

**Table S1.** Pea aphids examined in this study and their infection with *Rickettsiella*

No.	Local site (latitude, longitude)	Host plant	<i>Rickettsiella</i> infection	
			Diagnostic PCR	Real-time PCR
1	Sapporo, Hokkaido (N 43.01, E 141.41)	<i>Medicago sativa</i>	0/33 <sup>a</sup>	0/3 <sup>a</sup>
		<i>Trifolium repens</i>	0/24	0/2
		<i>Trifolium pratense</i>	0/21	0/2
2	Shichinohe, Aomori (N 40.71, E 141.13)	<i>Medicago sativa</i>	0/20	0/2
		<i>Trifolium repens</i>	0/34	0/3
		<i>Trifolium pratense</i>	0/15	0/2
3	Morioka, Iwate (N 39.76, E 141.13)	<i>Medicago sativa</i>	0/20	0/2
		<i>Trifolium repens</i>	0/20	0/2
		<i>Trifolium pratense</i>	0/12	0/2
4	Toyama, Toyama (N 36.63, E 137.12)	<i>Medicago sativa</i>	0/55	0/3
		<i>Vicia sativa</i>	0/34	0/3
		<i>Trifolium repens</i>	0/61	0/3
5	Yotsukaido, Chiba (N 35.69, E 140.15)	<i>Medicago sativa</i>	0/27	0/3
		<i>Vicia sativa</i>	0/40	0/3
		<i>Trifolium repens</i>	0/20	0/2
6	Shiojiri, Nagano (N 36.12, E 137.98)	<i>Medicago sativa</i>	0/20	0/2
		<i>Vicia sativa</i>	0/20	0/2
		<i>Trifolium repens</i>	0/20	0/2
7	Okazaki, Aichi (N 34.56, E 137.12)	<i>Medicago sativa</i>	0/28	0/3
		<i>Trifolium repens</i>	0/23	0/2
		<i>Trifolium pratense</i>	0/7	0/2
8	Nagakute, Aichi (N 35.16, E 137.07)	<i>Medicago sativa</i>	0/27	0/3
		<i>Vicia sativa</i>	0/18	0/2
		<i>Trifolium repens</i>	0/52	0/3
9	Bizen, Okayama (N 34.44, E 134.11)	<i>Medicago sativa</i>	0/2	0/2
		<i>Vicia sativa</i>	0/20	0/2
		<i>Trifolium repens</i>	0/28	0/3
10	Tottori, Tottori (N 35.23, E 134.12)	<i>Vicia sativa</i>	0/20	0/2
		<i>Trifolium repens</i>	0/20	0/2
		<i>Trifolium pratense</i>	0/19	0/2
11	Matsue, Shimane (N 35.45, E 133.09)	<i>Medicago sativa</i>	0/12	0/2
		<i>Vicia sativa</i>	0/20	0/2
		<i>Trifolium repens</i>	0/20	0/2
12	Kitakyushu, Fukuoka (N 32.48, E 130.54)	<i>Medicago sativa</i>	0/20	0/2
		<i>Vicia sativa</i>	0/52	0/3
		<i>Trifolium repens</i>	0/31	0/3
13	Nishihara, Kumamoto (N 32.40, E 130.60)	<i>Trifolium pratense</i>	0/11	0/2
		<i>Vicia sativa</i>	0/11	0/2
		<i>Trifolium repens</i>	0/11	0/2
14	Miyazaki, Miyazaki (N 31.49, E 131.26)	<i>Trifolium pratense</i>	0/12	0/2
		<i>Vicia sativa</i>	0/7	0/2
		<i>Trifolium repens</i>	0/20	0/2

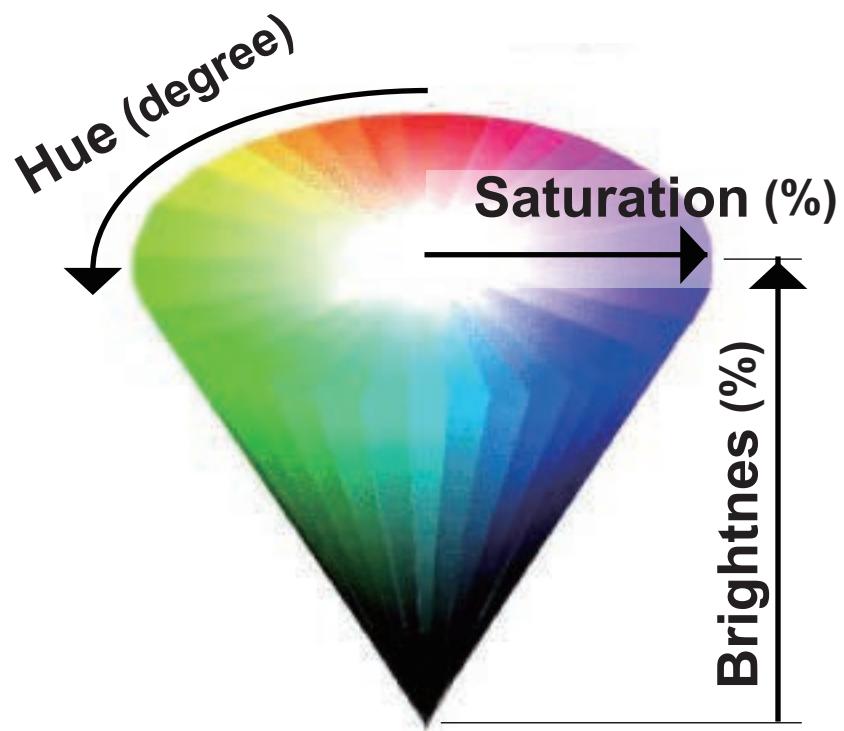
<sup>a</sup> Number of *Rickettsiella*-infected insects/number of insects examined.

