Supporting Information

Moran and Yun 10.1073/pnas.1420037112

SI Methods

Selection of Aphid Clones and Rearing Conditions. All aphids were grown in cup cages, as previously described (1), and were kept at 20 °C at 18 h of light, 6 h of dark, with the exception of heat treatments. Aphids were maintained as strictly parthenogenetic matrilines. The recipient *A. pisum* matriline contained *Buchnera* with a heat-sensitive genotype, and the donor matriline contained *Buchnera* with a heat-tolerant genotype. The relevant difference is the presence of either 10 or 11 adenines in a homopolymeric run within the spacer between the −10 and −35 binding sites in the heat shock promoter of *ibpA* (2). Because the homopolymer undergoes a relatively high rate of single-base deletions and the shorter *ibpA* promoter allele confers a fitness advantage at temperatures of 15–20 °C, the short allele often becomes fixed in laboratory lines kept at lower temperatures over a period of years, as previously documented (2). Initially, we verified the *ibpA* promoter genotypes of several laboratory matrilines, using PCR and sequencing, with methods and primers as in Dunbar and colleagues (2). In our matriline of LSR1, the same aphid clone used for full-genome sequencing by the International Aphid Genome Consortium (3), *Buchnera* had become fixed for the shorter, heat-sensitive allele. Correspondingly, females had low fecundity after heat treatment of 35 °C for 4 h as juveniles (2). This low fecundity reflects the loss of *Buchnera* under heat treatment: embryos not colonized by *Buchnera*, which fail to develop into viable progeny. Our *A. pisum* 5A retained the heat-sensitive *Buchnera* promoter, documented in this matriline 7 y previously. In contrast, a subline of 5A, *A. pisum* 5AY, had reverted from the heat-sensitive to the heat-tolerant *Buchnera* allele and had correspondingly acquired heat tolerance, indicated by the low effect of the heat treatment on fecundity. *A. pisum* 5AY is descended from a single *A. pisum* 5A female with a mutation in the carotenoid biosynthetic gene of the aphid causing change in body color from pink to yellow-green (4). We checked other *Buchnera* SNPs, using the previously sequenced genome for *Buchnera* strain 5A (GenBank accession no. CP001161); using the presence of polymorphisms unique to *Buchnera* 5A, we verified that this was the 5AY line (5). We chose *A. pisum* LSR1 as the recipient matriline and *A. pisum* 5AY as the donor matriline. LSR1 and 5AY body colors are pink and green, respectively, reflecting a difference in aphid genotype. Neither *A. pisum* LSR1 nor *A. pisum* 5AY has a facultative symbiont, as established previously and as verified by PCR assays, using universal bacterial primers spanning the spacer between 16S and 23S ribosomal RNA genes (6). *Buchnera* is nearly unique among known bacteria in not having the genes for 16S rRNA and 23S rRNA near one another.

PCR and Restriction Digest Screen for *Buchnera* Genotypes. Markers discriminating the *Buchnera* genotypes of *A. pisum* LSR1 and *A. pisum* 5AY were developed on the basis of single-nucleotide differences between the genomes, both available at GenBank (accession nos. NZ_ACFK01000001 and CP00161). Available sequence data for *A. pisum* LSR1 contain numerous ambiguous and erroneous base calls, so we used PCR and sequencing to verify SNPs distinguishing *Buchnera* of our LSR1 and 5AY matrilines. Position 293455 in the *A. pisum* 5A genome consists of an adenine that introduces a SalI restriction site, whereas *Buchnera* of *A. pisum* LSR1 has a cytosine at this position, eliminating this restriction site. Using forward primer 5′-CCATGGTGGGTAACTCTAGCA-3′ and reverse primer 5′-TTGGTGGTATCCATGACCCTA-3′ with perfect match binding sites in both lines, we amplified a 514-bp fragment that was digested by SalI to fragments of 363 and 151 bp in LSR1 but not digested in 5AY (Fig. 2).

Injection Protocol. Adults of the recipient line, *A. pisum* LSR1, were allowed to larviposit on several fava bean seedlings in cup cages overnight, and adults were removed, leaving 5–15 newborn nymphs per plant. On day 2, these plants and aphids were subjected to 4 h (set 1) or 5 h (set 2) at 34.5–35 °C, and returned to 20 °C. On day 3, these were used as recipients in microinjection experiments. The donor *Buchnera* were injected by crushing the abdomen of a fourth instar or young adult *A. pisum* 5AY female with a pestle and injecting ∼0.1 mL of this homogenate into the recipient aphid, using a syringe holding a fine glass needle, pulled from a microcapillary tube. The homogenate was used immediately after crushing the donor aphid, and the injected aphid was transferred to a fresh fava bean seedling and placed at 20 °C. Surviving recipients were monitored. Those that attained adulthood were placed on separate plants and allowed to reproduce, and their progeny (referred to as F1 individuals) were allowed to mature and reproduce and were then screened for the presence of the recipient and donor *Buchnera* genotypes, using the PCR and SalI restriction digest described earlier. 5AY and LSR1 individuals from the parent matrilines were used as controls.

Additional Heat Treatment of Lines with Mixed *Buchnera* Populations. Some lines retained both *Buchnera* types after experimental inoculation, and the mixture was maintained for several generations. A 4-h heat treatment was given to 2-d-old nymphs of these lines, and these aphids were allowed to mature and reproduce. Mother and progeny were screened for *Buchnera* genotypes, as described.

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