PNAS
www.pnas.org

Supplementary Information for

Strong within-host selection in a maternally inherited obligate symbiont: *Buchnera* and aphids

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Supplementary Methods

Aphid and *Buchnera* lines. The *Buchnera* haplotypes used in this study (LSR1, 5A, 5AY) are closely related: their 641 kb genomes differ at fewer than 25 sites, and whole genome phylogenies place them in an exclusively North American cluster that probably diverged following introduction of the matriline founder from Europe less than 150 years ago (cluster 2 in (1)). A single aphid genotype, *A. pisum* LSR1, was used for all experiments. Thus, *Buchnera* LSR1 was the "resident" haplotype, and two foreign *Buchnera* haplotypes, 5A and 5AY, were "donor" haplotypes injected to establish heteroplasmic lines. *A. pisum* 5A and 5AY descend from a single female collected in Madison, Wisconsin USA in 1999. *A. pisum* 5AY was derived from *A. pisum* 5A in 2001 (2). While *Buchnera* 5AY possesses the functional heat shock promoter for *ibpA,* a small heat shock protein, *Buchnera* 5A and *Buchnera* LSR1 are fixed for a single base pair deletion that lowers *ibpA* expression and thereby lowers thermal tolerance of these haplotypes (3, 4). The *Buchnera* $5A^{E}$ haplotype arose during the course of this study and is described in Results. All stock *A. pisum* lines were maintained as parthenogenetic females on seedlings of fava bean plants (*Vicia faba*) at 20°C and 16L:8D photoperiod.

Establishing heteroplasmic matrilines (5A-LSR1 and 5AY-LSR1). To establish matrilines heteroplasmic for *Buchnera*, we used a modified procedure based on a previously published protocol (4). Reproductive *A. pisum* LSR1 were placed on seedlings and allowed to deposit nymphs for 24 hours to generate age-controlled recipient aphids. At 4 days-old, recipient aphids were exposed to 35°C for 4 hours to reduce resident *Buchnera* LSR1 titer and were returned to 20°C for approximately 24 hours before injections were performed. On the day of the injections, adult donor aphids possessing *Buchnera* 5A or 5AY were ground individually in 30 µL of Buffer A (25 mM KCl, 10 mM $MgCl_2$, 250 mM sucrose, 35 mM Tris-HCl, adjusted to pH 7.5). Aphid homogenate was injected into the ventral abdominal segments nearest to the posterior legs, using glass needles pulled from 5.0 µL glass capillary tubes and loaded into a Narishige IM-400 microinjector. Injection settings were set to 0.037 mPa, 0.20 s, delivering approximately 0.1 µL of the homogenate per injection. Injections of *Buchnera* 5A and 5AY were performed on separate days.

Injected aphids were placed on fava leaves in Petri dishes for 24 hours and survivors transferred to seedlings. Offspring born 9 to 12 days after injection were transferred to individual dishes containing a single fava leaf in 1.5% agar, where they were allowed to mature to adulthood and reproduce for 1 day. Mothers were then screened for *Buchnera* 5A and 5AY using PCR and a diagnostic restriction digest, as previously described (4). Offspring from mothers that tested positive for both recipient *Buchnera* LSR1 and donor *Buchnera* 5A or 5AY were retained. We selected matrilines with roughly intermediate haplotype frequencies, based on visual estimates of band intensities following PCR and digest. For both combinations (5A-LSR1 and 5AY-LSR1), we selected a single female to initiate the experiment.

Measuring *Buchnera* frequencies in heteroplasmic matrilines over generations. We estimated frequencies in matrilines over five generations, by sampling females at the same developmental stage in each generation. The host genetic background was unchanging, since we worked with clonal generations. Each female was allowed to reach adulthood and reproduce for a single day. Aphids reproduce asexually and are viviparous, with each daughter undergoing prenatal development within the maternal ovariole. Progeny are infected by *Buchnera* from maternal bacteriocytes at an early embryonic stage and have a robust *Buchnera* population confined within their own bacteriocytes by the time of birth (5). After 1 day of reproduction, we harvested each mother and retained 3 of her progeny for the next generation, again allowing them to reach adulthood on separate plants and to reproduce for 1 day. Each aphid was confined to a separate clip cage on a leaf. We continued this for 5 generations (Fig. 1). In generation 5, only 2 offspring were sampled. Thus, each sampled female was treated as both a daughter and a mother in our analysis, except for the initial and final generations.

We established two haplotype combinations, one differing in the *ibpA* mutation and thus expected to be under temperature-dependent selection (5AY-LSR1), and one for which we expected no selection (5A-LSR1). For each combination, we imposed two temperature conditions,

constant 20°C (cool), and constant 20°C interrupted by 4 hours at 33°C during each 24-hour cycle for all 5 generations (heat). For the 5AY donor, we expected an advantage of *Buchnera* 5AY over *Buchnera* LSR1 under the heat condition, since 5AY has the responsive (long) *ibpA* promoter which increases *Buchnera* survival under heat challenge (3). Under constant 20°C, we had no prediction for selection within hosts, although the unresponsive (short) *ibpA* promoter was shown to be beneficial for host fitness at constant 20°C or constant 15°C (3). For the 5A donor, we did not expect a difference in fitness, as *Buchnera* 5A and *Buchnera* LSR1 both have the same *ibpA* promoter sequence, and their genomes differ in only 16 nucleotide differences, none of which disrupt genes (including 5 intergenic, 5 synonymous, and 6 nonsynonymous differences, Table S1).

