Intraspecific genetic variation in hosts affects regulation of obligate heritable symbionts

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Symbiotic relationships promote biological diversification by unlocking new ecological niches. Over evolutionary time, hosts and symbionts often enter intimate and permanent relationships, which must be maintained and regulated for both lineages to persist. Many insect species harbor obligate, heritable symbiotic bacteria that provision essential nutrients and enable hosts to exploit niches that would otherwise be unavailable. Hosts must regulate symbiont population sizes, but optimal regulation may be affected by the need to respond to the ongoing evolution of symbionts, which experience high levels of genetic drift and potential selection for selfish traits. We address the extent of intraspecific variation in the regulation of a mutually obligate symbiosis, between the pea aphid (*Acyrthosiphon pisum*) and its maternally transmitted symbiont, *Buchnera aphidicola*. Using experimental crosses to identify effects of host genotypes, we measured symbiont titer, as the ratio of genomic copy numbers of symbiont and host, as well as developmental time and fecundity of hosts. We find a large (>10-fold) range in symbiont titer among genetically distinct aphid lines harboring the same *Buchnera* haplotype. Aphid clones also vary in fitness, measured as developmental time and fecundity, and genetically based variation in titer is correlated with host fitness, with higher titers corresponding to lower reproductive rates of hosts. Our work shows that obligate symbiosis is not static but instead is subject to short-term evolutionary dynamics, potentially reflecting coevolutionary interactions between host and symbiont.

coevolution | maternal transmission | cytonuclear interactions | *Acyrthosiphon pisum* | *Buchnera aphidicola*

Symbioses between bacteria and animals enable the exploitation of novel environments by providing the genetic and phenotypic advantages needed to use previously unavailable niches (1–3). Some symbioses involve bacteria that are universal within individuals of a host species. Prominent examples are found in sap-feeding insects in the order Hemiptera, which depend on bacterial symbionts to synthesize essential amino acids and other nutrients that are absent or rare in their specialized diets of plant sap (1, 4, 5). In many of these insects, the vertical transmission of bacterial symbionts from mother to offspring over millions of years has resulted in very intimate associations such that neither organism is capable of growth and reproduction without the other (6–9). Although heritable symbionts play an integral role in the success of many major animal groups, obligate symbiosis also presents a major challenge to hosts. In addition to the metabolic costs of maintaining symbionts, hosts must organize and regulate symbionts within their bodies, and exchange molecules with them (10, 11).

For maternally transmitted symbioses, selection on both hosts and symbionts promotes cooperation and efficiency of the symbiosis so as to increase host fecundity, which is the basis for symbiont transmission. However, maternally transmitted symbionts are clonal and subject to high levels of genetic drift, leading to degradation of their genomes and potentially imposing fitness costs on hosts (9, 12–14). In addition, heritable symbiosis can be impacted by within-host selection that can lead to symbiont selfishness (15, 16). For example, a mutation in a symbiont that speeds replication could spread because of the increase in proportional representation in subsequent progeny of its host, even if the overall fecundity of the host is lowered. Thus, obligate symbiosis entails a balancing act between conflicting evolutionary forces and could instigate a coevolutionary arms race between host and symbiont (15). Potentially, genetic interactions involving symbiosis accelerate reproductive isolation and thus diversification (17). Indeed, interactions between organellar genomes (mitochondria and plastids) and host genotypes appear to often affect host fitness (e.g., refs. 17–20).

A model for heritable, mutually obligate symbiosis is the association of pea aphids (*Acyrthosiphon pisum*) and their maternally transmitted symbiont *Buchnera aphidicola* (Gammaproteobacteria). *Buchnera* is found in almost all aphids and provisions hosts with essential amino acids that are lacking or rare in the diet of plant phloem sap (5, 21, 22). *Buchnera* cells are located in the cytosol of specialized host cells called bacteriocytes, where each is enclosed in a host-derived membrane (4, 22–24). Host and symbiont functions must be coordinated to maintain an integrated system of nutrient biosynthesis and to ensure transmission to progeny, and some evidence supports host-control of the symbiosis (10, 21, 25). One might expect tight control of *Buchnera* numbers, given the essentiality of this symbiosis, but several reports suggest that regulation of the symbiosis is highly variable. Total number and pooled volume of bacteriocytes vary among field collected *A. pisum* clones differing in *Buchnera* haplotype (26), and *Buchnera* genome numbers vary among sibling *A. pisum* genotypes (27). Whether or how this variation affects host fitness has not been determined.

