

Figure S1: Facultative symbiont frequencies over time, graphed for each replicate alfalfa field, across our 2012 field season. Data points represent frequency in each field. Black lines, with open white circles, give the average frequency across all sampled fields from a given time.

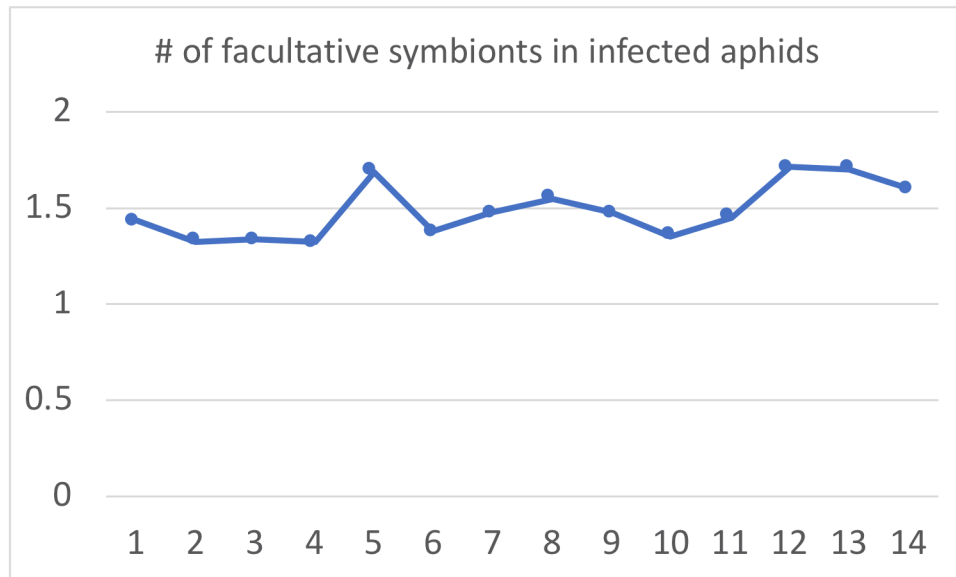


Figure S2: Average number of facultative symbionts in infected pea aphids, across 14 sampling times in 2012. Y-axis = # of symbionts per aphid. X-axis = sampling times, separated by 2-week intervals.