To estimate *Buchnera* haplotype frequencies, DNA was extracted from individual aphids, and PCR was performed using primers 5AL81_F and 5AL81_R (Table S2). These primers amplify an 81 base pair fragment containing a single nucleotide difference (A/C; #13 in Table S1). A two-step PCR using Phusion® HF DNA Polymerase (M0530S, NEB) was conducted. For the first step, the conditions were: 98°C for 30s; 25 cycles of 98°C for 10 s, 55°C for 20 s, and 72°C for 15 s; and 72°C for 5 min, followed by bead cleanup. The second step PCR used the Nextera XT index Kit v2 set A and set C with conditions: 98°C for 30 s; 10 cycles of 98°C for 10 s, 55°C for 20 s, and 72°C for 15 s; and 72°C for 5 min. After purification of PCR amplicons, concentrations were determined and libraries constructed. A total of 184, 178, 187 and 192 libraries from each of the four treatments were pooled and sequenced from both ends (2 × 150 bp) on an Illumina iSeq100 machine. We used Trimmomatic v0.38 to trim adaptors and remove low quality base pairs with the default parameters (6).

We sampled a total of 732 aphids which gave 165, 165, 182, and 175 mother-daughter pairs for the four combinations of haplotypes and environments: 5AY-cool, 5AY-heat, 5A-cool, and 5A-heat. In a few cases, aphids died before reproducing, causing lines to be terminated early. After filtering low quality reads, we obtained an average of 25,639 reads per aphid, with a range of 136 to 80,508 reads. A small proportion of reads (0.8%) passed the quality check but did not have an A or C at the variable site, and these were eliminated. For 97.3% of samples, read depth was sufficient to yield 95% confidence intervals with boundaries <1% from the estimated haplotype frequency. We performed the analyses described below with the full dataset and also with a trimmed dataset, where each individual was a mother to only one daughter, and samples with fewer than 80,000 reads were removed. This reduced the numbers of mother-daughter pairs to 72, 72, 77, and 74. Estimates for N_{SYM} and s_{SYM} were minimally affected, and so we report the results for the full dataset rather than the trimmed dataset.

Estimating N_{SYM} and s_{SYM} for *Buchnera*. We estimated the population size and relative fitness of *Buchnera* haplotypes within matrilines using likelihood. To derive the likelihood function, we assumed a highly simplified model for the evolution of its allele frequencies that captures the essential elements of selection and drift acting on *Buchnera* within its aphid host. The model does not account for details of the population dynamics of *Buchnera* within hosts, and the results are therefore approximate. It seems likely, however, that the errors introduced by the simplifications of the model are no larger than those resulting from the experimental paradigm used to obtain the data. The likelihood function derived here can be used to derive maximum likelihood estimates for s_{SYM}, the selection coefficient acting on a focal strain of *Buchnera*, and N_{SYM}, the effective population size of the bacterium within a host matriline, and confidence regions for those parameters.

In the text, we refer to N_{SYM} as the "bottleneck size" because the model developed below assumes that most drift occurs during the bottleneck that takes place when a mother aphid inoculates her offspring. In reality, drift also occurs in the bacterial population throughout the aphid's life, as subpopulations of symbionts are packaged into bacteriocytes where they replicate in independent subpopulations. The selection coefficient represents the cumulative effects of selection during the aphid's life and during transmission of the symbionts from a mother aphid to her offspring. Thus, it is best to regard N_{SYM} as an "effective population size" and s_{SYM} as an "effective selection coefficient". Importantly, both parameters describe the effect of drift and selection during a single aphid generation, not during a single bacterial generation.

Assumptions. We assume that the dominant event that causes random genetic drift in *Buchnera* is the bottleneck that occurs when an offspring aphid is inoculated with the bacterium by its mother and also the packaging into bacteriocytes that occurs during aphid development. To be specific, we assume that following the inoculation, the population of *Buchnera* within the offspring grows quickly to a sufficient size that drift is negligible relative to the bottleneck, and this assumption generally fits with empirical evidence (5). We assume that selection acts deterministically during the bacterium's phase in the host and/or during the transmission from mother to offspring. Finally, we assume that a mother inoculates each offspring with a random sample from her bacterial population.

Notation. We write the frequency of the focal strain of *Buchnera* in aphid *i* when it is first inoculated by its mother as p_i, and its frequency after selection has acted on the bacteria as p_i^*. When a mature aphid transmits *Buchnera* to the next generation, the expected frequency of the focal strain among her offspring is again p_i^*, but in any particular offspring the frequency will differ from that expectation because of drift that occurs during transmission. We denote the frequency of the focal strain in the j^{th} offspring of mother aphid *i* as p_{ij}. When that offspring matures into an adult, the frequency of the focal *Buchnera* strain in her will be p_{ij}^* (which will differ from p_{ij} because of selection).

