CWD Project Annual Report
2011-2012

Activities and Findings

Mark-recapture Estimation of Parameters in Disease Models

Estimating disease parameters in a mark-recapture study is inherently complex, due to missing data, a non-linear transmission function, and stochasticity of recapture probabilities, among other issues. To estimate the most basic parameters such as prevalence and transmission probabilities, which vary between capture periods and between deer, we developed a general, Bayesian, state-space approach. The model is hierarchical, the highest level comprised of parameter distributions, the next level representing the true (but unobserved) transmission model, and the final level modeling the observation mechanism. Extensive simulations have shown the approach to be flexible and robust under a wide variety of study types and underlying disease transmission models.

The most common mathematical descriptors of disease, the continuous time transmission rate and basic reproductive ratio (R_0), are typically estimable only in linear systems. We extended the use of our state-space model to solve this problem by first estimating the effective reproductive ratio and then using estimates of current transmission probabilities, prevalence, and assumed transmission model to recover the basic reproductive ratio. Again using simulation studies, we showed that we can recover conditions at disease introduction into the population (the conditions under which R_0 is defined) using only current parameter estimates, despite the non-linear transmission model (see Figure 1).

Bayesian estimation methods allow us to use existing information about disease parameters to inform prior distributions and incorporate covariate data collected from the deer. They also provide automatic credible intervals and approximate distributions for all estimates, including basic reproductive ratio.

The state-space formulation is general enough to be applied in many mark-recapture studies, not only for disease parameter estimation. And for disease estimation, the method requires only the correct establishment of transition and observation matrices. It can therefore be applied to frequency dependent or density dependent transmission, but also to any other reasonable (and perhaps more realistic) disease transmission dependencies. Model selection in the Bayesian context can also test and compare different possible disease dynamic model to determine which is most supported by the data.

Mark-recapture Model of Chronic Wasting Disease in Free-Ranging Mule Deer

The National Science Foundation (EF-0914489) is sponsoring research to improve our basic understanding of dynamics of free-ranging mule deer populations infected with chronic wasting disease (CWD). Our goal is to unify long-term population monitoring information (e.g. counts, hunter harvest) collected by the Colorado Division of Fish, Wildlife, and Parks with individual-level information on incidence, reproduction, and survival to determine effects of CWD on mule deer. We are attempting to learn about the transmission process and provide an estimate of the basic reproductive rate of the pathogen. Our research will help advise decision-makers in

managing infected mule deer populations and provide the scientific community with a case example for studying disease and population dynamics in wild populations.

We began an intensive mark-recapture study of free-ranging mule deer during January 2010. Deer were caught by helicopter net gunning and transferred to nearby processing locations. Animals were fit with a mortality sensing telemetry collar (Advanced Telemetry Systems, Isanti, MN) and age was determined. We collected blood sera and plasma for genetic characterization of the $PrnP^{CWD}$ gene and rectal mucosa associated lymphoid tissue (RMALT) to determine CWD infection status using IMH staining. Surviving deer were recaptured during subsequent winters to determine changes in CWD status (Table 1). All animals were handled in accordance with IACUC (11-2758A).

Field observers regularly monitored the survival status of deer and mortalities were investigated to determine the cause of death. We recovered 62 carcasses of previously tested deer and obtained diagnostic samples of retropharyngeal lymph node, tonsil, obex, or spinal column tissue from 20 of these mortalities. Postmortem samples recovered from two carcasses tested positive for CWD with both of these deer also testing positive on previous RMALT tests. The remaining 18 deer that were CWD negative during postmortem testing were also CWD negative during antemortem sampling.

We are the first to use hierarchical Bayesian mark-recapture models to estimate the probability of infection of female mule deer affected by CWD (Table 2). Our initial estimate of the posterior median and 95% credible interval of annual infection probability is 0.04 (0.02, 0.08; Figure 2). Recovering this parameter is an important first step in understanding the underlying transmission process.

In our hierarchical model formulation we differentiated between the true infection status (z_i) of each deer and our observation of infection status (y_i). We had to make this differentiation because all deer were not captured each year and some disease tests on individuals were inconclusive. Test results from 90 animals handled during February 2012 were also pending. We estimated annual CWD prevalence as a derived quantity in our Markov Chain Monte Carlo algorithm. This quantity was evaluated at each MCMC iteration and represented the quotient of the true number of live CWD infected deer and total surviving deer during January of each year. Therefore the derived value was discrete. CWD prevalence appears to be increasing in our sample of female deer (Figure 3).

