Interpretable Ordinal Analysis for Complex Designs in Cell and Molecular Biology

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The Challenge

- Complex phenotypes are commonly ordinally measured by scoring.
  - Rapid to Collect
  - Difficult to Analyze
  - Testing Assumptions[1]
- Ordinal Regression
  - Powerful
  - Uncommon[2]

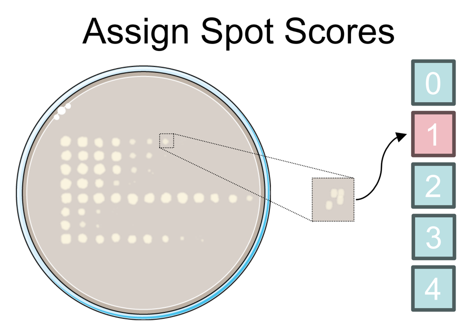

Figure 1. Example ordinal phenotype.

The Solution

Convert ordinal scores into a quantitative, interpretable metric we call MIC^*.

$$P(Y \leq 0 \mid \boldsymbol{X}, X_C = MIC^*) = 0.5$$

- Derived from ordinal regression model
- Biologically interpretable
  - *MIC = Minimum Inhibitory Concentration*

Statistical Framework

Proportional Odds (PO) Model:

$$\mathrm{logit}\big(P(Y_i \leq j)\big) = \alpha_j - \boldsymbol{X}_i\boldsymbol{\beta}$$

The MIC^* Metric:

$$MIC^* = g^{-1}\left(\frac{\alpha_0 - \sum \beta_k X_k}{\beta_C + \sum \beta_{Ck} X_k}\right)$$

Group Differences:

$$\Delta MIC^* = {MIC^*}_B - {MIC^*}_A$$

$$\Delta \log_2 MIC^* = \log_2 {MIC^*}_B - \log_2 {MIC^*}_A$$

Variance Estimation: $(g_A(\theta) = {MIC^*}_A)$

$$V(\Delta MIC^*) = (\nabla g_B - \nabla g_A)^T \sum (\nabla g_B - \nabla g_A)$$

The *ordinalMIC* Software Suite

Tool	Purpose	Benefit	Access
BLISS Web App	Blinded Scoring Assess Consistency	Reduce Bias Browser Based	lewislabUARK.github.io/BLISS
ordinalMIC R package	Perform Core Statistical Analysis	Powerful & Flexible	lewislabUARK.github.io/ordinalMIC
MICalculator Web App	Point-and-Click Tool for MIC* Analysis	Increased Accessibility	lewislabUARK.github.io/MICalculator

Table 1: Resources available for ordinal scoring and *MIC** analysis.

Assign Scores → Fit PO Model → Estimate MIC* → Analysis & Visualization

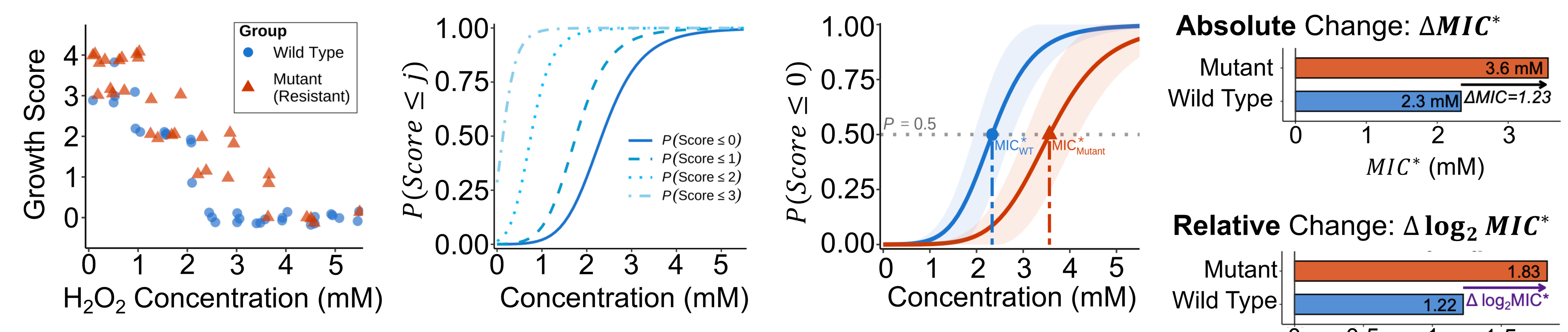

Figure 2. Example MIC* analysis process for group-wise comparison of MIC*.