Estimating the strengths of selection and drift. Our goal is to estimate the population size N_{SYM} and the selection coefficient s_{SYM} given the observed frequencies of the focal *Buchnera* strain in aphid mothers (the p_i^*) and in their mature offspring (the p_{ij}^*). We assume that the effects of drift are much larger than the errors in estimating the strain frequencies, so the latter can be ignored in our calculations.

The likelihood of the frequencies of the focal *Buchnera* strain observed in the offspring is

$$L(N_{\text{SYM}}, s) = \prod_{i,j} P\left(p_{ij}^* \mid p_i^*, N_{\text{SYM}}, s_{\text{SYM}}\right), \tag{1}$$

where $P\left(p_{ij}^* \mid p_i^*, N_{\text{SYM}}, s_{\text{SYM}}\right)$ is the probability that the frequency of the focal *Buchnera* strain in a mature offspring aphid is p_{ij}^*, given that its mother's frequency was p_i^* at the time that she transmitted the bacteria to that offspring. The probabilities for each transmission event are multiplied because we assume that the bacteria are sampled independently from the mothers.

Under our simple model in which drift in *Buchnera* occurs only during the transmission from mother to daughter aphid, the probability density appearing in Equation (1) is

$$P\left(p_{ij}^* \mid p_i^*, N_{\text{SYM}}, s_{\text{SYM}}\right) = B\left(N_{\text{SYM}}\, p_{ij} \mid N_{\text{SYM}}, p_i^*\right), \tag{2}$$

where $B\left(N_{\text{SYM}}\, p_{ij} \mid N_{\text{SYM}}, p_i^*\right)$ is the binomial probability density function with N_{SYM} trials each with p_i^* probability of success.

We now need an expression for p_{ij}, the frequency of the focal strain in offspring *ij* when she was first inoculated by her mother, in terms of p_{ij}^*, the frequency that we observe in her when she is an adult. From basic population genetics and a bit of algebra, we find that

$$p_{ij} = \frac{p_{ij}^*}{1+\left(1-p_{ij}^*\right)s}. \tag{3}$$

Substituting that result into Eq. (2), we have

$$P\left(p_{ij}^* \mid p_i^*, N_{\text{SYM}}, s_{\text{SYM}}\right) = B\left(\frac{N_{\text{SYM}}\, p_{ij}^*}{1+\left(1-p_{ij}^*\right)s} \mid N_{\text{SYM}}, p_i^*\right). \tag{4}$$

Last, there is an annoying minor technical complication. Because the binomial distribution is discrete, the probability density in Equation (4) is only nonzero when the first argument of *B*() is an integer. It is convenient to maximize the likelihood over continuously distributed parameters. We

can do so by simply omitting the factorial terms that appear in the binomial density function (since they do not depend on the parameters of interest):

$$\tilde{B}(i \mid n, p) \;=\; p^{i}(1-p)^{n-i}. \tag{5}$$

The likelihood function is then

$$L(N_{\mathrm{SYM}}, s_{\mathrm{SYM}}) = \prod_{i,j} \tilde{B}\left(\frac{N_{\mathrm{SYM}}\, p_{ij}^{*}}{1+\left(1-p_{ij}^{*}\right) s} \mid N_{\mathrm{SYM}}, p_{i}^{*}\right). \tag{6}$$

We found maximum likelihood estimates for N_{SYM} and s_{SYM} by maximizing Eq. (6) numerically, and determined the approximate 95% confidence regions by finding combinations of N_{SYM} and s_{SYM} whose likelihoods are within a factor of 20 of the maximum.

We used the same equation to estimate s_{HOST}, the magnitude of selection acting on hosts fixed for different *Buchnera* haplotypes. In this experiment, the value of N_{HOST} is not of interest, since it is determined by the experimental setup.

Resequencing *Buchnera* genomes. A previous study had determined that over 7 years in the laboratory, 2 new *Buchnera* mutations, both single nucleotide changes, arose and became fixed in two lines descending from an *A. pisum* 5A ancestor (1). The infrequency of new substitutions indicates that the genomes are generally stable and not undergoing rapid mutational change. Therefore, we expected the three matrilines (LSR1, 5A, and 5AY) to possess *Buchnera* with genomes near-identical to each other and to the *Buchnera* 5A and *Buchnera* LSR1 genome sequences previously deposited in GenBank (accessions GCF000174075.1 and GCF000121085.1). We resequenced *Buchnera* genomes for all experimental lines to verify genomic differences arising from subsequent mutations as well as potential errors in the publicly available sequences. We also resequenced at the end of the experiment, in generation 5, lines that were fixed for *Buchnera* 5A. All genomic differences are listed in Table S1; reconstruction of the genomic changes between different lines is shown in Fig. S1. Within the 641 kilobase genome, the greatest pairwise divergence was 21 differences, most being changes at synonymous or intergenic sites. There were 28 changes across all experimental lines, of which 16 of separated 5A from LSR1 and an additional 12 occurred within the 5A lab-reared matrilines. An additional 11 differences occurred between all resequenced lines and the GenBank sequence, suggesting errors in the public sequences. *Buchnera* 5A and *Buchnera* 5AY used in our experiments had diverged for 18 years and had fixed differences at 6 sites, including the recurring single base insertion/deletion in the promoter of *ibpA* for *Buchnera* 5A previously described (3).

