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No selection for cheating in a natural meta-population of rhizobia

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1 ABSTRACT

2 Whether natural selection favors ‘cheating’ in mutualisms is hotly debated. Gano-
3 Cohen *et al.* (2019a) report a negative correlation between fitness and mutualist
4 quality in rhizobia, suggesting that rhizobia evolve to cheat. However, re-analysis
5 of their data shows that the correlation is an artifact of unequal sampling across
6 populations.

7 MAIN TEXT

8 Measuring fitness is tricky, and measuring the fitnesses of two interacting organisms
9 is doubly difficult. We must rise to the challenge, though, if we want to understand
10 whether partners in mutualisms are under selection to ‘cheat.’

11 Selection for cheating should manifest as a negative genetic covariance between
12 an organism’s fitness and its quality as a mutualist, measured as the organism’s
13 effect on its partner’s fitness (Friesen, 2012; Porter & Simms, 2014; Jones *et al.*,

2015). Gano-Cohen *et al.* (2019a) conclude that rhizobia cheat based on finding a negative correlation between rhizobium fitness and symbiotic effectiveness (see their **Fig. 5**). They note that the pattern is largely driven by two strains, 155 and 156, that provide little benefit to hosts, but have high relative abundance in the field. However, my re-analysis of their rhizobium fitness data casts doubt on their results.

They collected *Acmispon strigosus* plants from six natural populations and isolated *Bradyrhizobium* strains from their nodules. They individually tested 26 strains against several *A. strigosus* genotypes in a large greenhouse experiment. They measured plant growth to quantify each strain’s symbiotic effectiveness, as well as nodule number and mass, which are common measures of rhizobium fitness (Heath & Tiffin, 2007, 2009; Porter & Simms, 2014; Batstone *et al.*, 2017). They report that plant growth was not significantly correlated with nodule number or mass across strains. So there was no evidence of selection for cheating when rhizobium fitness and mutualist quality were measured in a common environment—the greenhouse.

Gano-Cohen *et al.* (2019a) then used sequence data from a previous study to estimate strain frequencies at the field sites where the bacteria were collected. They report the frequencies of *Bradyrhizobium* chromosomal (CHR) and ‘symbiosis island’ (SI) haplotypes within each population. They found that strain frequencies in the field were negatively correlated with symbiotic effectiveness in the greenhouse, mostly because ‘ineffective’ strains 155 and 156 were locally abundant. But here is where the problems emerge.

I downloaded their data (Gano-Cohen *et al.*, 2019b) and discovered that for the population where strains 155 and 156 were sampled (YUC; **Fig. 1**), genotype frequencies were calculated from just two (SI frequencies) or seven (CHR frequencies) plants. Furthermore, highly unequal sampling results in differences in mean strain frequency among the six study populations. For both loci, genotype frequency is significantly negatively correlated with the number of plants sampled at a site (CHR: $R^2 = 0.175, p = 0.034$; SI: $R^2 = 0.207, p = 0.023$; **Fig. 1a,b**). In fact, these relation-

ships have slightly higher R^2 values than the negative correlations between genotype frequency and symbiotic effectiveness reported in the paper (CHR: $R^2 = 0.157$, SI: $R^2 = 0.178$). As the authors sampled more plants in a population, mean strain frequencies declined, suggesting that sampling additional plants regularly recovers novel rhizobia genotypes. This is further supported by plotting how the number of unique rhizobia genotypes increases as a function of the number of plants sampled (**Fig. 1c,d**); the curves do not plateau. Thus, high strain frequency is an artifact of inadequate sampling.

The authors sampled multiple nodules per plant, but their data shows nodules on the same plant are not independent samples of rhizobia in a population. Nodules on the same plant have fewer unique rhizobia genotypes than the same number of nodules chosen at random within a population (**Fig. 1e,f**). This suggests that plants ‘choose’ rhizobia genotypes in the wild, or that natural rhizobia populations are spatially structured (Akçay, 2017).

I accounted for differences in sampling effort among populations in two ways. My code is on GitHub (<https://github.com/drfreder/selection-for-cheating>). Briefly, first, I relativized rhizobium fitness within each population by dividing by the population mean, as is common when estimating selection gradients (e.g., Porter & Simms, 2014). This ensures each population has the same mean fitness. Relationships between genotype frequency and symbiotic effectiveness become non-significant, with R^2 values near zero (CHR: $R^2 = 0.025, p = 0.444$, SI: $R^2 = 0.000, p = 0.982$; **Fig. 2a,b**). Second, I re-sampled the data to equalize sampling effort, but not necessarily rhizobium fitness, across populations. One hundred times per population, I randomly chose two or four plants from each population to calculate SI and CHR genotype frequencies, respectively. These were the minimum number of plants used to calculate genotype frequencies in Gano-Cohen *et al.* (2019a). I then averaged genotype frequencies across iterations. This re-sampling also led to non-significant relationships between strain frequency and symbiotic ef-

fectiveness (CHR: $R^2 = 0.100, p = 0.116$, SI: $R^2 = 0.007, p = 0.702$; **Fig. 2c,d**).
These analyses shows that uneven sampling effort across populations gives rise to the
negative associations between strain frequency and symbiotic effectiveness reported
by Gano-Cohen *et al.* (2019a).

Mutualism researchers need to agree on how to measure selection on mutualist
quality in empirical studies, or we will not progress in our understanding of mu-
tualism evolution. I suggest measuring fitness and mutualist quality in a common
environment, ideally in the same experiment, because pooling across populations or
environments, or correlating fitness measures from one environment with trait values
measured in another, can produce misleading results. The results of Gano-Cohen
et al. (2019a) do not show that rhizobia are under selection to cheat, leaving the
field with few empirical examples of fitness conflict between partners in mutualisms
(Frederickson, 2017).

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Figure 1: Top: Correlations between rhizobium CHR (a) or SI (b) genotype frequencies and sampling effort. Points are colored by population, numbers indicate strain identity, and solid lines are significant linear regressions. In **b**, strain 156's SI frequency is hidden behind the dot for strain 155 because they have identical values; these strains were isolated from the same plant and share the same SI haplotype. **Middle:** CHR (c) or SI (d) genotype accumulation curves for the six study populations. Points and lines are colored by population (as in **a**), and error bars are standard deviations calculated from random permutations of the data using the *specaccum* function in the *vegan* package in R (Oksanen *et al.*, 2019). **Bottom:** Number of unique CHR (e) or SI (f) genotypes in nodules from the same plant (black) or the same number of nodules sampled at random within a population (blue). Each dot is a plant, and the solid black line is the regression line fit to the original data. Each blue line is a regression line resulting from sampling the same numbers of nodules at random from each population; randomization data points not shown. Data from Gano-Cohen *et al.* (2019b).

Figure 2: Top: Non-significant correlations between rhizobium CHR (a) or SI (b) genotype frequencies and symbiotic effectiveness after relativizing rhizobium fitness within populations. **Bottom:** Non-significant correlations between rhizobium CHR (c) or SI (d) genotype frequencies and symbiotic effectiveness after re-sampling to equalize sampling effort across populations. See text for details. In all panels, points are colored by population (as in **Fig. 1a**), numbers indicate strain identity, and dotted lines are non-significant linear regressions. Data from Gano-Cohen *et al.* (2019b).