Modelling Competition for Nutrients between Microbial Populations Growing on Solid Agar Surfaces

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1 RESULTS

1.1 Guessing

N_0 estimated from average final cell amounts. See formula in code for two N_0 estimation.

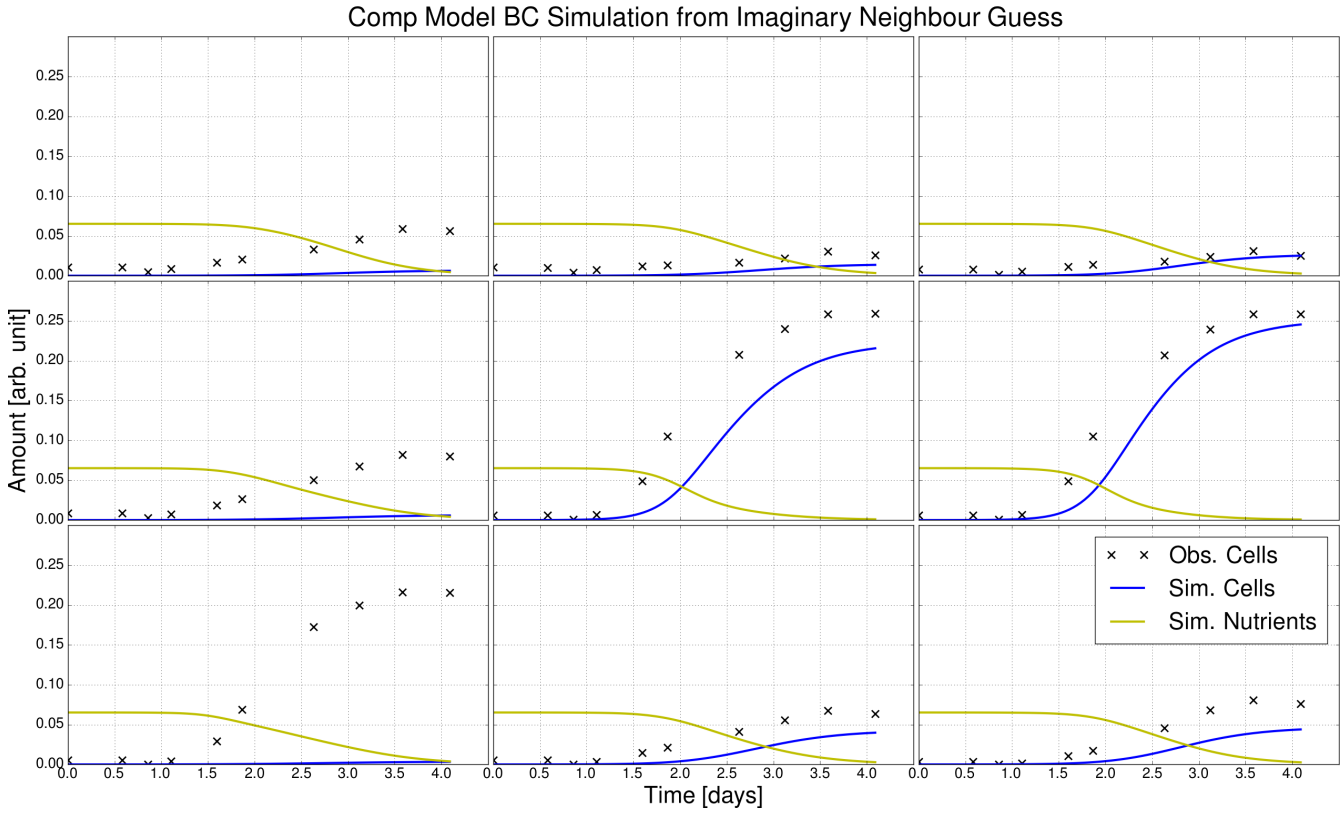

Figure 1: Competition model simulation using parameters from imaginary neighbour guessing. *Shows a 3x3 zone with top-left coordinate (5, 18) from P15 with background cdc13-1 at 27°C.*