Significance

Symbiosis between animals and bacteria enables the exploitation of new ecological niches and promotes diversification. Many insects have obligate heritable bacteria that provision nutrients enabling new lifestyles, such as sap-feeding. A model system for obligate symbiosis is the pea aphid and its heritable bacterial symbiont *Buchnera aphidicola*. Using this system, we address the extent of variation in host regulation of symbiont populations and find that pea aphids have extensive genetic variation in the ability to regulate *Buchnera* numbers. In addition, higher abundance of *Buchnera* is associated with lower reproductive rates in aphid hosts. Thus, we provide experimental evidence demonstrating that genetic variation in hosts affects regulation of symbiosis, suggesting fitness costs and potential negative consequences of obligate symbiosis.

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In this study, we examined how obligate symbiosis varies across genotypes within a single host species and how this variation is linked to host fitness. Specifically, we performed sexual crosses to generate distinct *A. pisum* clones of known relatedness and possessing known *Buchnera* haplotypes. We determined whether symbiont titer varies across these genotypes, and whether symbiont titer is related to host fitness. We find that *Buchnera* titer is significantly impacted by host genotype. We also find that symbiont titer is negatively correlated with overall host reproductive rate, suggesting a fitness cost associated with greater symbiont abundance. Thus, our findings show that the regulation of obligate symbiosis is dynamic within a host species rather than a static optimum. This variation in regulation of symbiosis is consistent with expected consequences of a coevolutionary arms race between host and symbiont.

Results

Establishment of Aphid Clones. To estimate the genetic basis of symbiont titer and its relationship to host fitness, we induced sexuals from four *A. pisum* clones, performed crosses, and generated sexually produced progeny belonging to full-sibling and half-sibling cohorts (*Materials and Methods*). Each progeny was an asexual female that gave rise to an asexual aphid line (clone), maintained long term in the laboratory under constant long-day conditions. To focus on the obligate symbiosis with *Buchnera*, any other (secondary) symbionts were eliminated before our experiments. Following sexual induction through exposure to short day length (27), clones LSR1 and 5AY, containing *Buchnera* haplotype B, produced both males and sexual females, whereas clones AustinC and TucsonC, containing *Buchnera* haplotype A, produced only sexual females. Thus, sexual females from all four clones were mated with either LSR1 or 5AY males to produce an F_1 generation that was then maintained as individual asexual clones under long-day conditions (Table 1). Each parental and progeny clone was grown as three replicate sublines for measurements of symbiont titer and host fitness.

***Buchnera* Titer.** To determine *Buchnera* titers for each clone, we used quantitative PCR (qPCR) to measure the ratio of the number of symbiont genome copies to the number of host genome copies, measured in fourth-instar juveniles, just before maturity. *Buchnera* titers varied among individuals representing each clone; this likely reflects various factors, including differences in quality of the food plants for each subline, differences in our exact timing of sampling, and environmental noise. Our sampling of fourth-instar juveniles corresponds to a phase of rapid growth of symbiont populations (28) and potentially adds to the sampling variation, because a few hours difference in sampling time may affect titer. Nonetheless, titers differed significantly among all clones (one-way ANOVA, $F_{41,\ 84} = 2.13$, $P < 0.01$) (Fig. 1*A*). Average titer varied by over 10-fold across clones, ranging from 7.2 in a TucsonC × LSR1 clone to 105.1 in an AustinC × LSR1 clone.