Sensitivity Analysis of R_0

SENSAI, the software package that automatically performs sensitivity and elasticity analysis for high-dimensional models, is now equipped with the capabilities of calculating the Next-Generation construction of R_0, if it is valid for the model. The equations are automatically tested to see if the definition of R_0 is a legitimate threshold index. The user is warned if a condition of the theorem on R_0 is not met.

Furthermore, the sensitivities and elasticities of R_0 to parameters, initial conditions, and a user defined parameter are automatically computed within SENSAI.

Consider the following SIR model with logistic growth for a demonstration. (See equations 1 and 2).

The plots of the elasticities of R_0 and the *QoI* are below (Figure 4 and 5). Elasticities are scaled versions of sensitivities.

The sensitivity and elasticity analysis of R_0 and any relevant quantities of interest *QoI* is fast and easy to implement in SENSAI.

The Next-Generation R_0 is probably the most widely used construction of R_0. There are, however, some models that do not have a valid Next Generation R_0 construction. For such models, other quantities of interest may be substituted to analyze the model sensitivity, such as the proportion of infected individuals.

Genetic Measurements

We are examining the role of genetics in creating heterogeneity in disease transmission by incorporating genotypic information as covariates in the analysis. First, we have determined tentative Prnp 225 genotypes of all captured deer using a simple restriction fragment analysis (Jewell et al., 2005). We have also begun sequencing the open reading frame of Prnp for all deer. This allows for confirmation of the S225F genotype and also provides data on the other known amino acid replacement in mule deer (D20G) as well as synonymous substitutions (393Y and 741Y) (Figure 6).

Genotypic information will be used in population genetic analyses and as covariates in the analysis of transmission heterogeneity. Genetic analyses will have high statistical power because hundreds of deer will be sampled. DNA has been extracted from blood using standard protocols; PCR protocols and scoring of alleles have been standardized (Jewell et al., 2005) and are running well in our hands.

Next, all deer will be genotyped for a series of nuclear microsatellite markers and mitochondrial DNA control region (mtDNA), to: 1) calculate relatedness among deer; and 2) assess movement of males relative to females. Microsatellite markers will be used to estimate relatedness of individuals within maternal groups. Tetranucleotide (CATC, TAGA) microsatellites, developed by the California Department of Fish and Game (GenBank AF102240–AF102260) are sufficiently diverse to allow accurate estimation of relatedness (Jones et al., 2000, 2002; Latch et al., 2008; Meredith et al., 2005). Each deer will be genotyped for at least ten markers, which will provide adequate power for both estimating relatedness between deer and determining population assignment. The ten markers should yield >99% probability of identity, calculated as the probability of (not) sampling the same multilocus genotypes within maternal groups, given the total probability of sampling any possible genotype twice (Hedrick, 2011). Because of maternal inheritance, mtDNA analysis will allow us to further distinguish between gene flow by male dispersal (deduced from microsatellite markers), and genetic structure and stability of local female groups (where microsatellites show no differentiation, but mtDNA does). The mtDNA control region is highly variable in cervid populations (Polziehn and Strobeck, 1998).

Molecular evolution of prions and the prion precursor gene, Prnp

Transmissible spongiform encephalopathies involve the fatal accumulation of misfolded proteins called prions (PrPD), which occur spontaneously in both humans and artiodactyls. Comparative analyses indicate that variation in the single-copy prion precursor gene (Prnp) is the result of purifying, balancing and/or positive selection. Thus, while PrP is constitutively expressed and prions occur in all vertebrates, it is unclear how evolutionary forces influence variation in Prnp and prions themselves. It is clear, however, that non-synonymous substitutions in the coding sequence of Prnp result in single amino-acid substitutions that alter the disease process, but that variation is also constrained by the native function of the cellular isoform (PrPC). What signatures of selection do these contradictory evolutionary forces leave on the Prnp gene? We have compiled available genetic data from a wide range of species and quantitatively analyzed dN:dS ratio within the entire coding sequence and across domains, as well as tested for positive selection at codon sites. We also performed a thorough literature review in an attempt to quantify variation within populations.

Education and Outreach

Our CWD study involves a rich collaboration among researchers, teachers and students at both the high school and college level. High school students participate in simulations of field work and laboratory tests during a "CWDeers Day" symposium at Colorado State University. We held two CWDeers Days last year – in October 2011 and March 2012. Students find deer collars hidden on campus using radio telemetry equipment, use micropipettors to load DNA samples for gel electrophoresis, and visit a wildlife research facility to understand CWD research projects in captive mule deer and mountain lions. In these and other activities, students enjoyed small group discussions with ecologists, veterinarians, geneticists, statisticians and college students who lead the breakout sessions.