Estimator Performance

MIC^* is *Powerful* Estimator

Monte Carlo Simulations (N=10^7) of factorial G×E design confirm framework's utility.

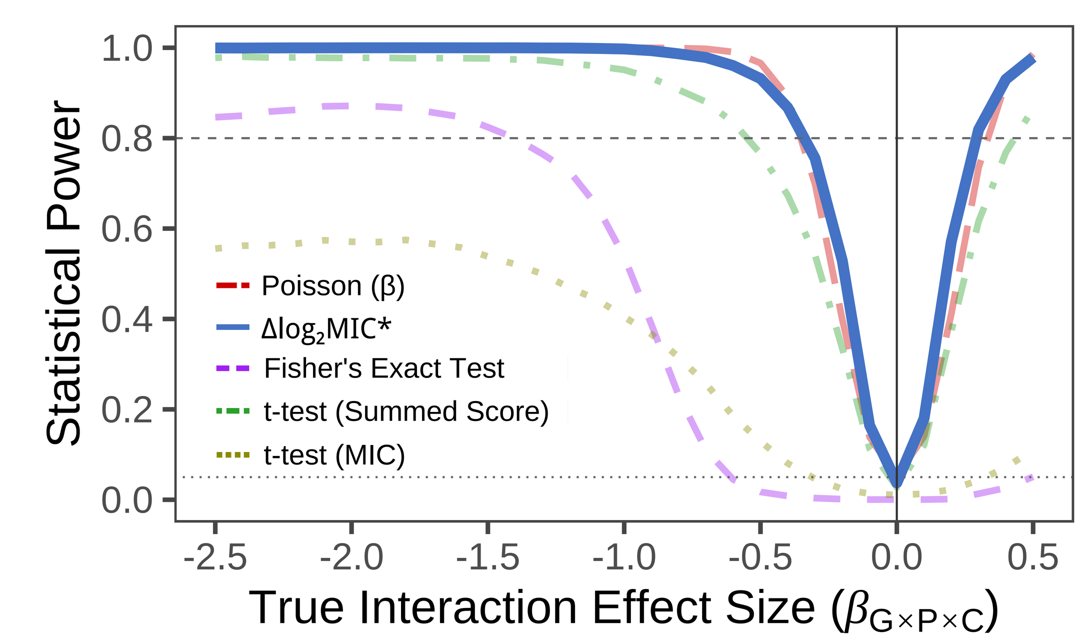

Figure 5: Power analysis for ΔMIC^* vs alternative analyses observed across a range of effect sizes.

- **Sensitive:** Achieves >80% power to detect 41% increase in expected count.
- **Specific**: Nominal Type I error rate (~5%)
- **Accurate:** Low bias with mean bias 0.0026 (IQR: -0.011 to 0.018) for MIC* estimates

Ex 1: Quantifying G×E

G×E = Genotype × Environment Interaction

Question: Does deletion of *CTT1* abolish salt-induced peroxide resistance in yeast?

