Supplemental online methods

Sequencing of *Buchnera* amino acid genes

Amplification primers (table S2) were designed using the sequenced genome of *Buchnera* APS (NC_002528.1) and synthesized by Eurofins-MWG Operon. PCR conditions were an initial denaturing step at 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 54-60°C depending on primer Tm for 30 s, 72°C for 2-4 min depending on amplicon length, and a final extension of 10 min at 72°C. Products were visualized on 1% agarose gel in TBE stained with ethidium bromide, and amplicon sizes were determined using the Invitrogen 1kb plus DNA ladder. Products were extracted from the gel using the Qiaquick Gel Extraction kit (Qiagen). When necessary, PCR products were cloned using the pGEM T-Easy kit from Promega. Clones containing the inserts were selected by blue/white screening on LB agar plates with 100μg ampicillin, 0.5 mM IPTG and 80μg X-gal. White colonies were grown to log phase in LB media with 100μg/ml ampicillin and plasmids were extracted with the 5-Prime Fast Plasmid Mini-prep kit. Sequencing primers (table S2) were developed based on the published genome sequence of *Buchnera* APS and were placed every 400-500bp along the amplicon. Sequencing was performed by the University of Arizona Genetics Core in Tucson, AZ, USA by the dideoxy chain termination (Sanger) method on an ABI 3700XL. Sequences were manually edited and assembled in Sequencher 4.7 and compared to the reference sequence of *Buchnera* APS using BLAST.

Establishment of F1 clones

Briefly, clones 5A, G19, G17, G6 and 8-10-1 were reared on *V. faba* with a 16:8 L:D photoperiod. Clone 5A was selected as its Buchnera genome has been sequenced.
previously (Moran et al 2009), revealing no obvious mutations likely to impact essential amino acid biosynthesis. Prior to induction of sexual aphids, clones G19, G17, G6 and 8-10-1 were cured of secondary symbionts by injection of 4th instar nymphs with injection of 100mg/µl ampicillin. Aphids were then allowed to deposit nymphs, which were also injected with ampicillin at the 4th instar. Offspring of these aphids were screened for secondary symbionts as previously mentioned and were determined to be free of secondary symbionts for two generations prior to sexual induction. Over five weeks, the photoperiod was adjusted by decreasing the light period by 15 minute increments until a 12:12 L:D photoperiod was achieved when the temperature was reduced to 15° C and male aphids began to develop only in clones 5A and 8-10-1, which were then isolated on fresh V. faba plants. The other clones tested are likely lost the ability to produce sexual aphids, and are thought to be anholocyclic. Oviparous females were isolated at the 4th instar to ensure they had not mated. Two to three male A. pisum from one clone were placed on a plant with 6 to 8 females from the other clone and allowed to mate and deposit eggs for two weeks. Eggs were collected and surface sterilized with 10% bleach solution and placed on wet filter paper in an 18 mm Petri dish then allowed to diapause for 60 days at 4° C in the dark. Emerging nymphs were separated onto individual plants to establish genetically distinct clones. Nymphs from each clone were screen by PCR to verify that they did not have a secondary symbiont, and were sequenced at the argC locus of Buchnera for a SNP which verified the maternal lineage of the clone.

Supplementary Results
**Examination of other EAA-dependent clones.** The Almota clone exhibited a percent mass attained of 61%, and was considered EAA dependent. This clone was also tested on individual essential amino acid deletion diets and required isoleucine, methionine, arginine and leucine (figure S2). Sequencing of the *Buchnera* genes underlying the biosynthesis of these amino acids and no mutations were found that would be likely to disrupt the production of these essential amino acids (mutations in promoter regions, non-synonymous mutations, or indels).

Clones G17 and 7-2-1 had similar performance on all diets lacking individual essential amino acids (figure 2). These results suggest that any mutation responsible for the observed performance of 7-2-1 is unlikely to reside in essential amino acid biosynthesis genes. The cause of these requirements may be due to disruption of an aphid or Buchnera transporter (either of amino acids or general metabolites) or of production of an upstream metabolite common to the biosynthesis of several essential amino acids such as glutamine. An alternative hypothesis would be that every amino acid biosynthesis pathway contains a inactivating mutation, which seems unlikely.

**Clone amino acid requirement and host plant.** There was a significant interaction of collection plant with treatment in the experiment (table S3, $F_{8,4025} = 3.038$, $p = 0.002$). Since collection plants were represented by few clones, this result may reflect differences in host genotype response to diet rather than a real effect of collection host plant.

**Clone age and EAA dependence.** There was no significant interaction between the “age” of a clone (how long it had been propagated in the laboratory) and the clone’s
performance on the EAA/NEAA dietary assay ($t_{8} = -0.27$, $p = 0.785$) suggesting that adaptation to laboratory environments did not systematically affect performance on artificial diet.