Mixed effects model for investigating dietary regimens intended to extend lifespan in *Caenorhabditis elegans*

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Abstract

In preliminary aging studies, animal models are used to investigate effects of treatments designed to extend length of life. One such model involves *C. elegans* (nematodes) and has been used in testing the effect of dietary regimens on lifespan or healthspan. Healthspan can be assessed via repeated measurements of pharyngeal pumping rate (PPR) which is recorded as a count of pharyngeal muscle contractions per minute and decreases as the animal ages. Thus, maintenance of a healthy PPR is associated with a longer lifespan. Using this study design, aging data are generated to simulate lifespan in *C. elegans*. The analytic data are modeled using two generalized linear mixed models. The first assumes a Poisson distribution for the PPR response and the second assumes a normal distribution. Hypothesis tests of equality of mean PPR between diet groups are performed via the two models and results are compared. *C. elegans* provide a novel, time- and cost-efficient experimental animal model for investigating aging. Here, we employ the generalized linear mixed model to promote a unified approach to researchers using *C. elegans* that is consistent with traditional experimental statistics.

Introduction

- *Caenorhabditis elegans*
  - ⇒ Free-living, non-parasitic, transparent nematode
  - ⇒ Time- and cost-efficient model system commonly used in laboratory studies
  - ⇒ Important for translational studies because genome is similar to humans
- Pharyngeal pumping rate (PPR)
  - ⇒ Involved in food intake in *C. elegans*
  - ⇒ Muscle contracts to draw in liquid in order to trap food particles
- PPR has been shown to be correlated with lifespan
  - ⇒ Decrease in pumping rate associated with aging
  - ⇒ Used as measure in survival and aging studies

Figure 1. Close-up image of *Caenorhabditis elegans*

Methods

- Compare aging of worms over time following administration of different diets
- Have small group sample sizes ($n < 10$)
- Typical analysis by laboratory researchers involves comparing mean PPR between groups at single time point using two-sample *t*-test
- Alternative: Repeated measures model to compare slopes between groups
  - ⇒ Distribution of the response?
    - Count data implies Poisson distribution
    - Normally distributed response is most common choice
      - ⇒ Fit both models and compare accuracy of *p*-values and estimated type I error rate of default *F*-test from PROC GLIMMIX in SAS 9.3 (SAS Institute, Cary NC).

Simulation

- Data set containing PPR data obtained from consulting project at Pennington Biomedical Research Center
  - ⇒ Worms added to wells on a 96 well plate and treatments administered
  - ⇒ Each treatment applied to only 9 replicates (wells)
- A simulation study was carried out to investigate properties of tests
  - ⇒ 5000 bootstrap re-samples generated using original data
  - ⇒ Accuracy of *p*-values
    - *p*-values should be UNI(0,1) under the null hypothesis
    - To evaluate, plot empirical CDF of p-values from 5000 simulations and compare closeness to UNI(0,1,) CDF

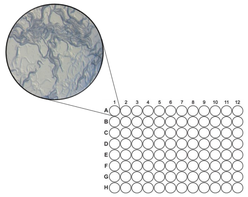

Figure 2. Diagram of a 96-well plate showing detail of one well containing multiple *C. elegans* specimens

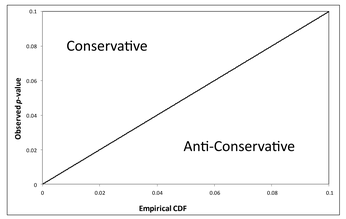

Figure 3. Description of layout of accuracy of *p*-values plots to aid in interpretation

Results

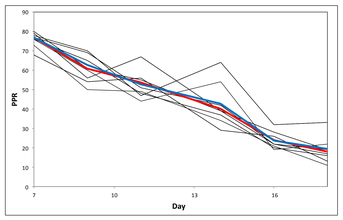

Figure 4. Black lines represent individuals from one bootstrap sample with the means of the treatment and control groups depicted in blue and red

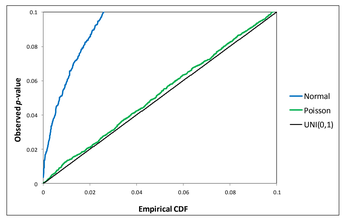

Figure 5. Accuracy of *p*-values from *F*-test under generalized linear model fitted with either Poisson or Normally distributed response (n=9)

Model	Type I Error
Normal	0.0069
Poisson	0.0479

Table 1. Estimated type I error rate from *F*-test under generalized linear model fitted with either Poisson or Normally distributed response (n=9)

Conclusions

- What is the best analytical method for comparing treatment groups in small sample aging studies using the *C. elegans* model and recording PPR as the primary outcome of interest?

  ⇒ Repeated measures model versus two-sample *t*-test
  - *t*-test: Comparing mean PPR between groups at a single time point during the experiment
    - ⇒ Lose information from data gathered at all other time points and on other covariates
  - RM model: Comparing rate of change in PPR between groups across entire experiment
    - ⇒ Allows researcher to adjust for other covariates of interest in the model
    - ⇒ Can perform post-hoc *t*-tests of mean PPR adjusted for all other model effects

  ⇒ Generalized linear model
  - Poisson vs. Normal
    - ⇒ Accuracy of *p*-values from *F*-test comparing slopes between treatment groups much higher under Poisson model
    - ⇒ Type I error rate under Poisson model very close to nominal level of 0.05
    - ⇒ *F*-test under the Normal model is highly conservative
    - ⇒ As sample size (number of replicates) is increased, accuracy of *p*-values from *F*-test under Normal model converges to that of the Poisson
    - ⇒ Repeated measures Poisson model appears to be the best choice for the researcher conducting this type of experiment

References

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