To determine whether *Buchnera* titer differs because of aphid genotype, we compared titer among the 32 progeny clones with *Buchnera* haplotype A (from maternal AustinC and TucsonC) and among the 6 progeny clones with *Buchnera* haplotype B (from maternal LSR1 and 5AY). Clones with *Buchnera* haplotype A differed in *Buchnera* titer (one-way ANOVA, $F_{31,\ 64} = 2.02$, $P < 0.01$). Clones with *Buchnera* haplotype B did not differ significantly (one-way ANOVA, $F_{5,\ 12} = 0.31$, $P > 0.05$), potentially because of the small sample size. Therefore, aphid genotype may have an effect on symbiont titer.

We further tested progeny lines with *Buchnera* haplotype A, for effects of parentage, either maternal or paternal, on symbiont titer. We found significant effects of maternal clone and of paternal clone (two-way ANOVA, maternal: $F_{1,\ 64} = 33.75$, $P < 0.0001$, paternal: $F_{1,\ 64} = 4.98$, $P < 0.03$). The effect of parentage supports some heritability of titer. We tested for differences among full-sibling progeny clones, using the two largest sets of full-sibling clones (from AustinC × 5AY and AustinC × LSR1 crosses) and found no significant differences (one-way ANOVA, $F_{15,\ 32} = 0.92$, $P > 0.05$ and $F_{12,\ 26} = 0.62$, $P > 0.05$, respectively).

Clone Fitness. To estimate differences in host fitness, we measured developmental time, adult mass, and fecundity of all *A. pisum* progeny clones (Fig. S1 and Table S1), and calculated reproductive rates (Fig. 1*B* and Table 1). Comparing across all progeny clones, there were significant differences in all fitness measures: adult weight (Kruskal–Wallis, $\chi^2 = 94.81$, df = 37, $P < 0.0001$), development time (Kruskal–Wallis, $\chi^2 = 70.41$, df = 37, $P < 0.001$), time to first reproduction (Kruskal–Wallis, $\chi^2 = 83.01$, df = 37, $P < 0.0001$), fecundity measured as number of offspring produced in 6 d (Kruskal–Wallis, $\chi^2 = 107.33$, df = 37, $P < 0.0001$), and reproductive rate (as calculated from developmental time and fecundity; Kruskal–Wallis, $\chi^2 = 121.39$, df = 37, $P < 0.0001$). All fitness parameters also significantly differed among progeny from different maternal clones and among progeny from different paternal clones (Table S1). Full-sibling clones from AustinC × 5AY and AustinC × LSR1 crosses varied significantly for multiple fitness parameters, including reproductive rate (Kruskal–Wallis, $\chi^2 = 45.91$, df = 15, $P < 0.0001$ and $\chi^2 = 30.03$, df = 12, $P < 0.01$, for AustinC × 5AY and AustinC × LSR1 crosses, respectively).

Relationship of Symbiont Titer to Host Fitness. To determine how symbiont titer may relate to host fitness, we tested for correlations between titer and development time, adult mass, and fecundity of all *A. pisum* progeny lines (Fig. 2 and Fig. S2). Comparing across progeny clones, symbiont titer was significantly correlated with development time (Spearman's $R = 0.238$, $P < 0.001$), time to first

Table 1. Experimental sexual crosses conducted and average *Buchnera* titer and host fitness measurements of progeny clones produced from each cross

Parentals		Progeny	Symbiont	Average					
Mother	Father	Total	*Buchnera* haplotype	*Buchnera* titer	Days to adult	Adult weight (mg)	Days to first reproduction	Total offspring (over 6 d)	Reproductive rate
AustinC	5AY	16	A	47.4 ± 5.1	7.45 ± 0.13	0.239 ± 0.01	9.45 ± 0.17	51.97 ± 1.72	5.02 ± 1.21
AustinC	LSR1	13	A	59.1 ± 6.1	7.24 ± 0.12	0.264 ± 0.01	9.26 ± 0.14	54.00 ± 1.73	5.29 ± 0.19
TucsonC	5AY	1	A	16.2 ± 6.5	7.00 ± 0	0.260 ± 0.08	8.83 ± 0.43	56.17 ± 8.17	7.72 ± 0.86
TucsonC	LSR1	3	A	18.9 ± 8.5	7.17 ± 0.25	0.276 ± 0.02	8.92 ± 0.33	60.45 ± 3.95	6.10 ± 0.39
5AY	5AY	0	B	—	—	—	—	—	–
5AY	LSR1	4	B	36.2 ± 6.7	6.79 ± 0.22	0.297 ± 0.02	8.46 ± 0.28	57.96 ± 1.54	6.14 ± 0.32
LSR1	LSR1	0	B	—	—	—	—	—	—
LSR1	5AY	2	B	34.4 ± 12.4	6.8 ± 0.25	0.305 ± 0.05	8.7 ± 0.31	60.67 ± 5.37	6.28 ± 0.56