High school teachers participate in research discussions at monthly campus meetings and also participate in the field research. Teachers enjoy helping with data collection and support duties once the helicopter pilot arrives with a deer or two. Teachers also travel with field technicians to track deer mortality signals, as well as live animals. Being imbedded in the CWD project in so many authentic ways helps teachers understand the science in the investigation and invigorates them to share cutting edge research and knowledge with their high school students.

Project team members also held two public information meetings for the community who lives in the research area over the past year – one in November 2011 and one in May 2012. We also meet with our landowner advisory committee every quarter.

References

Hedrick, P., 2011. Genetics of populations. Jones & Bartlett Learning.

Jewell, J., M. Conner, L. Wolfe, M. Miller, and E. Williams, 2005. Low frequency of prp genotype 225sf among free-ranging mule deer (odocoileus hemionus) with chronic wasting disease. JOURNAL OF GENERAL VIROLOGY 86:2127–2134.

Jones, K., K. Levine, and J. Banks, 2000. Dna-based genetic markers in blacktailed and mule deer for forensic applications. CALIFORNIA FISH AND GAME 86:115–126.

Jones, K., K. Levine, and J. Banks, 2002. Characterization of 11 polymorphic tetranucleotide microsatellites for forensic applications in California elk (cervus elaphus canadensis). MOLECULAR ECOLOGY NOTES 2:425–427.

Latch, E. K., R. P. Amann, J. P. Jacobson, and O. E. Rhodes, Jr., 2008. Competing hypotheses for the etiology of cryptorchidism in sitka black-tailed deer: an evaluation of evolutionary alternatives. ANIMAL CONSERVATION 11:234–246.

Meredith, E., J. Rodzen, K. Levine, and J. Banks, 2005. Characterization of an additional 14 microsatellite loci in California elk (cervus elaphus) for use in forensic and population applications. CONSERVATION GENETICS 6:151–153.

Polziehn, R. and C. Strobeck, 1998. Phylogeny of wapiti, red deer, sika deer, and other North American cervids as determined from mitochondrial dna. MOLECULAR PHYLOGENETICS AND EVOLUTION 10:249–258.

Tables

Table 1: Total numbers of free-ranging mule deer captured by helicopter net gunning and transferred to nearby processing locations to determine CWD infection status. Numbers in parentheses indicate deer handled during previous years.

Year	*Fawn*	*Female (>1.5)*	*Male (>1.5)*
2010	15	125	0
2011	5	165 (89)	10
2012	7	118 (118)	17
Total	27	408 (207)	27

Table 2: Marginal posterior distributions of parameters of a hierarchical Bayesian mark-recapture model for free-ranging female mule deer affected by CWD.

Parameter	*Mean*	*SD*	*Median*	*95% credible interval*	
infection	0.04	0.02	0.04	0.02	0.08
survival (susceptible deer)	0.80	0.02	0.80	0.75	0.85
survival (infected deer)	0.65	0.12	0.65	0.39	0.86
postmortem sampling	0.73	0.02	0.73	0.69	0.77
antemortem sampling	0.33	0.06	0.33	0.22	0.45