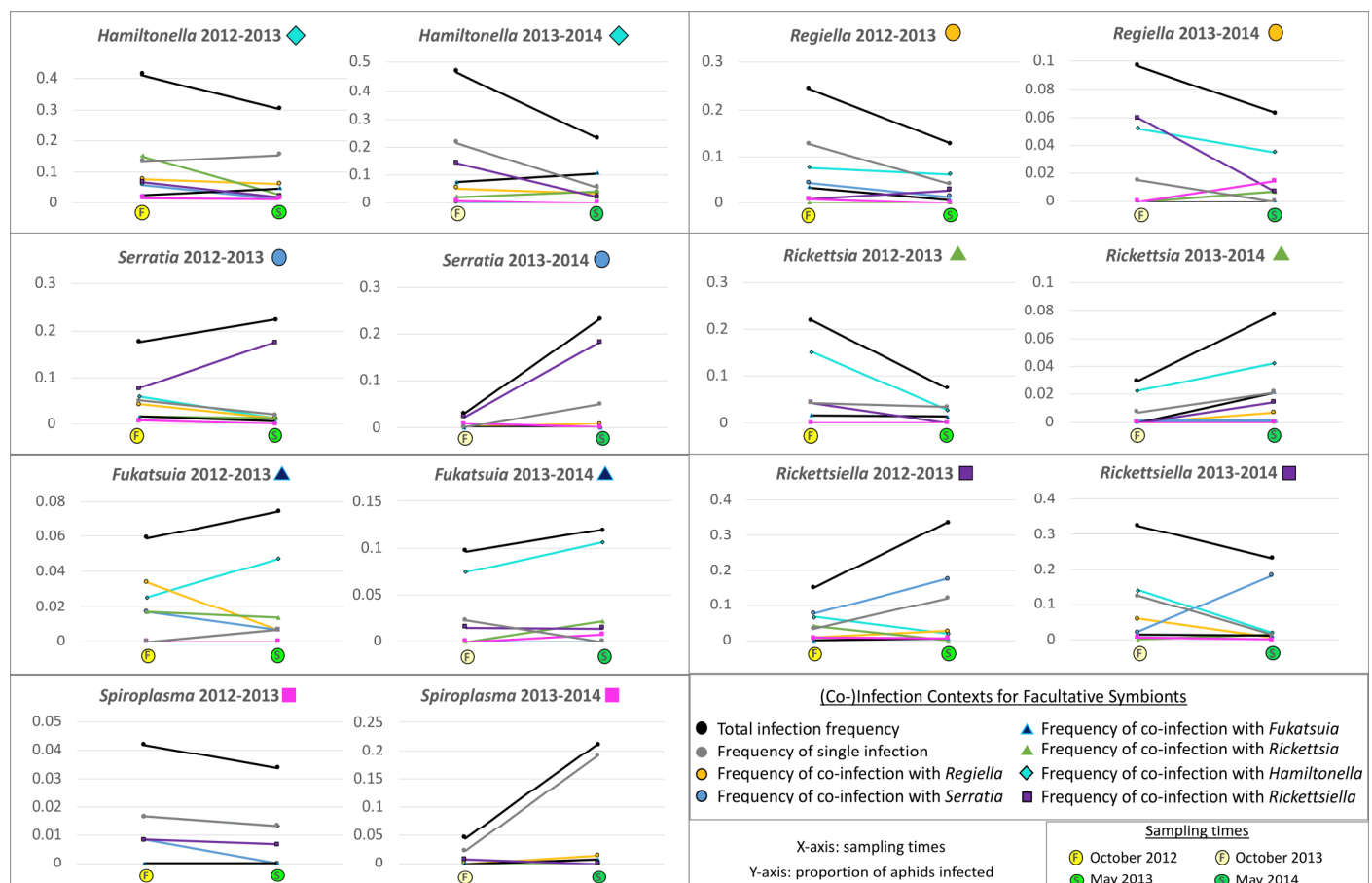


Figure S3: Co-infection contexts for facultative symbionts across two overwintering periods. Shown here are results of diagnostic PCR screening for each of seven facultative symbionts – in total (pooled across fields – black lines) and in relation to the presence of other symbionts in the same aphid hosts (colored and gray lines). Y-axis: proportions of aphids harboring the particular symbiont species, or those exhibiting the specific (co-)infection type. Note, that for the present figure, y-axis scales differ across the varying symbionts. Also, time points on the x-axis are separated by 2 weeks, spanning late April through late October. We use the same color scheme for fall (“F”) and spring (“S”) collections adopted for **Fig. 5**.

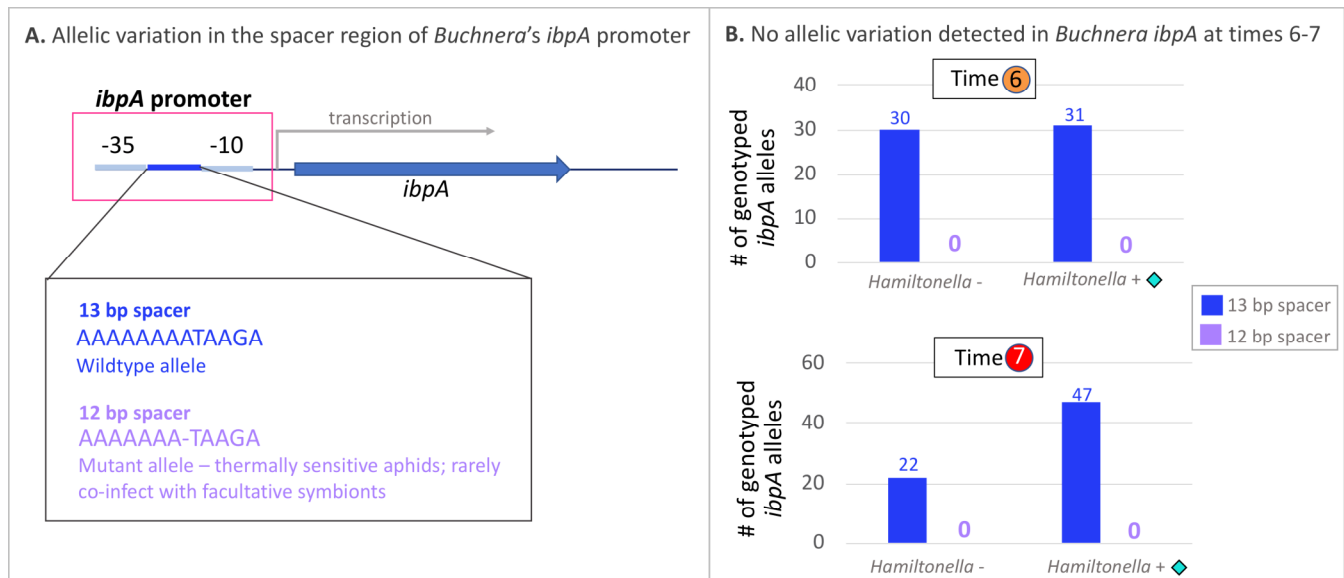


Figure S4: The absence of a thermally sensitive *ibpA* promoter allele in obligate *Buchnera* symbionts across times 6-7, argues against selection on the thermal properties of this obligate symbiont as the driver of a large *Hamiltonella* shift. Analyses performed to further assess whether *Hamiltonella*'s rise in frequency during a hot time period in 2012 (times 6-7; Smith et al. 2021) was a product of hitchhiking. A) *Buchnera*'s *ibpA* gene promoter region shows allelic variation in some pea aphid populations. Aphids harboring *Buchnera* with the wildtype (13 bp) variant have greater thermal tolerance than those with the mutant (12 bp) variant, and also greater tendencies to live with facultative symbionts like *Hamiltonella*. B) But genotyping of *Buchnera* from n=61 and n=69 aphids at Times 6 and 7 uncovered only the wild type allele.