**Table S2.** Primers and probes used in this study

Target symbiont	Target gene	Primer or Probe name	Sequence(5' -3')	Product size (kb) /fluorechrome at 5' end	References*
<b>Primers for cloning and sequencing</b>					
Eubacteria	16S rRNA	16SA1	AGAGTTGATCMTGGCTCAG	1.5	1
		16SB1	TACGGYTACCTGTTACGACTT		1
	16S rRNA	16SA1	AGAGTTGATCMTGGCTCAG	0.94	1
		γ940R	ACATGCTCCACCGCTTGTG		2
<b>Primers for specific PCR detection<sup>†</sup></b>					
<i>Buchnera</i>	16S rRNA	Buch16S1F	GAGCTTGCTCTCTTGTGGCAA	0.43	3
		Buch16S1R	CTTCTGCGGGTAACGTCACGAA		3
<i>Serratia</i>	16S rRNA	16SA1	AGAGTTGATCMTGGCTCAG	0.48	1
		PASScmp	GCAATGTCTTATTAAACACAT		4
<i>Regiella</i>	16S rRNA	U99F	ATCGGGGAGTAGCTTGCTAC	0.22	5
		16SB4	CTAGAGATCGTCGCCTAGGTA		6
<i>Hamiltonella</i>	16S rRNA	PABSF	AGCACAGTTACTGAGTTCA	0.22	5
		16SB4	CTAGAGATCGTCGCCTAGGTA		6
<i>Rickettsiella</i>	16S rRNA	RCL16S-211F	GGGCCTTGCCTCTAGGT	0.26	7
		RCL16S-470R	TGGGTACCGTCACAGTAATCGA		7
	gyrB	RclGyrB-AF1	GAGGCCACTGAAATCCGCTTTATCC	0.12	7
		RclGyrB-AR1	GGCAACGCCAGAATTAGGAATGAG		7
<b>Probes for <i>in situ</i> hybridization</b>					
<i>Buchnera</i>	16S rRNA	ApisP2	CCTCTTTGGGTAGATCC	AlexaFluor 488 or 555	8
<i>Rickettsiella</i>	16S rRNA	RCL1252R	TCGCGGGTTGGCTTCCT	AlexaFluor 555	7
<i>Hamiltonella</i>	16S rRNA	BTH	CCAGATTCCCAGACTTTACTCA	AlexaFluor 488	9