Figures

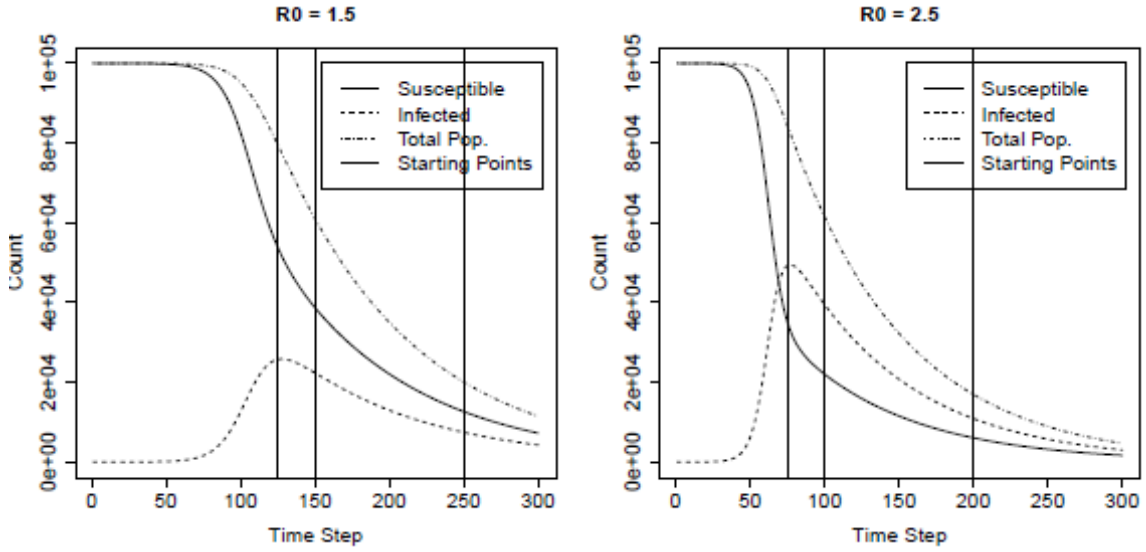

Figure 1: A basic *SI* model simulated for two values of R_0. Mark-recapture samples were simulated at different time steps (the vertical lines) to ensure effectiveness of the method under varying scenarios.

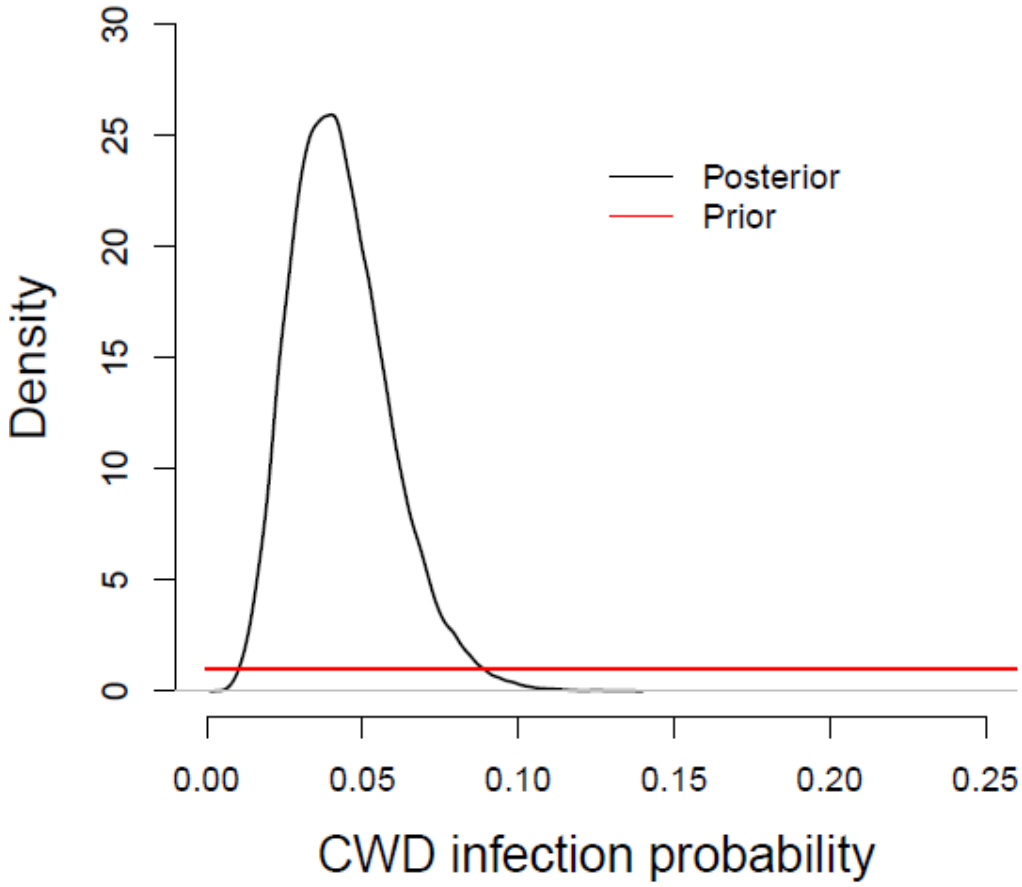

Figure 2: Posterior distribution of the annual probability of infection of adult female mule deer during January, 2010-12.