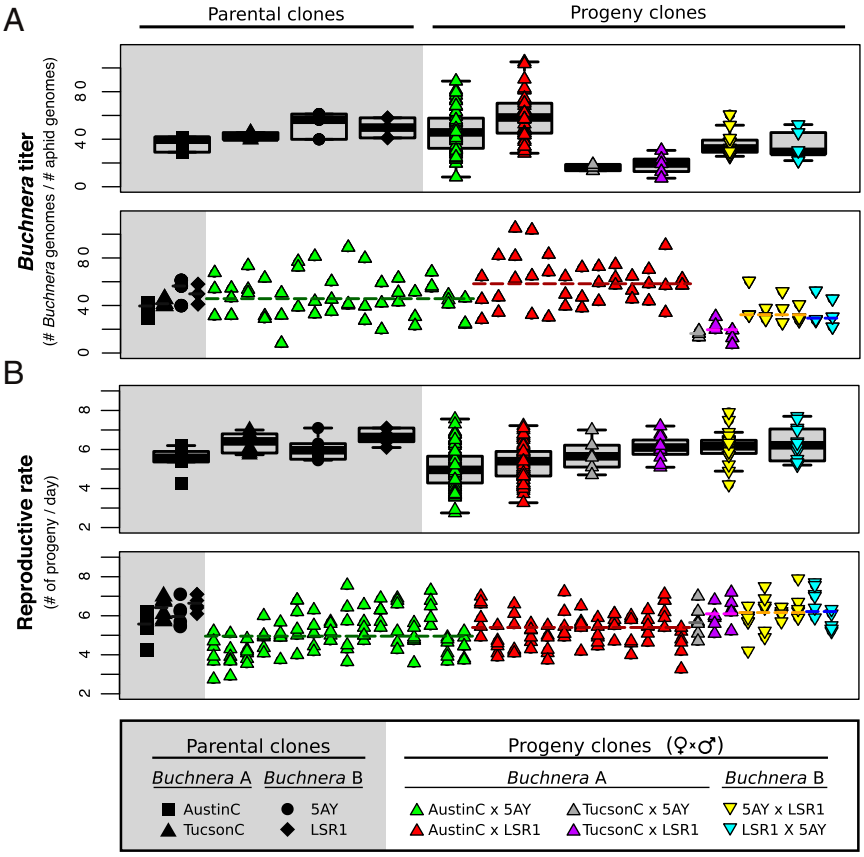

Fig. 1. Measurements of *Buchnera* titer and of reproductive rate of *A. pisum* parental clones and F_1 progeny clones generated from experimental crosses. (*A*) Titer for parental clones and for progeny clones grouped by sibships (*Upper*) and for each subline (*Lower*). Dashed line represents median titer for each sibship. (*B*) Reproductive rate of parental clones and progeny grouped by sibships (*Upper*) and for each subline (*Lower*). Dashed line represents the median for each sibship.

reproduction (Spearman's $R = 0.241$, $P < 0.001$), and number of progeny produced (Spearman's $R = -0.156$, $P < 0.02$), but not to adult mass (Spearman's $R = 0.036$, $P > 0.05$). We combined developmental time and fecundity to obtain a single measure of reproductive rate and observed a highly significant relationship between titer and reproductive rate (Spearman's $R = -0.228$, $P < 0.001$) (Fig. 2). Thus, a higher *Buchnera* titer is associated with longer time to reproductive maturity as well as fewer offspring.