Measuring aphid fitness parameters. We measured fitness parameters for *A. pisum* LSR1 fixed with *Buchnera* $5A^{E}$ to *A. pisum* LSR1 fixed with *Buchnera* LSR1, using cages and plants as for the earlier experiments. To control for subline effects, we first established 4 sublines of the *Buchnera* LSR1 line and 6 sublines of the *Buchnera* $5A^{E}$ line and grew sublines independently for 3 generations before the experiments began. We then allowed adult females to deposit progeny overnight on host plants, removed the mothers, and followed the progeny. We recorded the number of days to first reproduction and weight on the first day of reproduction. We placed these young adults in Petri dishes with fresh fava leaves with petioles embedded in agar, changing the leaves every 2 days. We allowed them to reproduce for 7 days, counting and removing progeny daily. Fecundity was scored as number of progeny in the first 7 days of reproduction. Experiments were conducted at constant 20°C and under a 16L:8D photoperiod. Only 1 aphid died during this experiment, and we obtained data for 59 and 60 individuals for the LSR1 and $5A^{E}$ matrilines respectively.

Estimating between-host selection using population-level competition. To compare the fitness of *A. pisum* LSR1 fixed with *Buchnera* $5A^{E}$ to *A. pisum* LSR1 fixed with *Buchnera* LSR1, we set up a population-level competition experiment between aphids fixed for these two haplotypes

with *A. pisum* host genotype LSR1 (Fig. 5). For each haplotype, 3 sublines were again established and maintained separately for 3 generations. Sublines were paired for each LSR1-5A^E subline combination, to establish 9 distinct competition pairs, and 2 replicates were maintained per pair, giving 18 replicate populations. Each replicate was initiated with 5 reproductive, 10-day-old females from each haplotype. Aphid populations were maintained in closed containers with two *V. faba* plants per container, at 20°C and under a 16L:8D photoperiod, in growth chambers of the same models as for the within-host experiment. After 14 days, all aphids were gently removed from plants, collected in a Petri dish, weighed, mixed, and 200 mg transferred to a new container with fresh plants. Remaining aphids were frozen at -20°C for DNA extraction. Samples consisted of mixed-aged pools of aphids, since females reproduce continuously, with reproductive periods longer than time from birth to first reproduction. Collections and transfers were repeated every 7 days. At 10 weeks, collection was discontinued for 6 of the 18 populations, due to limitations on laboratory work during the covid-19 pandemic shutdown. Remaining populations were maintained for an additional 2 weeks, to 12 weeks in total. Additionally, one timepoint at week 7 for population 6 was not collected for sequencing because the total weight collected was 200 mg and all aphids were used for transfer (top right, Fig. S4). The total number of population samples was 203.

To estimate haplotype frequencies in each population sample, we weighed 100 mg of frozen aphids from the sample (~100 aphids of varying ages) and extracted DNA using the Qiagen DNeasy Blood & Tissue Kit. We then used AccuStart II PCR SuperMix to perform nested PCRs. The first PCR used primers IGR-FW and IGR-RV (Table S2) amplifying a 136 bp region for *Buchnera* LSR1 and a 149 bp region for *Buchnera* 5A^E, spanning the 13-nucleotide insertion (#3 in Table S1). We used the following cycling conditions: 94°C for 3 min, 94°C for 30 sec, 54°C for 30 sec, 72°C for 1 min, repeat steps 2-4 for 29 additional cycles, and 72°C for 10 min. The second PCR was used to append Nextera primers and was performed with the following cycling conditions: 94°C for 3 min, 94°C for 20 sec, 55°C for 30 sec, 72°C for 1 min, repeat steps 2-4 for 9 additional cycles, and 72°C for 10 min. Bead cleanup was used to clean PCR products between and after nested PCRs. Products were then quantified and combined for 2x150 bp paired-end sequencing on the Illumina iSeq 100 system. PhiX was spiked-in at 30% to increase base diversity and ensure more accurate barcode assignment. Sequencing reads were aligned to the sequence for *Buchnera* 5A^E and *Buchnera* LSR1 or *Buchnera* 5A^E variants were quantified using FreeBayes (7).

To validate our approach, we included a standard curve with 0:10, 1:9, 2:8, 3:7, 4:6, 50:50, 6:4, 7:3, 8:2, 9:1, and 10:0 of *Buchnera* 5A^E: *Buchnera* LSR1 (Fig. S3). We used 7-day-old aphids and combined 10 aphids total for each point. These samples were extracted, amplified, and sequenced similarly to the experimental samples. We found a reasonably tight fit (Fig. S3). Some noise likely reflects barcode-hopping during the Illumina sequencing; also, the *Buchnera* 5A^E frequencies are slightly underestimated, probably because the slightly shorter amplicon was disfavored during PCR. However, these slight errors would apply equally to all of our samples, which were all sequenced on the same Illumina iSeq run, so the comparisons are generally robust. A maximum likelihood estimate of s_{HOST} across the weekly time frame was obtained using Eq. 1. In this case N_{HOST} is not of interest since it merely reflects the sampling scheme of our experiment as well as the error in estimating frequencies from the pooled aphids.

