infectious strains has been discussed in the context of immunity [9], little has been done in modeling the infection of multiple viruses at the microscopic level. To date, it has not been clear how to compare the infectivity rates of multiple viruses when the cells are infected by more than one virus.

To study the virus–virus and virus–cell interaction in this situation, [5] designed a dual-color tag system to investigate the interaction of two viruses. A *DsRed2* gene encoding for the red fluorescent protein (RFP) expression cassette was inserted into a wide host-range *Autographa californica* nucleopolyhedrovirus (AcMNPV) at the polyhedrin locus (vAcDsRed2). An enhanced green fluorescent protein (EGFP) gene expression cassette was inserted at the *gp*37 locus and the *p*10 locus of the narrow host-range *Thysanoplusia orichalcea* NPV (ThorMNPV) and *Spodoptera exigua* NPV (SeMNPV) to produce vTHGFP and vSeGFP, respectively. In this experiment, a certain amount of Sf21 and Hi5 cells were exposed to two types of viruses, namely, the AcRed (vAcDsRed2) and AcGFP (vTHGFP or vSeGFP) simultaneously. The study was terminated after a specific duration of the exposure, e.g. two hours. When AcRed infected a cell, the cell displayed a red color. In contrast, when AcGFP infected a cell, the cell presented green color characteristics. Usually, the infection by one virus precludes the infection by the other virus. However, if both AcRed and AcGFP virus arrive at a cell within a very short time window, then it is possible that the cell could be infected by both. When both the AcRed and AcGFP viruses infected a cell, it displayed yellow. The cell did not display any color in fluorescence microscopy (i.e., it was "blank") if it had not yet been infected by either virus by the end of the experiment. Data were collected after a specified exposure duration by taking a photograph of a collection of cells and counting the numbers of cells that displayed red, green, yellow or were blank.

The methods in the present paper were developed in response to questions raised by Professor X. Cheng (private communication) regarding the new test system described above. Our goal is to lay the groundwork for estimating the viral infectious rates along with the time window in which a cell might be infected by two viruses. We also test if the infection rate of the AcRed virus is the same as that of the AcGFP virus. Because data for such analyses are not yet available, we use simulated data to investigate the proposed analytic framework. The rest of the paper is organized as follows: in Section 2, we present a framework to model the simultaneous attack of two viruses on a cell. The virus infectivity rates and the time window which allows multiple infection of a cell are estimated and hypothesis tests concerning these parameters are also discussed.

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In Section 3, a simulation study is presented with discussion. Section 4 concludes the study and discusses some possible future work.

2. Modeling of the simultaneous attack of two viruses on a cell

2.1. Assumptions

Typically the number of viruses is orders of magnitude larger than the number of cells, hence the number of viruses affects the infection rate. As shown in [10] and illustrated in [6], the exponential distribution describes the waiting time between events in a Poisson process in which events occur continuously and independently at a constant average rate. Therefore, in this particular study, the time until a viral infection occurs is modeled using an exponential distribution for both AcRed and AcGFP viruses, assuming the virus-specific infectivity rates to be constant, i.e., $T_i \sim \exp(\lambda_i),\ i = R, G$, where T_R represents the infectivity time for an AcRed virus, and T_G is the infectivity time for an AcGFP virus. Then the probability density functions for T_R and T_G are

$$f(t_i) = \lambda_i e^{-\lambda_i t_i}, \tag{1}$$

where $\lambda_i > 0$ and $i = R, G$. Here we build the model by assuming that the investigator decides to terminate the study after a certain time period of exposure, denoted by τ. Also we assume the numbers of red, green, yellow and non-infected cells are recorded and known. Finally, we assume the existence of a time window Δ when simultaneous infection by both viruses is possible if $|T_R - T_G| \leqslant \Delta$.

2.2. Model

The results of the experiment are classified into four categories: "red," "green," "yellow," and "blank." If we assume that the two infectivity times T_R and T_G are independent, then the joint density function of T_R and T_G is

$$f(t_R, t_G) = f(t_R) f(t_G) = \lambda_R \lambda_G e^{-\lambda_R t_R} e^{-\lambda_G t_G}. \tag{2}$$

The probability of the four different infectivity outcomes for each cell can be derived using the joint density function. Fig. A.1 illustrates the case in which exposure is terminated at $\tau = 2$ hours. For example, the red region represents the experimental result that a particular cell displays a red color, corresponding to the case in which (1) AcRed infects the cell before the termination of the study ($T_R < \tau$ hours) and (2) the time T_G until infection by AcGFP either exceeds the lesser of $T_R + \Delta$ (the end of the time interval during which a cell is susceptible to infection by two viruses) and τ. Therefore, in the general case with termination time τ, the probability π_R that a cell displays red is given by

$$\pi_R = \Pr[(T_R < \tau) \cap (T_G < \tau) \cap (T_R < T_G - \Delta)] + \Pr[(T_R < \tau) \cap (T_G > \tau)] \tag{3}$$

$$= \frac{\lambda_G}{\lambda_R + \lambda_G} e^{\lambda_R \Delta} e^{-\tau(\lambda_R + \lambda_G)} + \frac{\lambda_R}{\lambda_R + \lambda_G} e^{-\lambda_G \Delta} - e^{-\tau(\lambda_R + \lambda_G)}. \tag{4}$$