Discussion

As for any obligate, heritable symbiosis, aphids are strongly selected to maintain their *Buchnera* and to ensure transmission to progeny. Past studies have made it clear that severe reduction or loss of *Buchnera* is highly detrimental to aphid growth and reproduction (29, 30). Similarly, selection generally favors *Buchnera* mutations that increase host fecundity, because *Buchnera* relies on host reproduction for its own persistence. Such selection for mutualistic traits appears to underlie many observed features of the *Buchnera* genome, including retention of amino acid biosynthetic pathways (24), and amplification of some amino acid biosynthetic genes on plasmids (31). Aphids also exhibit symbiotic adaptations including bacteriocyte-specific expression of genes involved in amino acid metabolism and altered function of specific amino acid transporters within bacteriocytes (21, 25, 32). Nonetheless, there is a zone of potential coevolutionary conflict. A *Buchnera* mutant with elevated replication rate could spread by being transmitted disproportionately to progeny, even if it depresses host fecundity. This potential for the spread of "selfish" symbiont mutations is enhanced if the inoculum size is large (16, 33). This conflict between levels of selection could lead to a coevolutionary arms race, because selection will act on hosts to counter the impact of symbiont selfishness.

If these dynamics are affecting an obligate symbiosis, one expectation is intraspecific variation affecting the regulation of the symbiosis. Because aphids have a clonal life-cycle phase, genotypes can be reared in independent clonal sublines, providing an unusual opportunity to measure genotypic variation. We found that symbiont titer, measured as genome copy number, is surprisingly variable among host genotypes, and that average titer can vary several-fold among clones harboring the same *Buchnera* haplotype (Fig. 1).

A second expectation, if coevolutionary dynamics are affecting an obligate symbiosis, is that symbiont regulation affects host fitness, and specifically that overreplication of symbionts can lower host fitness. In our experiments, higher *Buchnera* titer is associated with lower performance of aphids for all three of our performance measures (fecundity, developmental time, and time to first reproduction) (Fig. 2 and Fig. S2). The negative correlation between symbiont titer and overall reproductive rate (Fig. 2) raises the possibility of a fitness cost of greater symbiont abundance, at least under our laboratory conditions. Thus, our findings are consistent with the possibility that the intimate obligate relationship between heritable symbionts and hosts entails a coevolutionary arms race.

However, our results do not demonstrate a causal relationship between high symbiont titer and aphid fitness, and several alternative explanations for the correlation are possible. Specifically, higher *Buchnera* titers might increase aphid fitness under