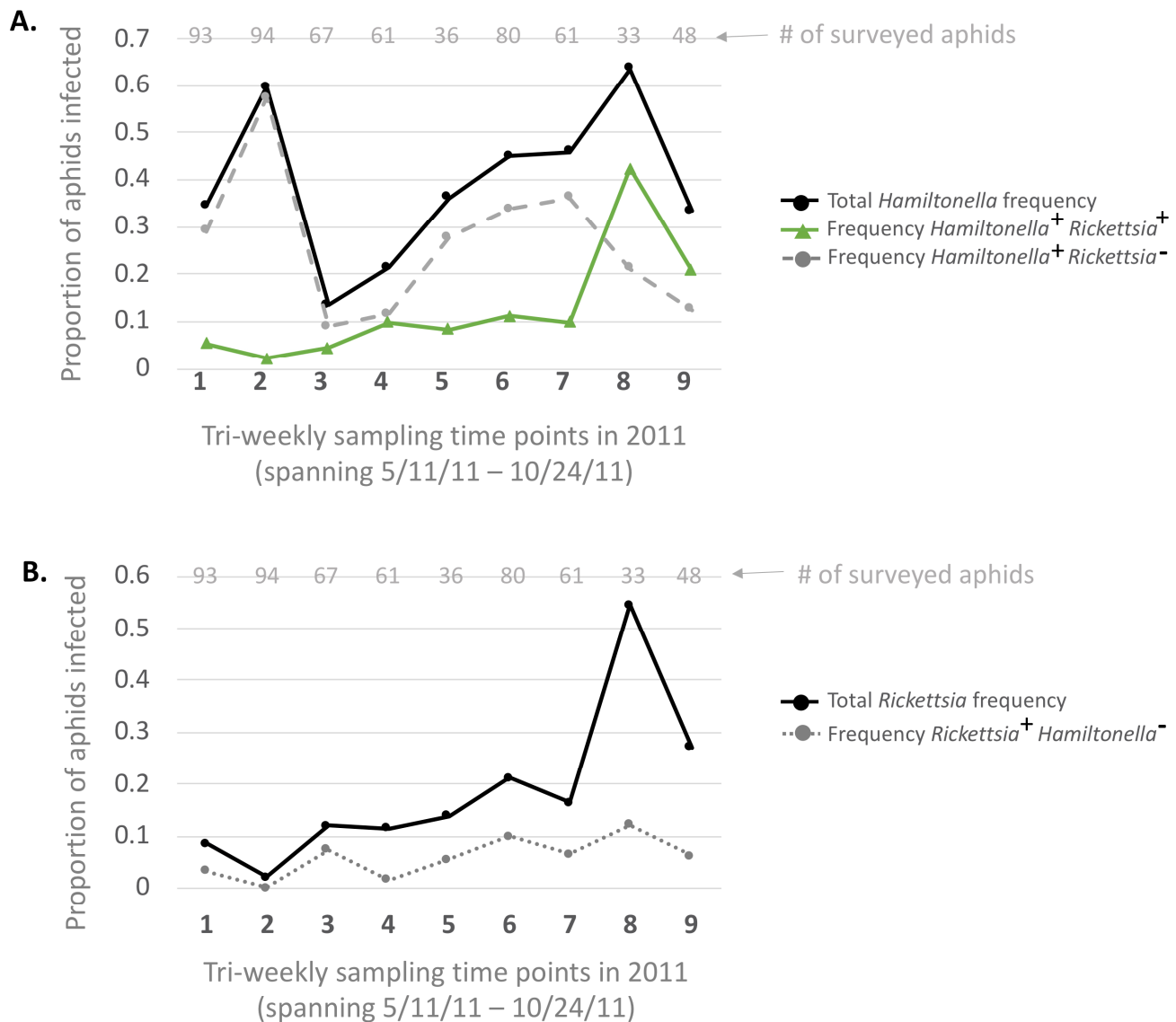


Figure S5: Data from 2011 collections in nearby southeastern Pennsylvania alfalfa fields reveal potential selection on the *Hamiltonella*-*Rickettsia* co-infection in the later periods of the growing season. Data taken from Appendix S1 of Smith et al. 2015 [64]. Only aphids screened for both *Hamiltonella* and *Rickettsia* in alfalfa fields of southeastern Pennsylvania were included. Graphing scheme follows that used for **Figures 4 & 5**. Panel A: *Hamiltonella*-focused plots. Panel B: *Rickettsia*-focused plots. Patterns across time points 7 & 8 (September 12 – October 3) show: 1) large increases in the frequencies of both symbionts (+17.7% for Ham; +38.2% for Rick); 2) strong increases in the frequencies of aphids harboring both symbionts (+32.6% for Ham+Rick+ aphids); and 3) no strong increases in frequencies of aphids harboring one symbiont without the other (-14.9% for Ham+Rick-; +5.6% for Rick-Ham+). Combined, these patterns suggest selection on the *Hamiltonella*-*Rickettsia* co-infection unfolded between times 7-8. By similar logic it may have also been selection against this co-infection between time points 8 & 9, although the evidence is slightly less convincing.