Similarly, the probabilities that a cell displays a green color, yellow color and does not display any color (blank, not infected by either virus) are

$$\pi_G = \frac{\lambda_R}{\lambda_R + \lambda_G} e^{\lambda_G \Delta} e^{-\tau(\lambda_R + \lambda_G)} + \frac{\lambda_G}{\lambda_R + \lambda_G} e^{-\lambda_R \Delta} - e^{-\tau(\lambda_R + \lambda_G)}, \tag{5}$$

$$\pi_Y = 1 + e^{-\tau(\lambda_R + \lambda_G)} - \frac{\lambda_R}{\lambda_R + \lambda_G}\left[e^{-\lambda_G \Delta} + e^{\lambda_G \Delta} e^{-\tau(\lambda_G + \lambda_R)}\right] - \frac{\lambda_G}{\lambda_R + \lambda_G}\left[e^{-\lambda_R \Delta} + e^{\lambda_R \Delta} e^{-\tau(\lambda_R + \lambda_G)}\right], \tag{6}$$

$$\text{and} \quad \pi_B = 1 - \pi_R - \pi_G - \pi_Y = e^{-\tau(\lambda_R + \lambda_G)}. \tag{7}$$

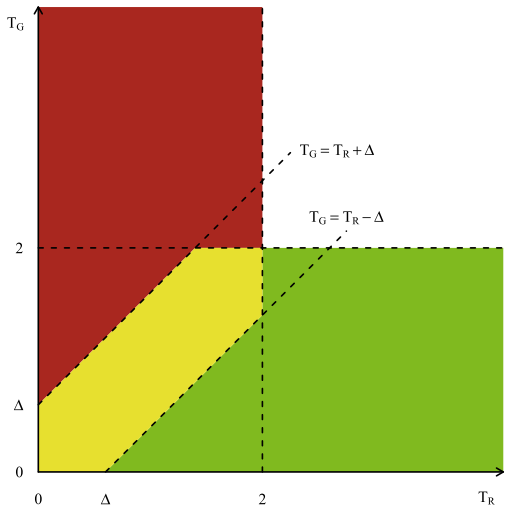

Fig. A.1. Illustration of a cell's possible infectivity outcomes based on attack times by two viruses. The color of the region (red, green, or yellow) indicates the cell's display color under fluorescence microscopy. Here, T_R is the time of attack by the AcRed virus and T_G is the time of attack by the AcGFP virus. Δ is the length of the window in which infection by both viruses is possible. Finally, $\tau = 2$ h is the total experimental time. If no color is present in the graph, i.e., $T_R > 2$ and $T_G > 2$, then the cell is classified as blank.

Detailed derivations of these probabilities are provided in Appendix A.

Since the probability for each outcome to be observed is known, the numbers of red, green, yellow and non-infected cells are naturally modeled using a multinomial distribution with probabilities π_R, π_G, π_Y and π_B. The multinomial likelihood is defined by

$$L(\lambda_R, \lambda_G, \Delta; n, n_R, n_G, n_Y, n_B) = \binom{n}{n_R \quad n_G \quad n_Y \quad n_B} (\pi_R)^{n_R} (\pi_G)^{n_G} (\pi_Y)^{n_Y} (\pi_B)^{n_B}, \tag{8}$$

where $n_B = n - n_R - n_G - n_Y$, n is the total number of cells at risk at the beginning of the study, and n_R, n_G, n_Y are the number of cells displaying red, green and yellow respectively at the end of study.

2.3. Inference

The main objective is to estimate the viral infectious rates (λ_R and λ_G) along with the time window (Δ). Since the likelihood of the observed data (n, n_R, n_G, n_Y, n_B) has been formulated in the previous section as a function of the parameters of interest $\boldsymbol{\theta} = (\lambda_R, \lambda_G, \Delta)$, computing the maximum likelihood estimator (MLE) for $\boldsymbol{\theta}$ is a natural solution. The first partial derivatives and second partial derivatives with respect to the parameters of interest are derived in order to achieve the optimization of the likelihood using Newton's method.

A test of equal infectivity rates might be of interest, i.e., we may wish to test if the infectious rate of the AcRed virus is same with that of the AcGFP virus: $H_0 : \lambda_R = \lambda_G = \lambda$. A likelihood ratio test can be constructed using a test statistic

$$G_1^2 = -2 \log\left[\frac{L_{01}(\hat{\lambda}, \hat{\Delta}_{01})}{L_1(\hat{\lambda}_R, \hat{\lambda}_G, \hat{\Delta}_1)}\right], \tag{9}$$

where L_{01} is the likelihood evaluated when the two viruses are assumed to have the same infectivity rates and $\hat{\boldsymbol{\theta}}^* = (\hat{\lambda}, \hat{\Delta}_{01})$ is the MLE under this assumption; L_1 is the likelihood evaluated when the two viruses may have different infectivity rates and $\hat{\boldsymbol{\theta}} = (\hat{\lambda}_R, \hat{\lambda}_G, \hat{\Delta}_1)$ is the MLE. The hypothesis of equal infectivity is