1.2 Competition Model Fitting to P15

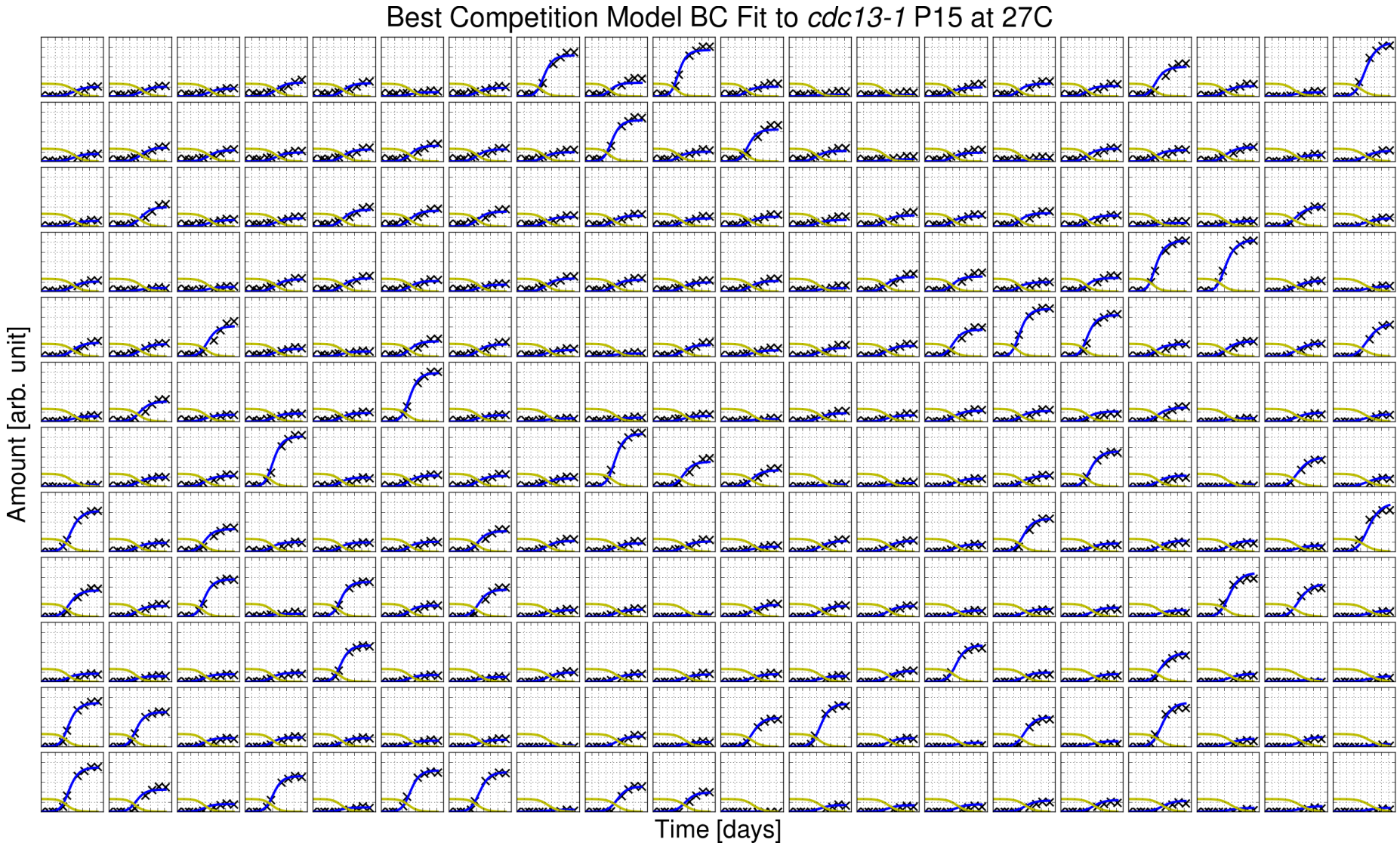

Figure 2: Fit of the competition model to a QFA plate. *Data is for a 16x24 format plate (P15) with a background mutation cdc13-1 incubated at 27°C. The plate contains 6 repeats of 50 genetic strains randomly arranged across the internal cultures. Repeats of a single strain are used for all edge cultures (removed in the plot). Model output for state variable, cell population size (blue curve), is fit to observed data (black crosses). Model predictions for unobserved variable (nutrient amount) are also plotted (yellow).*

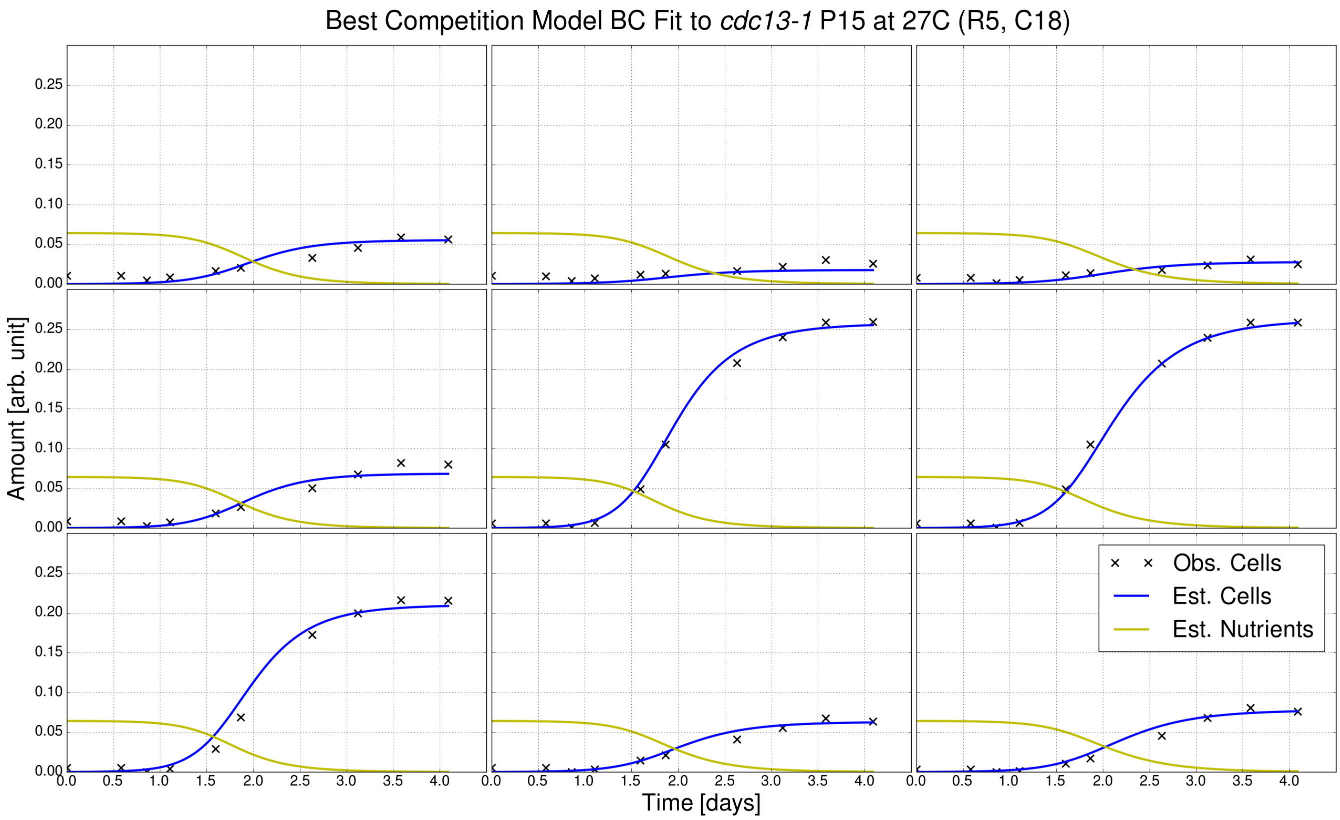

Figure 3: A 3x3 zone from Figure 2 with top-left coordinate (5, 18).

Some text Some text.

