Supplementary Data

Hamiltonella symbiont titre

While the majority of data for this study was collected in 2011, quantification of symbiont populations with increasing maternal age was carried out in 2012. It was noted at the start of the year that *Hamiltonella* densities for the *Hamiltonella* 1 line were significantly lower than previously recorded. To confirm when this drop in symbiont density had occurred, historic samples of adults collected from the *Hamiltonella* 1 line were screened for symbiont quantification using qPCR (as described in Methods Section 2.5), along with samples of the new *Hamiltonella* 2 line. For *Hamiltonella* 1, samples were screened from 2008, 2010, 2011 and 2012. For *Hamiltonella* 2, samples were screened from 2012. Three biological replicates were screened per line and year, although for 2011 only one suitable historic sample was available.

There was no significant difference in the secondary symbiont populations of *Hamiltonella* 1 samples collected in 2008, 2010 or 2011 (Figure S1, ANOVA, $p = 0.598$), and these were grouped together for subsequent analysis. There was a significant effect of sample on *Hamiltonella* density (ANOVA, $F_{2,8} = 199.22$, $p < 0.0001$), with the *Hamiltonella* 1 2012 samples having significantly lower ($p < 0.0001$) and the *Hamiltonella* 2 line having significantly higher ($p = 0.0418$) *Hamiltonella* densities than the pooled historic *Hamiltonella* 1 samples (Figure S1).

Figure and Table Legends

Figure S1. Variation in *Hamiltonella* 1 symbiont density in historic aphid samples from 2008-2012, compared to the *Hamiltonella* 2 line sampled in 2012. Symbiont density is

24 calculated as number of symbiont gene copies divided by number of aphid gene copies in
25 the same sample (error bars are ±1 s.e.m). Asterisks indicate statistically significant
26 differences.
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28 **Table S1**. Primer sequences used for qPCR of pea aphid primary and secondary
29 symbionts, plus the primer for the Ef1-α endogenous control aphid gene.
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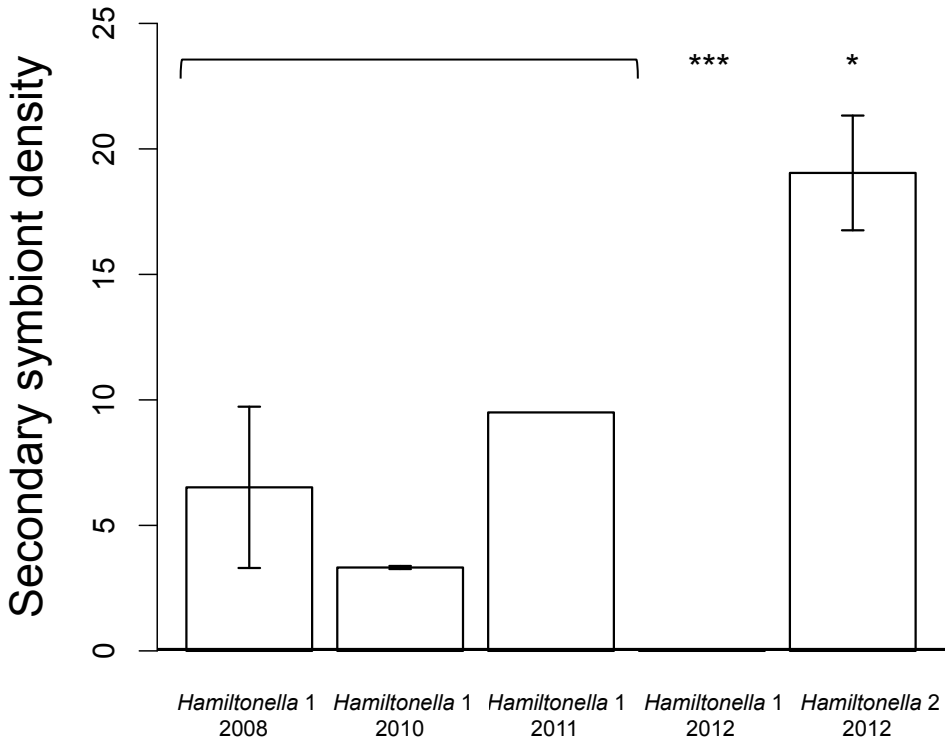

31

32 Figure S1

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Primer name	Primer sequence	Target
rpsL1	F: GCTGAATTAGGCTTTTTAGGTGTAG	*Buchnera aphidicola*
	R: CCTGCACAATCTAAGGAGCC	
U70	F: GATTTTCGCTTTCTCTGCTG	*Regiella insecticola*
	R: ATACCCATCTCGGTGGTG	
T70	F: GGTTCAGAAAAAAGTGGCAG	*Hamiltonella defensa*
	R: CGAGCGAAAGAGGAGTGA	
R70	F: TGGCGGGTGATGTGAAG	*Serratia symbiotica*
	R: CGGGATAGTGGTGTTTTTGG	
Ef1-α	F: CTGATTGTGCCGTGCTTATTG	Aphid endogenous control
	R: TATGGTGGTTCAGTAGAGTCC	

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35 Table S1

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