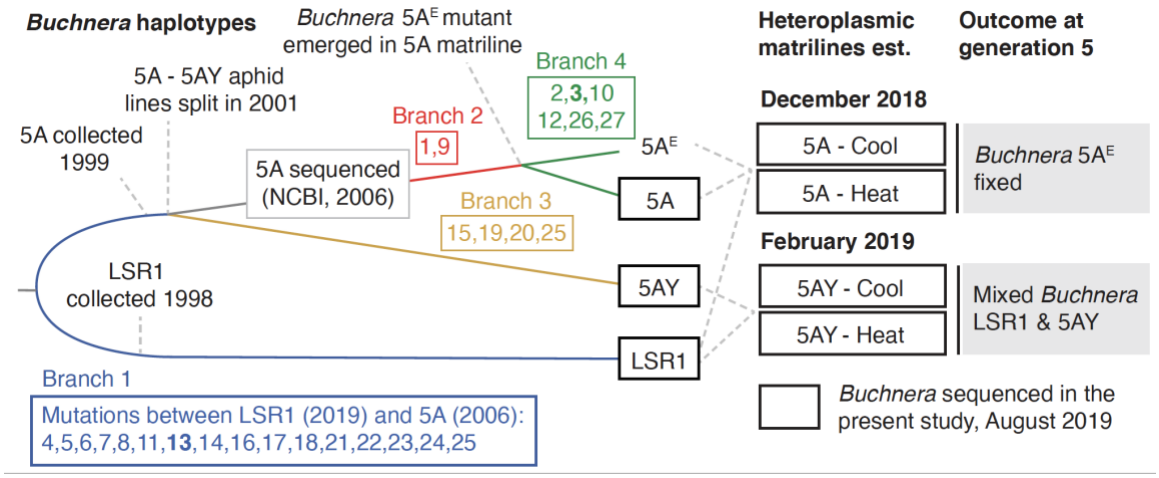

Fig. S1. Chronology of sequence changes occurring in the experimental *Buchnera* lines. Numbers refer to individual mutations, which are listed in Table S1. 5A, 5A^{E}, 5AY, and LSR1 refer to the *Buchnera* genotypes used in the experiments. Bolded numbers indicate mutations used to identify the haplotypes in sequencing runs.

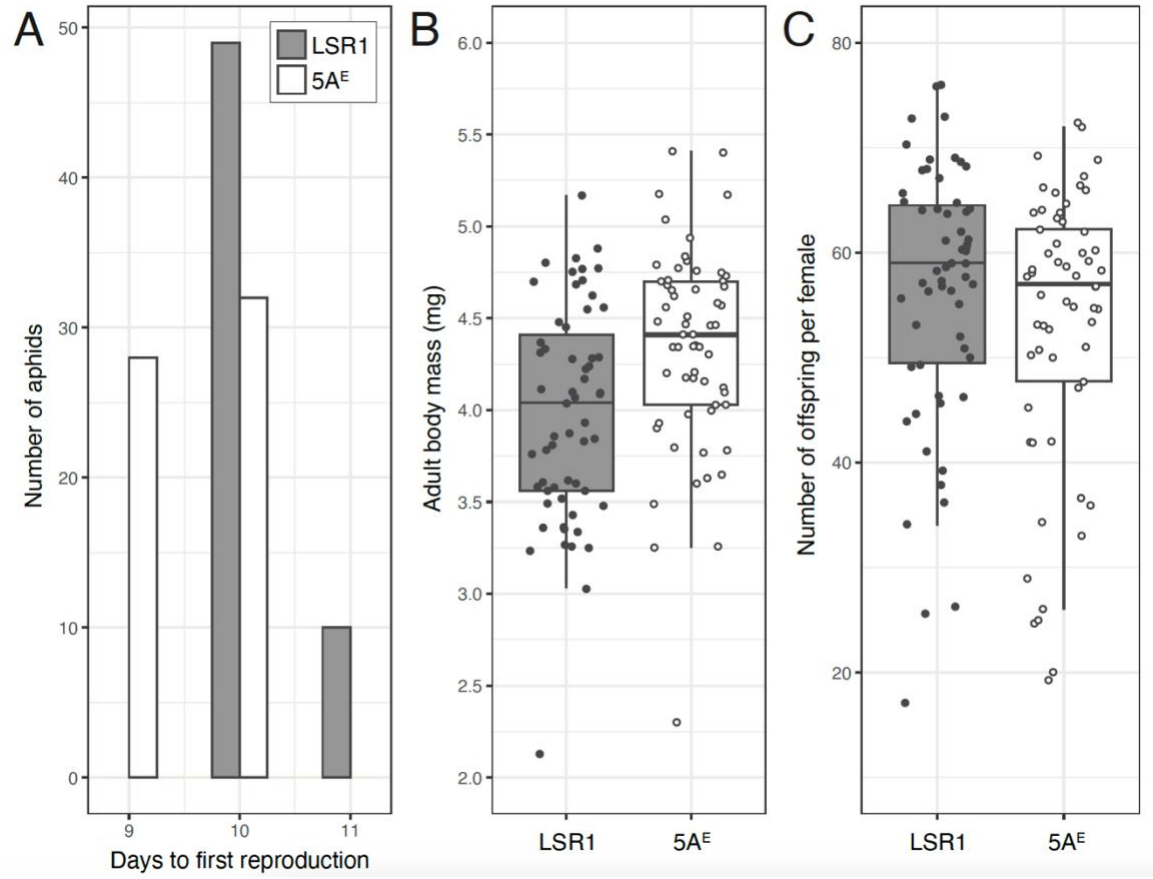

Fig. S2. Estimates of fitness-related parameters for aphid lines fixed for *Buchnera* haplotypes 5A^E or LSR1. Plots show (A) time in days from birth to first reproduction, (B) body mass on first day of adulthood, and (C) number of offspring produced in the first 7 days of reproduction. For B and C, dots represent measurements from individual aphids and boxplots represent median, IQR, and 95% CI. N=60 and 59 for 5A^E and LSR1, respectively.