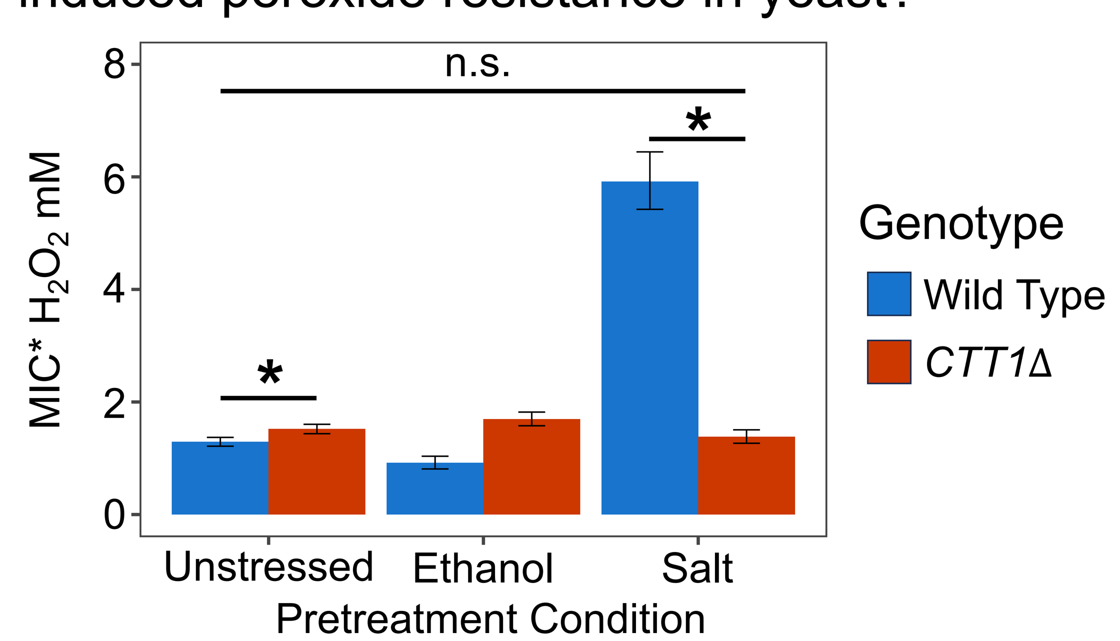

Figure 3. MIC^* for H_2O_2 resistance of Wild Type and *CTT1Δ* mutants following salt pretreatment.[3] Error bars represent 95% CI, * denotes p < 0.05.

Finding: Yes. Resistance phenotype for salt is completely dependent on the gene *CTT1*. $\Delta MIC^* = 0.09$ (95% CI: -0.04 to 0.23 mM H_2O_2)

Ex 2: Trait Mapping

Question: Can spot assays quantify ethanol-induced peroxide resistance inheritance?

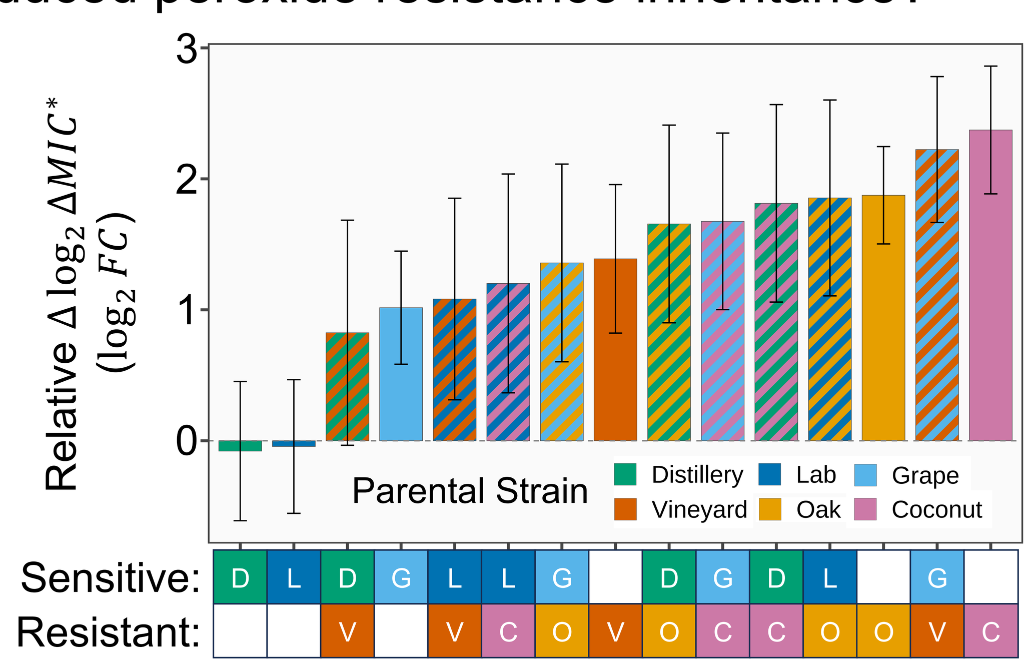

Figure 4. MIC^* for H_2O_2 resistance of wild yeast strains and crosses following ethanol pretreatment versus no pretreatment. Error bars represent 95% CI

Finding: MIC* reveals majority exhibit an intermediate phenotype, while the Grape × Vineyard cross exhibited enhanced resistance.

Take-Home Messages

1. **Stop Summing Scores.** Conventional analyses of ordinal data are underpowered
2. **Translate Scores to Biology.** The MIC* framework converts ordinal scores into intuitive quantitative metric.
3. **New Tools Available to Help.** We provide complete, open-source workflow for research

References

[1] Agresti, A., 2010. *Analysis of ordinal categorical data.* John Wiley & Sons.

[2] Agresti, A. and Tarantola, C., 2018. Simple ways to interpret effects in modeling ordinal categorical data. *Statistica Neerlandica*, *72*(3), pp.210-223.

[3] Scholes, A.N., Stuecker, T.N., Hood, S.E., Locke, C.J., Stacy, C.L., Zhang, Q. and Lewis, J.A., 2024. Natural variation in yeast reveals multiple paths for acquiring higher stress resistance. *BMC biology*, *22*(1), p.149.

Acknowledgements

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