1.3 Evaluating the treatment of boundaries

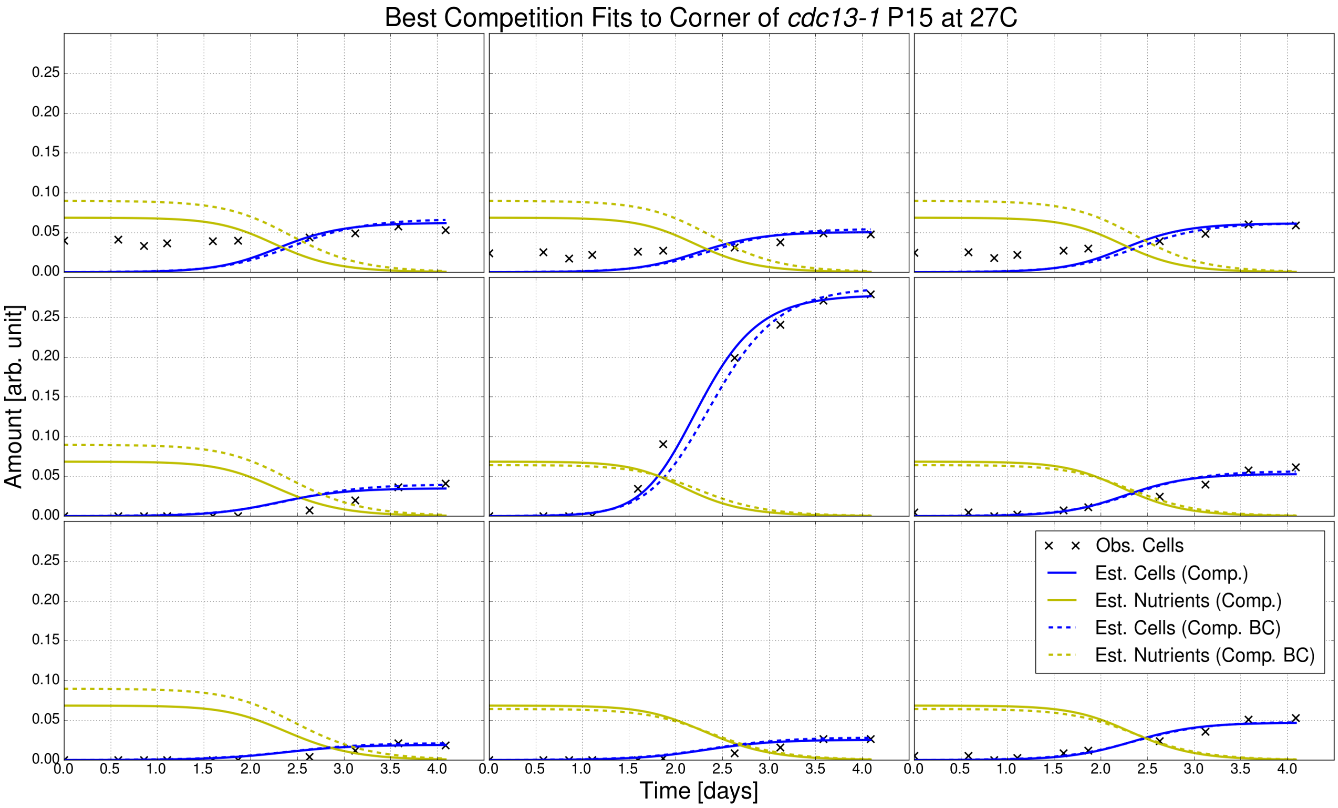

Figure 4: Treatment of boundary conditions in fits of the competition model. *The top left corner of a 16x24 QFA plate fitted with two versions of the competition model, the first has a single initial nutrient amount for all cultures, the second has a separate initial nutrient amount for edge cultures.*

Table 1: Average error in objective function for one a two N_0 parameter competition models. *Values are for the same fits as in Figure 4 and have been scaled by 10^4. Averages are for cultures belonging to the areas indicated by the column "Cultures". "Next to edge" refers to cultures one in from the edge. "Internal" refers to all cultures but the edge.*

Cultures	One N_0	Two N_0
Edge	35.9	36.5
Next to edge	9.54	7.98
Internal	6.67	6.30
All	12.4	12.2

Table 1

Some text Some text.

1.4 Agreement of b rankings

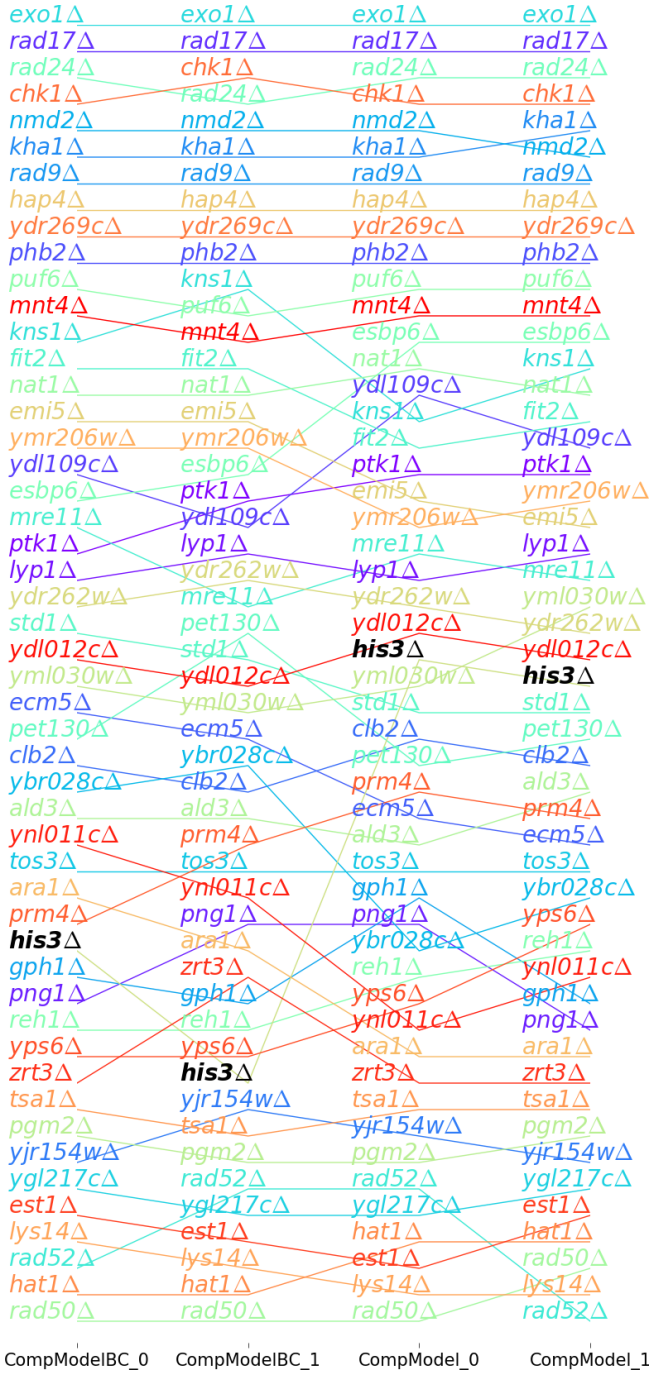

Figure 5: Comparison of b ranking for the best five competition model fits to P15. *Ranking is calculated from the mean b estimate from the six repeats or each strain.*

