Measuring aphid clonal line abundance after experimental evolution

Lucas Nell

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Introduction

Experimental evolution studies have been instrumental in informing our understanding of the processes shaping evolution (Elena and Lenski, 2003). Most of such studies have been carried out in microbes (reviewed in Dettman et al., 2012; Jerison and Desai, 2015), and have provided insights on such diverse and fundamental themes as historical contingency, evolutionary innovation, parallel evolution, and adaptation (Blount et al., 2008; Barrick et al., 2009; Toll-Riera et al., 2016; Voordeckers and Verstrepen, 2015; Gerstein et al., 2012).

Similar experiments have been carried out in invertebrates (Gompert and Messina, 2016; Chandler, 2014; Burke et al., 2010; Kang et al., 2016; Rouchet and Vorburger, 2014), although such studies are comparatively rare. Experimental evolution studies in insects typically utilize limited numbers of clonal or inbred lines (e.g., Rouchet and Vorburger, 2014; Kang et al., 2016) and characterize experimental populations by either (a) measuring the distribution of specific phenotypes (e.g., Rouchet and Vorburger, 2014) or (b) sequencing pooled DNA ("Pool-seq"; e.g., Burke et al., 2010). The former requires that the researcher manipulate the environment such that a specific phenotype is predicted to change. Moreover, if starting experimental populations have a continuous distribution of phenotypes or if any significant degree of phenotypic plasticity exists, this method is not likely to provide accurate estimates of how distributions of individuals change through time.

Pool-seq, however, is an accurate, cost-effective method to measure allele frequencies in populations (Gautier et al., 2013; Futschik and Schlötterer, 2010) and to identify loci associated with traits (Rubin et al., 2010; Bastide et al., 2013). However, Pool-seq's advantageous accuracy-to-cost ratio is only present when there are many pooled individuals (> 40) and when depth of coverage is high ($> \times 50$). Because sequencing error is difficult to distinguish from rare alleles, Pool-seq is not ideal when trying to detect these low-frequency alleles (Schlötterer et al., 2014). Additionally, Pool-seq of whole-genome sequencing provides much unnecessary information if an association study is not the ultimate goal.

One way to reduce genome complexity is to use restriction site-associated DNA sequencing ("RADseq"). RADseq approaches use restriction enzymes to break apart the genome at specific locations determined by the enzyme's binding site sequence. Although some use RADseq to refer

to one specific methodology, here I use the more inclusive definition of RADseq by Andrews et al. (2016), who define RADseq as all methods "... that rely on restriction enzymes to determine the set of loci to be sequenced" (Andrews et al., 2016, p 81). Genotyping-by-sequencing ("GBS") is a particularly low-effort, low-cost RADseq approach that requires no specialized equipment for sample preparation (Elshire et al., 2011).

Herein I will assess pooled GBS as a method to assess the abundance of clonal lines of aphids after experimental evolution. Aspects of this specific experiment that make it suitable for pooled GBS are as follows:

- Each experimental population will contain $\gg$1,000 individuals when allele frequencies are sought at the end of the experiment. Individual-based estimates (e.g., using microsatellites) would require 50–100 individuals to be sampled from the population (See Fig 1), which would take huge amounts of preparation time.
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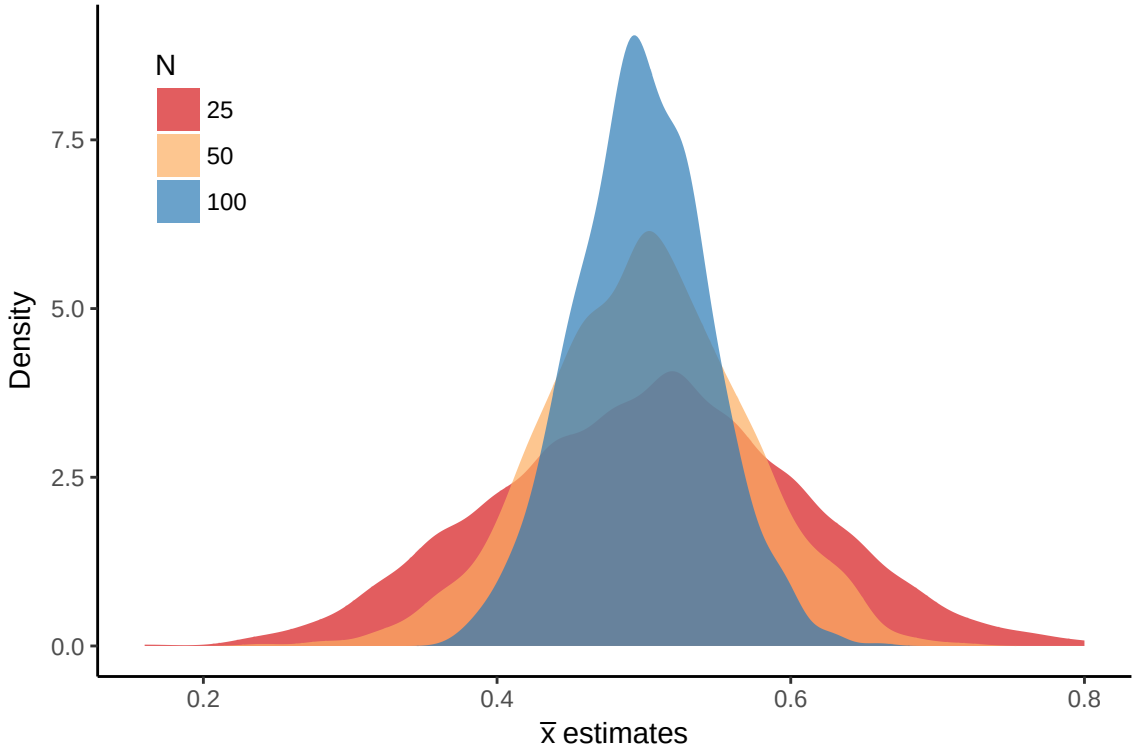