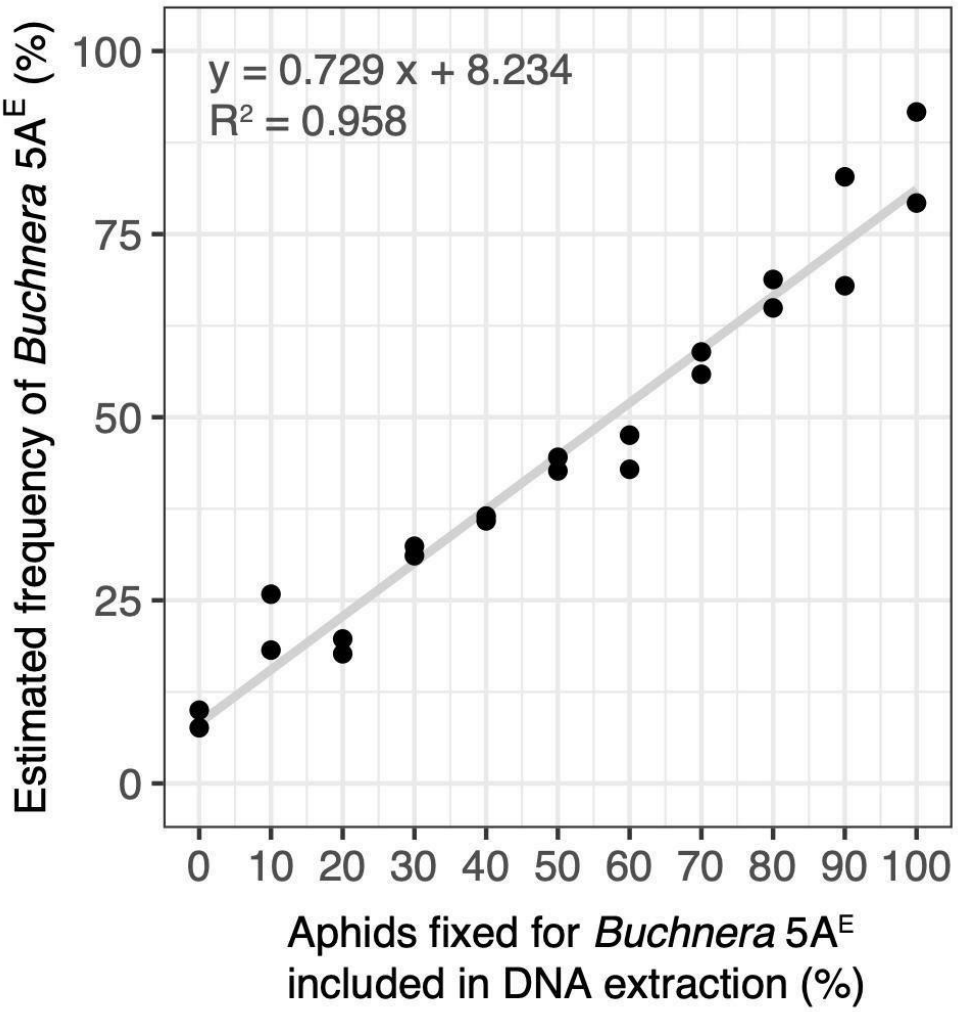

Fig. S3. Standard curve for estimating *Buchnera* haplotype frequencies in successive population samples, in the host-level selection experiment. Known ratios of aphids with each *Buchnera* haplotype were combined into a sample. The estimated frequencies of *Buchnera* $5A^E$ were obtained in the same sequencing run used for the experimental samples.