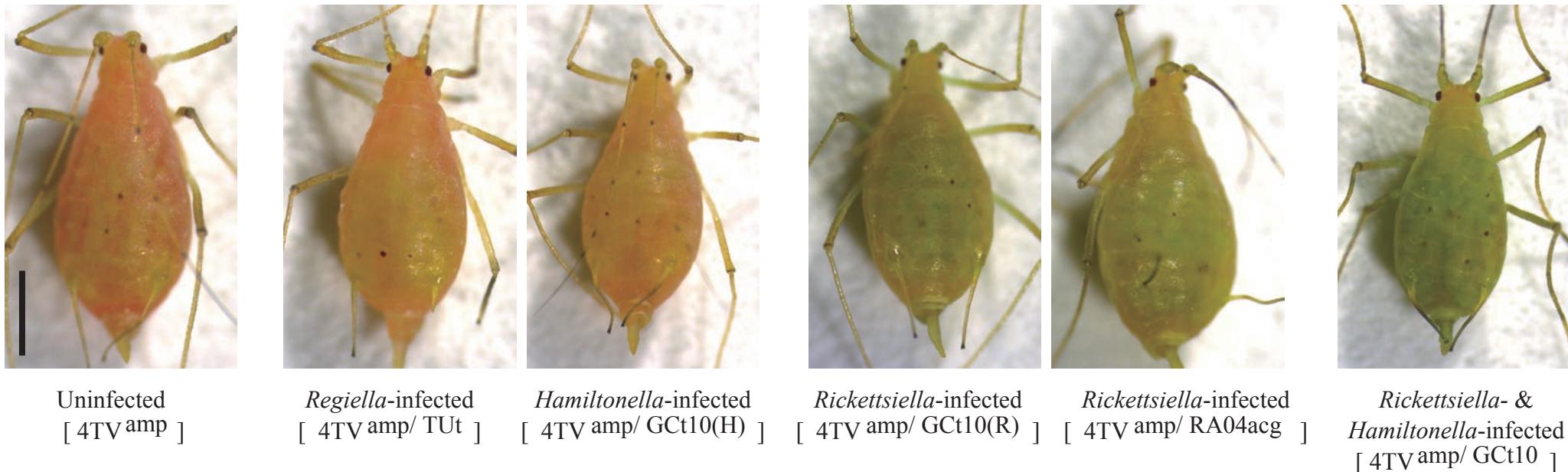
\* References: 1, Fukatsu and Nikoh (1998) *Appl. Environ. Microbiol.* 64, 3599; 2, this study; 3, Tsuchida *et al.* (2002) *Mol. Ecol.* 11, 2123; 4, Fukatsu *et al.* (2000) *Appl. Environ. Microbiol.* 66, 2748; 5, Sandström *et al.* (2001) *Mol. Ecol.* 10, 217; 6, Tsuchida *et al.* (2005) *Microb. Ecol.* 49, 126; 7, Tsuchida *et al.* (2010) *Science* 330, 1102; 8, Koga *et al.* (2003) *Proc. R. Soc. Lond., B, Biol. Sci.* 270, 2543; 9, Gottlieb *et al.* (2008) *FASEB J.* 22, 2591. <sup>†</sup>*Rickettsiella*-specific primer sets targeting 16S rRNA gene and *gyrB* gene were used for both diagnostic PCR and real-time PCR detection. The other primers were used for diagnostic PCR only.

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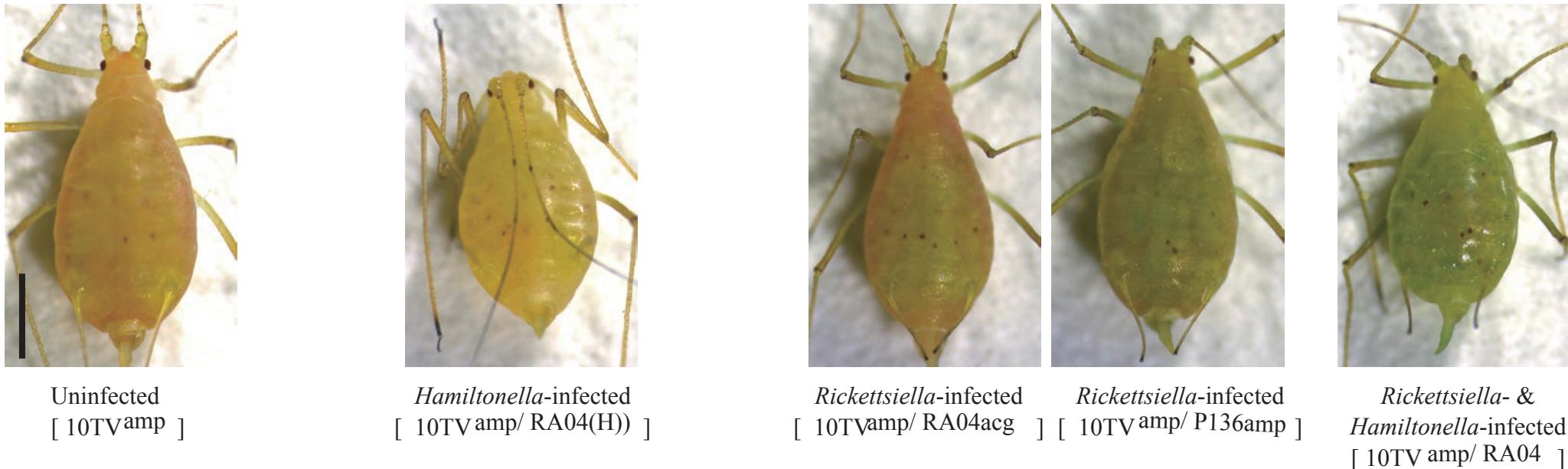


**Fig.S1.** HSB color model to evaluate aphid body color. According to this model, any color is represented by three factors, hue, saturation, and brightness. Hue indicates the property of colors, measured in angular degrees starting and ending at red (0 or 360 degrees). Pure green hue is 120 degrees. Saturation represents the purity of the color, measured in percent from center of the cone (0% = no color) to the surface (100% = full color). Brightness is measured in percent from black (0) to white (100).

(A)



(B)



**Fig. S2.** Different color-changing effects of *Rickettsiella* strain and other facultative symbionts on the pea aphid of identical genetic background: (A) 4TV<sup>amp</sup> and (B) 10TV<sup>amp</sup>. Bars = 1 mm.