Figure 1: Simulated estimates of mean genotype abundance in a population for a given sample size. Samples were randomly drawn from a population of 1,000 with $\mu = 0.5$. N is the number of samples drawn from the population, and distributions are for 1,000 simulations. See here for this figure's code.

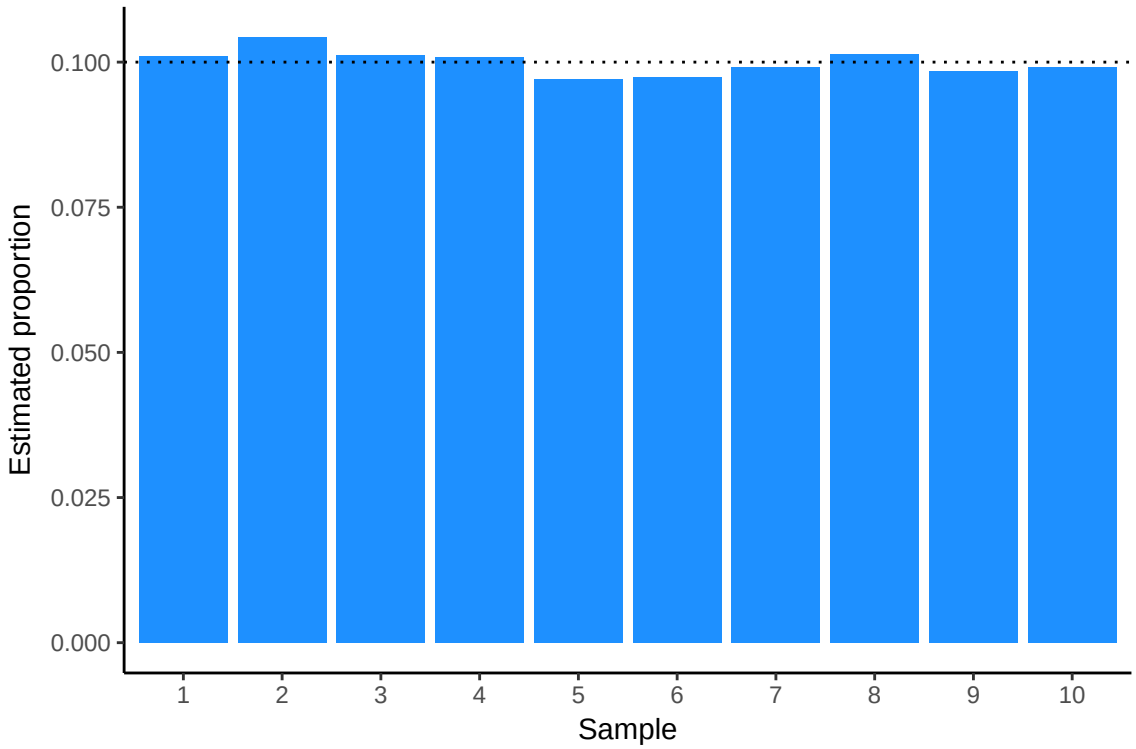

Figure 2: Estimated proportions from simulated data for 10 samples. The dotted line represents the actual abundance, which is the same for all samples.

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