1.5 Comparison of fitness ranking

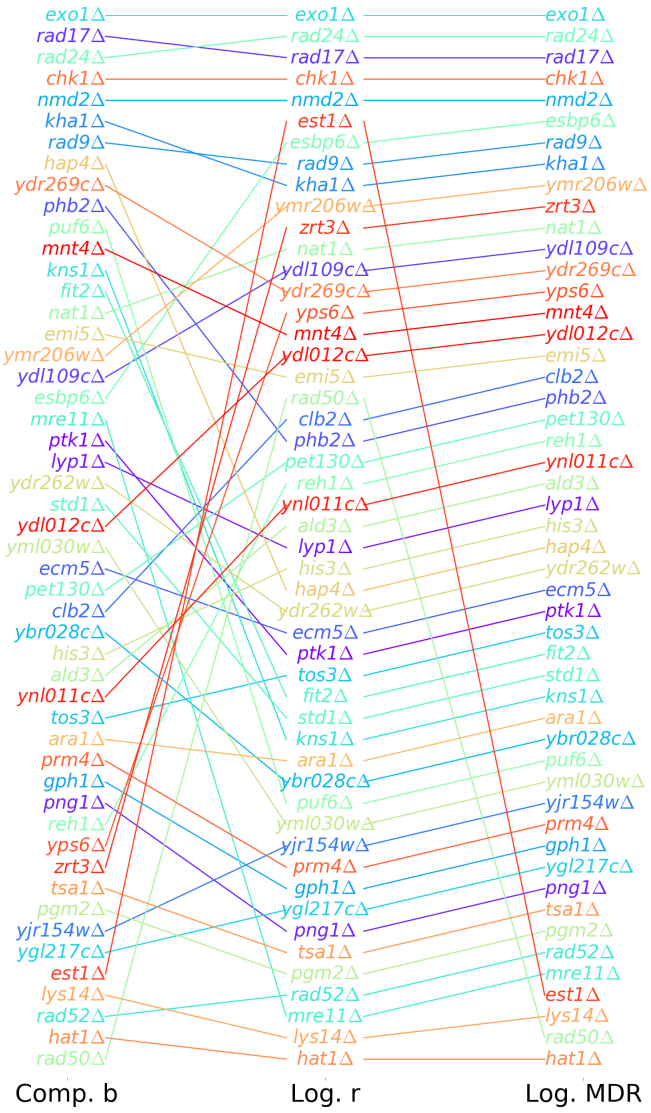

Figure 6: Comparison of r ranking for fits of the competition and logistic model to P15. *Competition model r was converted from b, N_0, and C_0 from the best competition model estimate. Logistic r was taken from fits using the QFA R package which makes heuristic checks for slow growing cultures.*

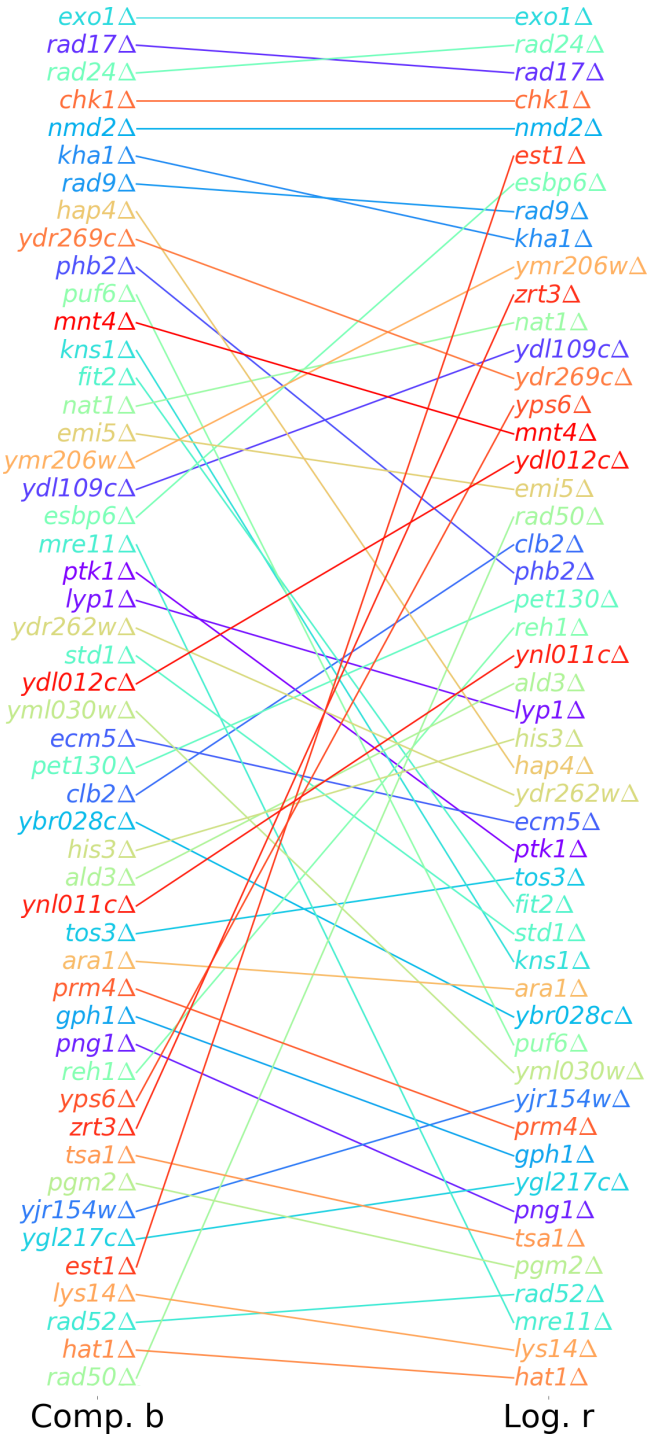

Figure 7: Comparison of r ranking for fits of the competition and logistic model to P15. *Fitnesses of genetic strains are ranked most to least fit from top to bottom. Competition model r was converted from b, N_0, and C_0 from the best competition model estimate. Logistic r and MDR were taken from logistic model fits using the QFA R package which makes heuristic checks for slow growing cultures.*

1.6 Comparison of Variation in Fitness Estimates

Use repeats on plate 15 (6 per deletion) to calculate coefficient of variation (COV) of estimated r or MDR.