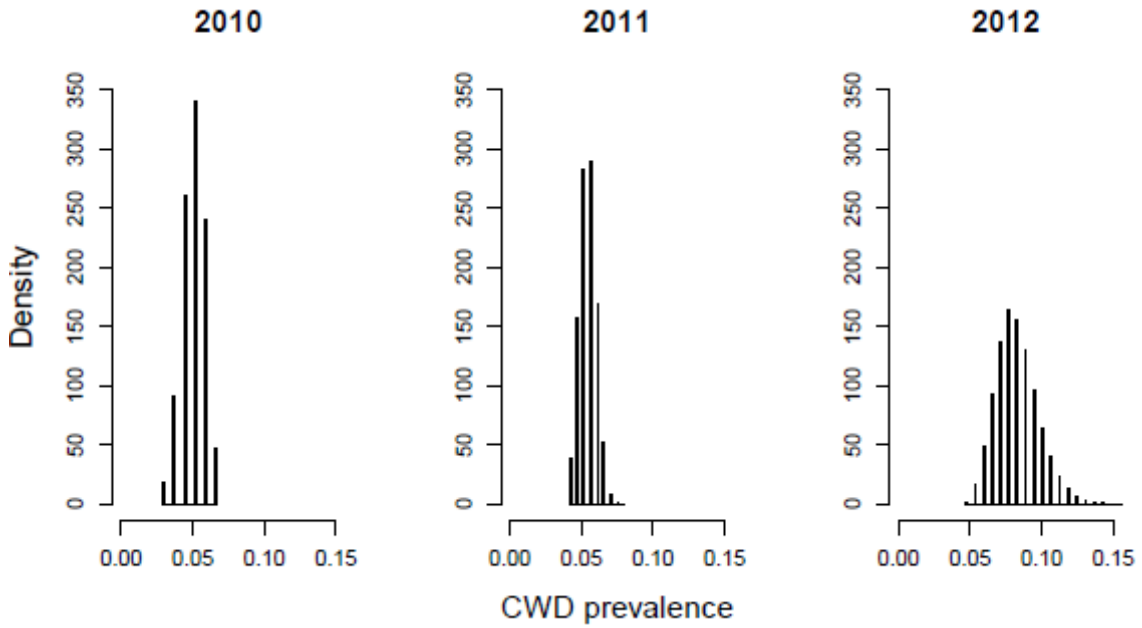

Figure 3: Estimates of CWD prevalence among adult female (>1.5) mule deer during January, 2010-2012 from our MCMC algorithm. Prevalence is discrete since it depends on the number of live CWD infected deer.

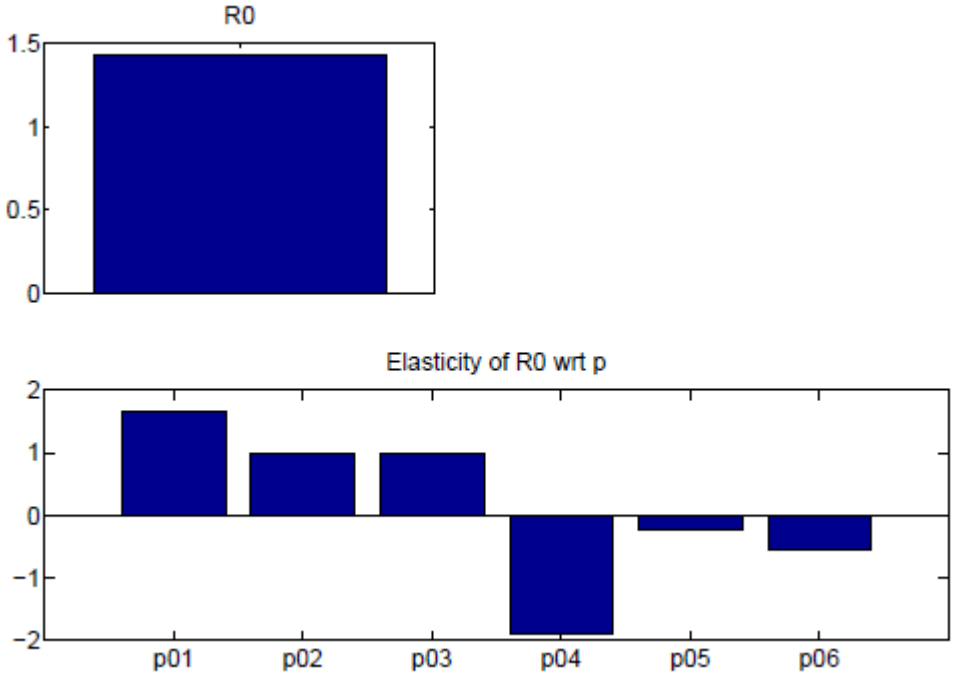

Figure 4: R_0 and elasticities to the 6 parameters from the SIR model.