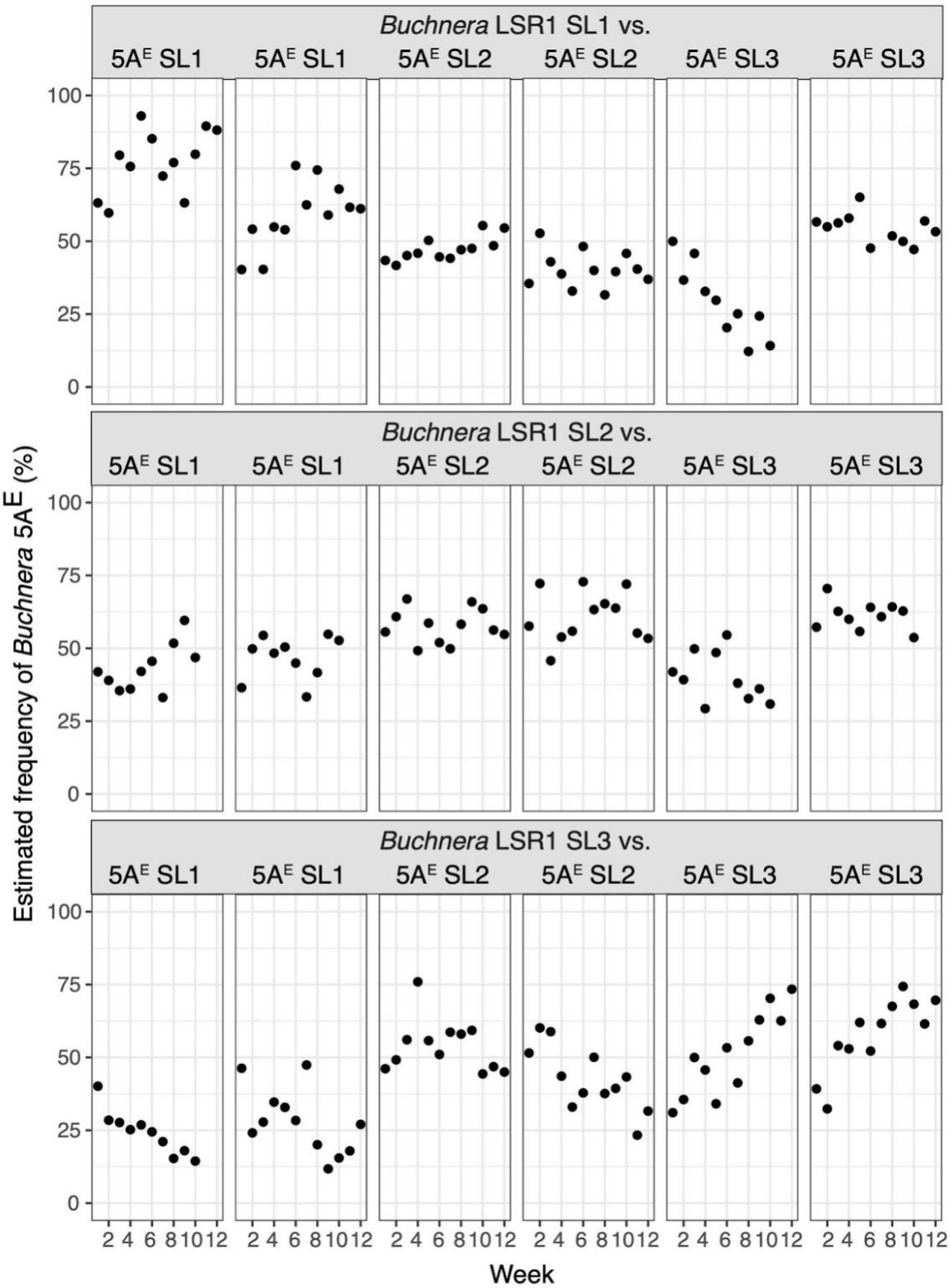

Fig. S4. Weekly changes in frequencies of *Buchnera* haplotypes 5A^E and LSR1 in the host-level selection experiment. "SL" refers to aphid subline within each of the two aphid lines. Each panel shows a continuous experiment, initiated with equal numbers of aphids with the two *Buchnera* haplotypes.

Table S1. Genomic sequence differences among *Buchnera* haplotypes used in experiments.

Number	Branch [+]	*Buchnera* 5A (NCBI) position[++]	Aphid lines with 1st state	Aphid lines with 2nd state	Mutation[+++]		Annotation	Gene	Description of Gene Product
1	B2	12,880	5AY, LSR1	5A, $5A^E$	G→T	R	R347S (CGT→AGT)	*dnaA*	chromosomal replication initiator protein DnaA
2	B4	16,577	5A, 5AY, LSR1	$5A^E$	T→G	R	F523C (TTT→TGT)	*yidC*	membrane protein insertase YidC
3*	B4	21,004-21,016	5A, 5AY, LSR1	$5A^E$	$(CTTTTCATATAAA)_{1→2}$	I	intergenic		heat shock protein GroEL / elongation factor P
4	B1	30,443	LSR1	5A, $5A^E$, 5AY	T→C	I	intergenic		Cof-type HAD-IIB family hydrolase/ 5-methyl-tetrahydropteroyl triglutamate--homocysteine methyltransferase
5	B1	53,411	LSR1	5A, $5A^E$, 5AY	G→C	S	G9G (GGG→GGC)	*argB*	N-acetylglutamate kinase
6	B1	64,877	LSR1	5A, $5A^E$, 5AY	$(A)_{7→6}$	I	intergenic		3,4-dihydroxy-2-butanone-4-phosphate synthase / D-glycero-beta-D-manno-heptose-7-phosphate kinase
7	B1	79,118	LSR1	5A, $5A^E$, 5AY	C→T	S	L137L (CTA→TTA)	*fliG*	flagellar motor switch protein FliG
8	B1	83,872	LSR1	5A, $5A^E$, 5AY	C→T	R	I160T (ACA→ATA)	*fliM*	flagellar motor switch protein FliM