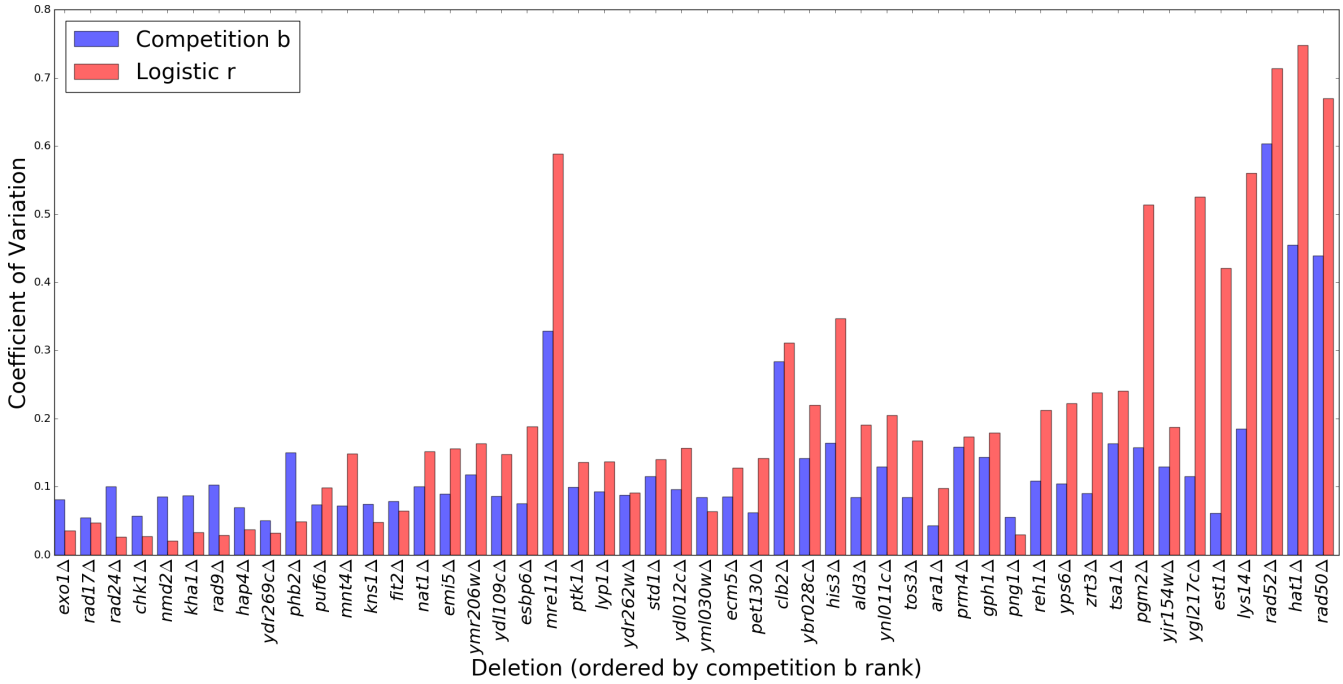

Figure 8: Coefficient of variation of r estimates. *Strains are ordered left to right along the horizontal axis by highest to lowest competition model r ranking. Fits are for the competition model, the QFA R logistic model, and the logistic equivalent model.*

1.7 Cross-plate validation

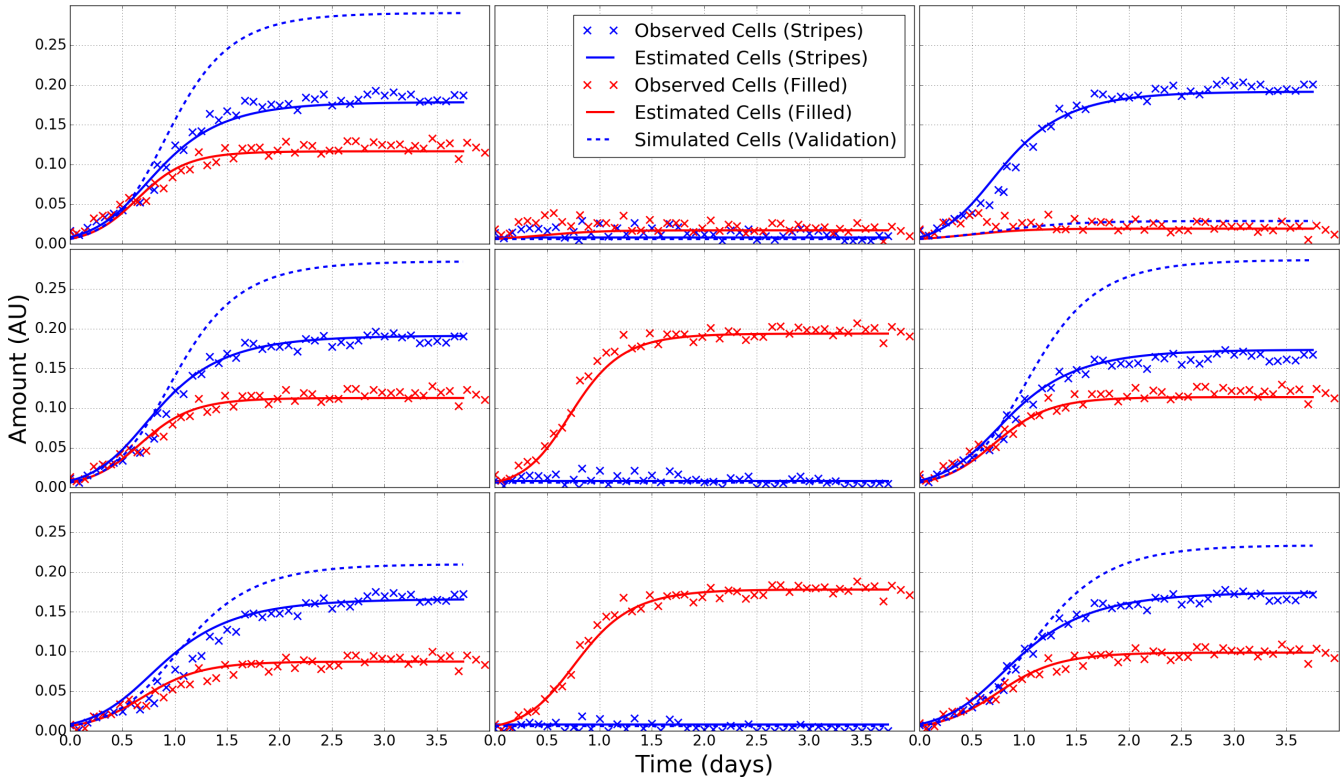

Figure 9: Calibration and validation of the competition model. *I fit the competition model to the 16x24 format "Stripes" and "Filled" plates in Figure* **??**. *The plot shows cell measurements and estimates for both plates for a 3x3 section with top left coordinates (R9, C10). I took the parameters estimates for the "Filled" plate (calibration) and set growth constant, b, to zero for cultures in the empty columns of the "Stripes" plate. I then simulated using these parameters to produce the dashed blue curve (validation). If the model is working correctly, the dashed blue curve should resemble the "Stripes" data (blue crosses).*