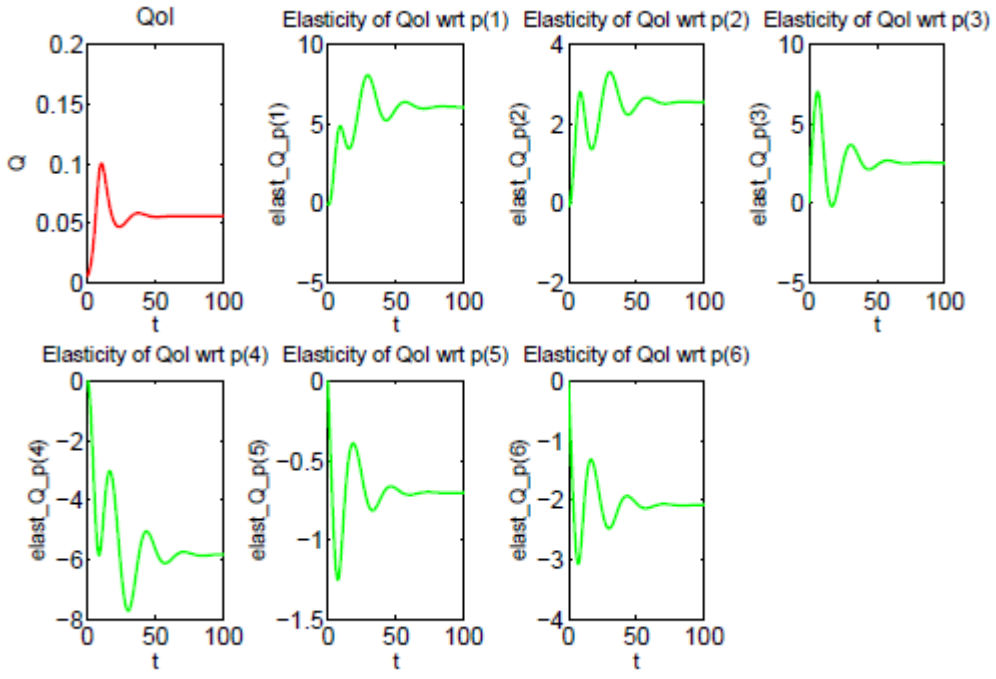

Figure 5: *QoI* (prevalence) and its elasticities to parameters.

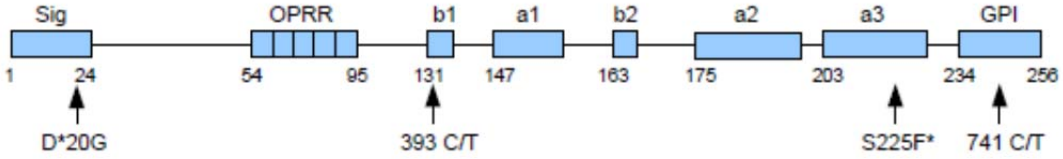

Figure 6: The mule deer prion precursor gene, *Prnp*. Blue boxes represent protein domains from an alignment with structure work on the murine protein (Riek 1997). From N- to C-terminal these include the signal sequence, octapeptide repeat region, beta-pleated sheet 1, alpha helix 1, beta-pleated sheet 2, alpha helix 2 and 3 and the glycophosphoinositol anchor. Arrows show all polymorphisms within our study population. Amino acid substitutions are listed AA codon# AA and synonymous substitutions are listed bp# nt/nt. Amino acids previously reported associated with disease resistance are marked with *.

Equations

$$\frac{dS}{dt} = rN\left(1 - \frac{N}{K}\right) - \beta SI - \delta S,$$
$$\frac{dI}{dt} = \beta SI - \gamma I - \mu I - \delta I,$$
$$\frac{dR}{dt} = \gamma I - \delta R,$$

Eq. 1

where $N = S + I + R$ at any time t, r is the per capita growth rate, K is the carrying capacity, β is the infection rate, δ is the natural death rate, γ is the recovery rate, and μ is the disease-specific

death rate due to the infection. The Next Generation R_0 for this model is computed along with the quantity of interest (*QoI*), the proportion of infected individuals. In this example,

$$R_0 = \frac{\beta K(r-\delta)}{r(\gamma+\mu+\delta)} \qquad QoI = \frac{I(t)}{S(t)+I(t)+R(t)}$$ Eq. 2