9	B2	128,266	LSR1, 5AY	5A, 5A^{E}	G→A	R	M146I (ATG→ATA)	*ydiK*	AI-2E family transporter
10	B4	143,766	LSR1, 5A, 5AY	5A^{E}	C→T	I	intergenic		glycine--tRNA ligase alpha/ endonuclease
11	B1	198,431	LSR1	5A, 5A^{E}, 5AY	A→G	R	E32K (AAA→GAA)		DUF2076 domain-containing protein
12	B4	283,458	5A, 5AY, LSR1	5A^{E}	C→T	S	L135L (TTG→TTA)	*era*	GTPase Era
13**	B1	293,455	LSR1	5A, 5A^{E}, 5AY	C→A	S	R280R(CGC →CGA)	*rnb*	exoribonuclease 2
14	B1	316,271	LSR1	5A, 5A^{E}, 5AY	C→T	R	T284I (ACA→ATA)	*ispG*	4-hydroxy-3-methylbut-2-enyl diphosphate synthase (flavodoxin)
15	B3	332,611	5A, 5A^{E}, LSR1	5AY	$(A)_{10\rightarrow9}$	F	coding (360/1452 nt)	*phrB*	deoxyribodipyrimidine photo-lyase
16	B1	381,302	LSR1	5A, 5A^{E}, 5AY	C→A	R	S717I (AGC→ATC)	*rne*	ribonuclease E
17	B1	396,685	LSR1	5A, 5A^{E}, 5AY	T→C	I	intergenic		nicotinate phosphoribosyltransferase/ dihydroorotate dehydrogenase
18	B1	426,309	LSR1	5A, 5A^{E}, 5AY	$(T)_{10\rightarrow11}$	I	intergenic		23S rRNA (uridine(2552) -2'-O) -methyltrans-ferase/ transcription elongation factor GreA
19	B3	509,090	5A, 5A^{E}, LSR1	5AY	G→A	R	V68I (GTA→ATA)	*ribD*	5-amino-6-(5-phosphoribosylamino) uracil reductase

20	B3	532,105	5A, 5A^{E}, LSR1	5AY	G →A	S	L502L (TTG→TTA)	*smdB*	multidrug ABC transporter permease/ATP-binding protein
21	B1	568,745	LSR1	5A, 5A^{E}, 5AY	T→C	I	intergenic		FKBP-type peptidyl-prolyl cis-trans isomerase/ aspartate aminotransferase family
22	B1	571,078	LSR1	5A, 5A^{E}, 5AY	G→A	S	V246V (GTG→GTA)	*tsgA*	MFS transporter
23	B1	576,416	LSR1	5A, 5A^{E}, 5AY	T→C	S	L230L (TTA→TTG)	*deoD*	purine-nucleoside phosphorylase
24	B1	609,063	LSR1	5A, 5A^{E}, 5AY	T→C	R	I528V (ATC→GTC)	*pgi*	glucose-6-phosphate isomerase
25**	B1, B3	615,570	5AY	5A, 5A^{E}, LSR1	$(A)_{11\rightarrow10}$	I	intergenic		Heat shock promoter for heat-shock protein IbpA
26	B4	pTrp 597	LSR1, 5A, 5AY	5A^{E}	T→C	S	G444G (GGT→GGC)	*trpE*	Anthranilate synthase subunit
27	B4	pTrp 2,494	LSR1, 5A, 5AY	5A^{E}	GTGGG→ GT-GG	I	intergenic		Large intergenic region between anthranilate synthase subunit copies

* Mutation #3, insertion, was polymorphic in founder female 5A^{E} and fixed during the course of the experiment (by generation 5). This mutation was used to distinguish aphids fixed for different *Buchnera* haplotypes in the between-host selection experiment.
** Mutation #13, a single base polymorphism, was used to distinguish *Buchnera* LSR1 from *Buchnera* 5A and 5AY in the within-host selection experiments.
***Mutation #25, a single base deletion in the *ibpA* heat shock promoter, was fixed independently in 5A and LSR1 and reverted in 5AY.
$^{+}$ Branch refers to the lineage in which the mutation occurred, shown in Fig. S1. Branch 4, with 4 mutations, leads to *Buchnera* 5A^{E}.
$^{++}$ NCBI accession CP001161.1 for *Buchnera aphidicola* str. 5A (*Acyrthosiphon pisum*); for the 2 plasmid mutations, numbering is based on NCBI accession NC_002252.
$^{+++}$ Mutation types: R=amino acid replacement, S=silent, F=frameshift, I=intergenic

Table S2. Primers used to amplify *Buchnera* polymorphic sites diagnostic for experimental haplotypes.

Primer name	Sequence (5'-3')*	Haplotypes distinguished
5AL81_F	ACCCCGTAATTTATCAGAAGA	LSR1 versus 5A/5AY (within-host selection experiment)
5AL81_R	AAAACCGTAATATGACATGC	
IGR-FW	TCGTCGGCAGCGTC agatgtgtataagagacag**agaagactatactttactttattt**	LSR1 versus $5A^{E}$ (between-host selection experiment)
IGR-RV	GTCTCGTGGGCTCGG agatgtgtataagagacag**ctatgatatattctcatacaacc**	

* CAPITALS indicate overlap sequences (TCGTCGGCAGCGTC, GTCTCGTGGGCTCGG)
bold type indicates adapter trimming sequence (agatgtgtataagagacag)

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