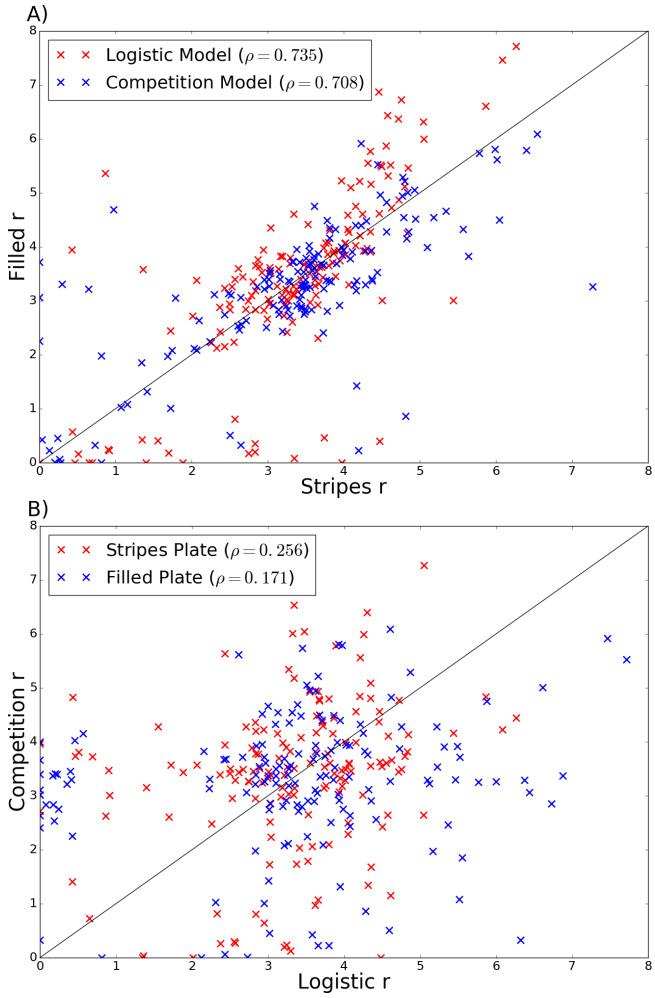

Figure 10: Correlation of r estimates for "Stripes" and "Filled" plates. *A) Correlation of r estimates between plates for logistic and competition models. B) Correlation of r estimates between logistic and competition models for both plates. I fit the competition model and independent model to the "Stripes" and "Filled" plates in Figure* **??**. *I converted competition model b to logistic model r. I only used data for cultures that were common between the two plates common and removed edge cultures. The Pearson correlation coefficient, ρ, is shown in the legends. The line $y = x$ is also plotted.*

1.8 Towards a genetic algorithm

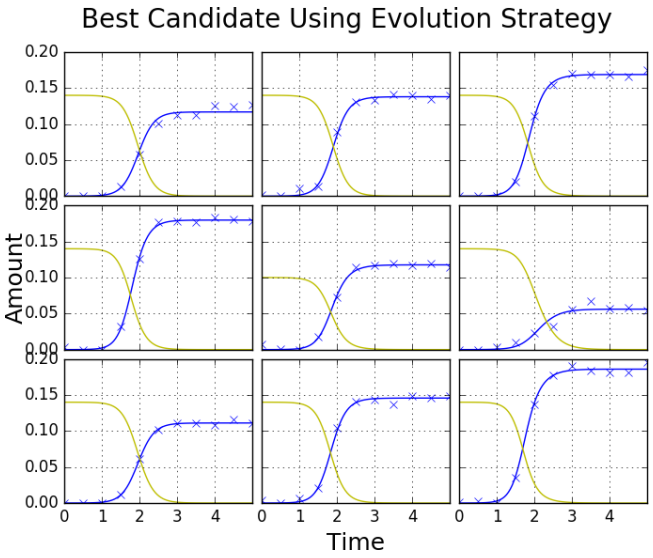

Figure 11: *Genetic algorithm fit to a 3x3 simulation. MIGHT TAKE A LITTLE BIT OF WORK TO REPRODUCE AND COULD USE PARAMETERS FROM THE BEST P15 FIT RATHER THAN JUST PICKING/RANDOMIZING. NEED TO CHECK THAT PLATE LEVEL PARAMETERS WERE ALSO EVOLVED.*

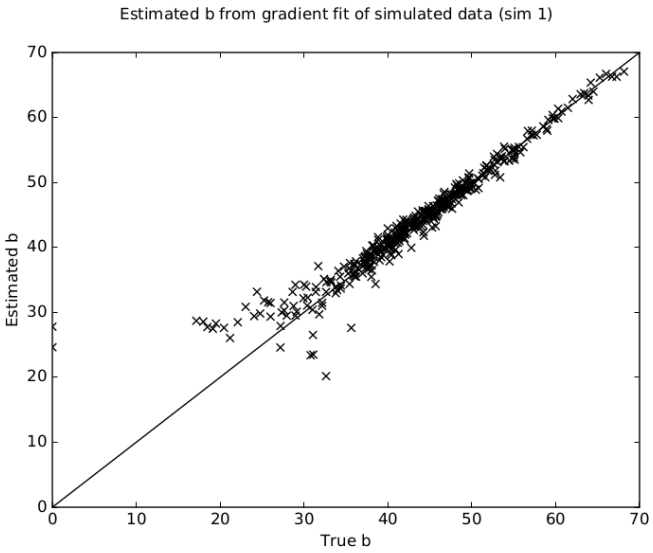

Figure 12: *Recovery of true b values from a gradient method with fixed plate level parameters. I simulated timecourses from the best five (which model? all BC?) fits to p15, fixed the true plate level parameters, and used a gradient method to recover b. This plot shows the worst case from the five sets of values.*

2 DISCUSSION

Fits of the competition model (see Figures 2 and 3) use less parameters and are qualitatively better than fits of either the logistic or generalised logistic model from the QFA R package (**??**). Competition model ranking of growth estimates for repeats on P15 (see Figure 6) agree with the logistic model rankings from **?** for the fastest and slowest growers (and with rankings from independent spot tests (refs) CHECK THIS). However, there is much disagreement in the rank of other

strains. (Could also do with the correlation plot for P15). The reliability of growth estimates was not improved using the competition model in for the fastest growing strains on P15 (see Figure 8). This may be due to the effect of noise dominated cell observations from slow growing cultures on collectively fit parameters. The Logistic model, on the other hand, was fit to cultures individual. The greater reliability of estimates for slow growing cultures could be entirely due to collective fitting rather than to correcting for competition. (Could do with p-values on figure order of HIS3 is changed; also bold HIS3 everywhere) Unfortunately, the change in order for middle rankings is unlikely identify new genetic interactions because significance is determined by comparison to a neutral deletion also in this range. Similarly, although improved, uncertainty in estimates for the slowest growers is still much higher than for fastest meaning that the power to infer genetic reactions is not dramatically different.

Fitting the logistic model to slower growing cultures requires heuristic checks to correct for confounding between r and K. The QFA R implementation appears to have some issues. The strains *est1*Δ and *rad50*Δ have dramatic changes in ranking between logistic model r and MDR (see Figure 6). In independent spot tests (ref) these are very sick strains and I confirmed this in the raw QFA images by visual inspection. High r and low K have been erroneously fit to both strains. This is corrected for when converting to MDR which agrees with the competition model ranking and independent validation and is more similar to the fitness measure ($MDR \times MDP$) used in the original analysis by **?**. For other cultures it appears that encroachment of fast growing cultures into neighbours is not being picked up by Colonyzer. In logistic fits some growth curves are still in the exponential phase at the end of observations and this may be another fitting issue. If repeated, the experiment of **?** should be run with a lower concentration of nutrients in the agar so that the stationary phase can be reached before cultures start to merge. *mre11*Δ is a weak growing strain (ref validation) which was misclassified as healthy by the competition model but not the logistic model. One repeat contained unusual heterogeneity which may be natural or be the results of contamination. (Should take median value next time). It may be that the failure heuristic checks is leading to inaccurate estimates of fitness for many strains. From visual inspection of two other strains with noticeable disagreement, *hap4*Δ appears to be healthier than *zrt3*Δ which agrees with the competition model but not the logistic model. Although the precision of estimates is similar for both models, the competition model appears to be more accurate. Unfortunately, I lack independent data for validation of the middle strains.

Recent work Herrmann and Lawless suggests that direct measures of C_{t_0} may not be reliable due to heterogeneity between starting cells in the same culture; many inoculated cells do not grow and only the fastest growing cells contribute significantly to the final population. A plate level C_{t_0} also seems inappropriate but having extra parameters for the staring cell density of each culture is undesirable. Only a small amount of nutrients is used when cultures are small. Therefore, cultures could be grown for a short time before making direct cell density measurements that may be more accurate. QFA inocula use cells taken from the stationary phase where there might be more heterogeneity (ref). It may be possible to increase the reliability of fitness estimates by taking inocula from the exponential growth phase or using a higher starting density to average out effects.

//Noise affecting competition model//

I also had some thoughts about why the competition model might have higher variance. The competition model has to deal with noise of the slowest growing cultures which affects both plate level parameter estimates and the b estimates of faster growing neighbours. I.e. sharing plate level parameters gives us more information about slower growing cultures but adds noise to our estimates of faster growing cultures. For the logistic model, each growth curve is fit independently and so fast growing cultures are less affected by noise and may have less variance than competition model estimates. Conversely slow-growing cultures are dominated by noise and this requires us to use heuristic checks (accuracy is actually affected more than precision). This may be why we see more variance in the b estimates of fast growing cultures from the competition but less for medium and slow growing (probably also less overall). If we had a plate with no very slow growing cultures dominated by noise perhaps we would see less variance in the fastest growers from the competition model. We could probably use the filled plate to check this.

In light of heterogeneity. For independent fits may start with inoculum density below the level of detection and take time zero the time when cells first reach some observable level. I.e. different t_0 for each culture.

(Wait until discussion: Recent work by Herrmann and Lawless - issues with plate level C_{t_0} - Could approximate cells as not starting to grow until levels become detectable at t_i (OK if assume only small diffusion before this point)).

Possible to have fast nutrient limited diffusion without nutrinet competition and poisoning by ethanol. Separable.

Fitness ranking from competition model fits may be better than from logistic model fits (Will comparing stripes rankings reveal anythin?). However, we cannot quantitatively compare fitness estimates between plates because we are not finding global minima. Work has begun to develop a genetic algorithm to do so. I am not convinced that this will succeed because growth is systematically overestimated when we move from the filled to striped plate for all of the current best parameter solutions. This suggests an issue with the modelling approach; below I suggest ways in which this could be improved. In any case, qualitative cross-plate validation using order of fitness ranking may still be better (for the competition model).

The first thing to notice about QFA data - from P15, the striped plate, and the filled plate - is the characteristic endpoint in growth on each plate (experiments could be designed to study variation in timescales over regions of a plate by inoculating cultures in columns left-to-right according to fitness). This suggests a plate-level or region-level growth-limiting effect. // Could this conceivably be an experimental limitation

such as the drying out of an agar plate over time? // Comparison of the striped and filled data, shows that cultures grow larger when neighbours are removed and this suggests a direct interaction between cultures. The strongest candidates are competition for nutrients and growth limiting signalling such as ethanol poisoning. It is possible that other growth limiting effects may exist and could confound any attempt to fit a model which accounts for just one of these. It makes sense to investigate each likely effect in turn to determine its contribution and to start by validating the independent limit.

There is a characteristic timescale for the cessation of growth which I believe to be caused by an interaction between cultures.

The an experiment to test the length-scale of the competition interaction could inoculate a gradient of fast to slow growing cultures across a plate and observe local differences in endpoint.

Spots can grow after a long time. Must be nutrients remaining or an encroachment? I have an image for the stripes plate showing cultures growing and believe after a very late stage. I need to check the data images but this may just be encroachment of another culture.

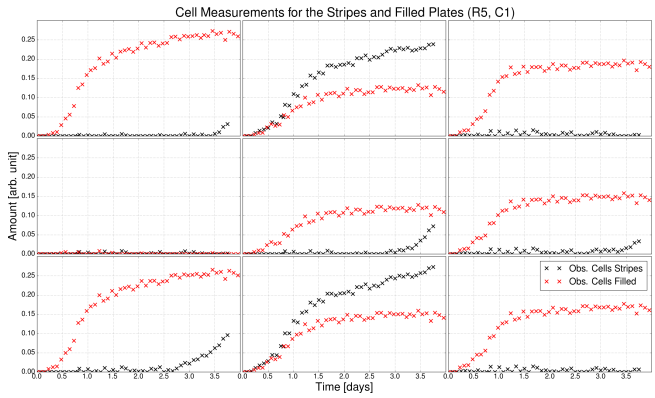

Figure 13: *Observed cells for (R5, C1) 3x3 zone of Stripes and Filled plates showing (for Stripes data) slow growing cultures starting to grow after faster growing cultures have reached the stationary phase.*

We have only studied data where cultures are grown in an array on solid agar where we cannot validate the independent limit. In this limit, our model says that nutrients can only be converted to cells and all cultures starting with the same amount of nutrients will reach the same final cell density. This ignores metabolism which may differ between strains. Cell arrest could also limit growth (and this may occur in different strains at different rates). If present, differences in such effects could account entirely for differences in final cell density. However, they are unlikely to be the only effect, because this would not lead to the observed characteristic endpoint in growth. Using one-culture spot tests (in a perti-dish on ager?) or liquid cultures we can grow cultures independently and validate the independent limit. A current issue with methods for estimating fitness, is that identical strains grow differently on agar or in liquid culture leading to different fitness rankings (cite). This problem need not affect our validation as we can simply define a culture to have different parameters for growth in either medium. A greater difference may be caused by the dimensionality of the environment. Mass action kinetics is derived for reactions in a three-dimentsional (gas or fluid?) (Guldberg and Waage C.M. Guldberg and P. Waage, Studies Concerning Affinity, C. M. Forhandlinger: Videnskabs-Selskabet i Christiana (1864), 35) and this approximation is more valid for liquid cultures than for cultures spotted onto a surface. I suggest to study first the more ideal case of liquid cultures and later see if the model holds for cultures grown on a surface. If it does not, it may be necessary to use a fractal kinetics model (I have references for this from the proposal) or, if the reaction is diffusion limited, consider a more detailed model of nutrient diffusion.

// Model equations for metabolism.//

Our model splits the agar into a grid with volume discretised per culture. In the stripes validation, we overestimate the effect of diffusion when neighbouring cultures are removed. I believe that we are not accurately capturing the point at which growth becomes diffusion limited and that nutrients are well approximated as being evenly distributed withing the spatial scales that we model. A diffusion equation model could capture the local distribution of nutrients around a culture when the stationary phase is reached. Reo and Korolev (2014) use the diffusion equation (with Neumann and Dirichlet boundary conditions) to simulate nutrient dependent growth of a single bacterial culture on a pertri dish in two-dimensions. They create a sink for nutrients from culture growth and equate the flux of nutrients through culture area with the rate of increase in culture size. They model culture area as varying and keep culture density constant. This model could be adapted for QFA by keeping culture area constant and allowing culture density to vary. A mass action kinetic model of reaction (**??**) could be used for culture growth and the nutrient sink. Simulating or fitting this model could help us learn more about diffusion in QFA experiments. It is probably computationally unfeasible to use such a detailed model to fit a whole plate. However, if necessary, it may be possible to use a finer grid to increase compartmentalisation of nutrients to capture spatial heterogeneity in the distribution of nutrients around a culture and the effect of diffusion limited growth. This could extend the validity of the competition model over a larger range of variability in culture growth rates (for instance when some cultures are left empty and others are very fast growing.)

It would also be useful to determine experimentally how nutrients are distributed throughout the agar at the stationary phase. Gaps could be left in an array of cultures and only inoculated once the stationary phase is reached. If they grow then nutrients remain. (Sill require simulations to see distribution across depth). This could be extended by growing a single column of identical strains and, after the stationary stage has been reached, inoculating identical strains on the same plate at different distances from the row.

//Talk about an improvement to the imaginary neighbour model.//

Nutrients (sugars, nitrogen, etc.) in QFQ agars are of a standard composition, designed to reduce the excess of any single nutrient (check QFA paper and cite). ((back-

ground) What is the nutrient? Nitrogen is only used to build molecules for new cells, whereas sugars are also used for metabolism.) For modelling nutrient limited growth, especially across plates, it would be useful to know the identity of the limiting nutrient and ensure that it is always the same. We could achieve this using a different formula of agar.

The design of the stripes validation experiment could be improved. Rather than filling gaps with cultures not present on the stripes plate, and for which we have no b estimates, we could fill with repeats of the cultures already present on the stripes plate. (I'm not sure it makes any difference actually whether we validate from one direction to the other). It would also have been helpful to have repeats to study differences in COV between the competition and independent models. In order to make sure that competition effects were present in data, we made a drastic change between the stripes and filled plates. This provides a stern validation. The model assumes that competition effects are present whenever there is a difference in final cell amounts between cultures. We could have first validated the model against a smaller change, by varying between slower and faster growing cultures rather that none and very strong growing cultures. If the model works well between such plates it may work well for the majority of QFA experiments which typically have smaller differences between cultures than the data we study. If we did want to test the in an extreme case we could have inoculated fast growing cultures next certain strains and not others to try to induce a change in ranking for which the competition model might compensate better than the logistic model.

//Signalling//

If we find that competition for nutrients is not a significant effect, for instance if growth becomes diffusion limited before nutrients from neighbours can be accessed, then we could instead model signalling by ethanol as the interaction effect. This may be modelled similarly to how we are already modelling nutrient diffusion.

//Signalling equation//

If there is any combination of competition, metabolism, signalling, or arrest contributing significantly to differences in the growth of cultures and the interaction between neighbours then it will be difficult to separate them when fitting a model to data. We may have to develop ways to calibrate effects in isolation (e.g. by adding/measuring ethanol?) and use this information when fitting to high-throughput data.

It is quicker to fit to small zones of a plate but as these have a larger proportion of edge cultures boundary conditions become important. In current data (e.g. P15), different cultures surround the edge and this makes accurate fitting difficult. As a result we must work with larger zones that take longer to analyse. We could surround small 3x3 and 4x4 zones with an empty ring and only need to consider net flux of nutrients across the boundary and not local variation due to different cultures surrounding the zone. We could also surround with the same, low-variance, strain to reduce net flux.

//Stochastic effects.//

Unpublished work by Hermann and Lawless has investigated heterogeneity between cell lines within single QFA spots. They have found that a single or small number of extremely fast growing cell-lines come to dominate the population of a single culture. The implication is that cultures with a lower starting cell densities are likely to have greater variance between repeats. We could use higher starting cell concentrations to reduce this variance but then we study less of the growth phase. It may be possible to reduce heterogenetity by inoculating from the exponential growth phase rather than the stationary phase and still study full growth curves. (Unless mean population growth constant is being studied...) Staring cell densities should ideally be as close to the lowest resolvable level as possible.

//Ways to measure C_0// There is a confounding effect between initial cell density and b value with may justify using initial cell densities slightly above the minimum detectable level. Hetoregeneity within cultures is an issue again here and cell density would effectively be lower than the measured value, e.g., if most inoculated cells are dead or slow growing.

To fit growth curves more accurately QFA has begun using the generalised logistic model (cite). Fitness estimates (MDR*MDP or MDR?) from this model have higher coefficient of variation than those from either the standard logistic or competition model. (accuracy and precision? Could for instance a step function be less variable than the standard logistic model?) Although the fits to data are qualitatively worse, it may be advisable to revert to the standard logistic model.

The logistic model requires different K parameters (N_0 for log. eq.) to be fit for each culture. The competition model shares information about N_0 between cultures and therefore has 383 fewer parameters for a full plate (Could this also explain the higher variance for the fastest growers?). For the slowest growing cultures, noise is more dominant and there is a confounding effect between r and K. To deal with this, the QFA R package uses heuristic checks. In the case of *est1*Δ, this has led to a dramatic disagreement in estimated fitness with the competition model. The estimate from (which model)? agrees better with existing biological knowledge (/independent spot experiments?). (Is the competition model then useful?)

//QFA R is fixing C_0 rather than fitting (I used a grid))//

//Improvement to imaginary neighbour guessing//