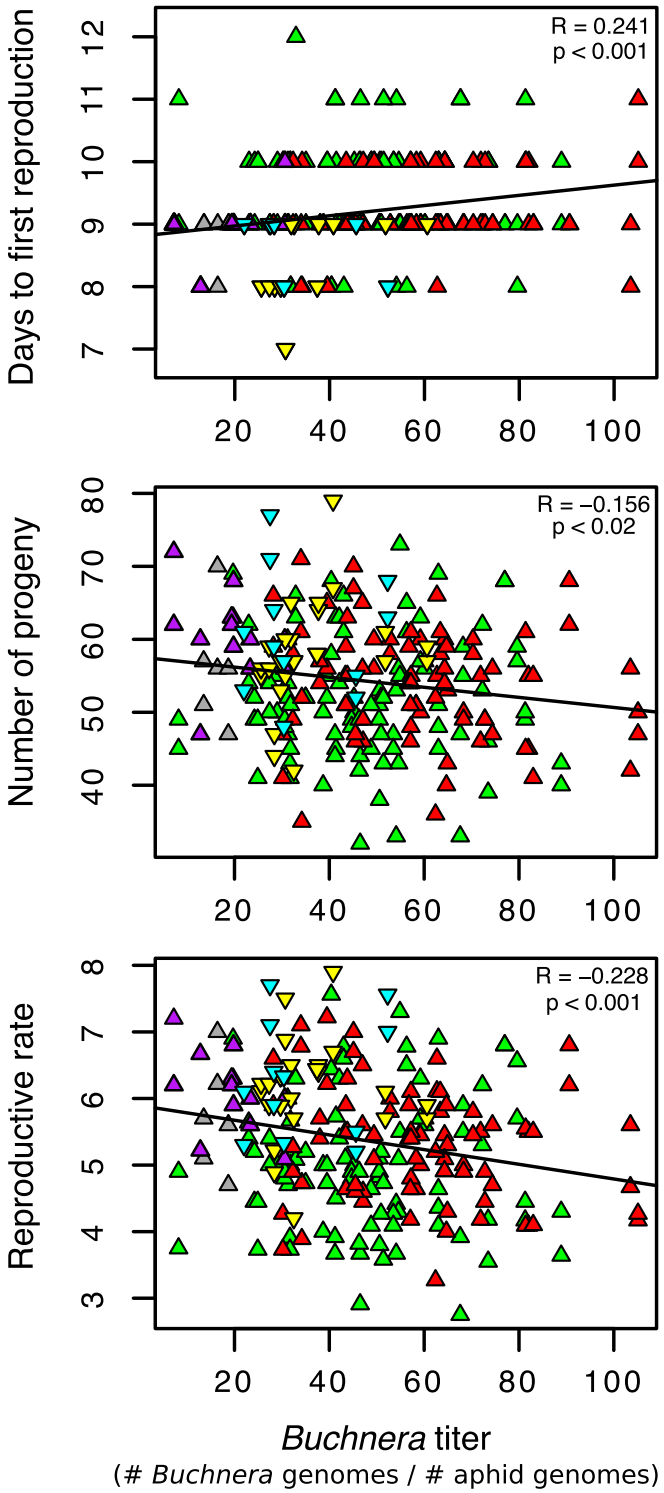

Fig. 2. Correlation of *Buchnera* titer and host fitness measurements, including days to reproduction, number of progeny, and reproductive rate for sublines of *A. pisum* clones. Refer to Fig. 1 for key.

conditions of low nutrient availability or heat exposure, which can kill *Buchnera* cells (29, 34). Our laboratory conditions may remove the pressure to have large numbers of *Buchnera*, as we provide highly nutritious host plants (fava bean seedlings) and a constant favorable temperature. Potentially, the correlation could reflect differences in compartmentalization of *Buchnera* between maternal and embryonic bacteriocytes, which might vary with aphid growth rate, thus impacting the ratio of *Buchnera* to aphid genomes. Weighing against this explanation are the results of Simonet et al. (28), who found that only a small proportion of the *Buchnera* cells within a fourth-instar *A. pisum* (the stage we measured) are within the embryos. Nonetheless, explanations such as these, in which the aphid genotype has independent effects on *Buchnera* titer and on fecundity, cannot be ruled out as possible explanations for the correlation.

We were unable to test directly for an effect of *Buchnera* genotype on titer, or on host fitness, because any effect of the *Buchnera* haplotype is confounded with aphid genetic contributions. We note that the three progeny clones with the TucsonC *Buchnera* haplotype A show the lowest titers and some of the highest reproductive rates (Fig. 1 and Table 1). (Reciprocal crosses to distinguish effects of host and symbiont genomes were not possible because TucsonC parental clones failed to produce males.)

Our findings reveal that symbiont titer is variable and depends at least in part on host genotype. The mechanisms by which aphids control their symbiont populations are not known. In some other symbioses, modified elements of the innate immune system play a role, as for the symbionts of *Sitophilus* grain weevils, in which antimicrobial peptides, typically associated with defense against pathogens, function to confine mutualistic symbionts within bacteriocytes (35). In *A. pisum*, short peptides resembling antimicrobial peptides are the most highly overexpressed genes in bacteriocytes, and show specific patterns of expression in these cells or in the nearby sheath cells, suggesting a role in controlling *Buchnera* (36). Other genes with potential roles in controlling *Buchnera* are several genes that were laterally transferred from bacterial genomes into the genome of an ancestral aphid, and that are highly overexpressed in bacteriocytes (32, 37). Some of these laterally transferred genes encode lysozymes that could act to lyse symbiont cells as part of a control mechanism. Another bacterial origin aphid gene up-regulated in bacteriocytes encodes the protein RlpA, which is imported into *Buchnera* cells, where it may act as an effector for symbiont regulation (38). Together, these aphid genes may form part of a system for recognizing, controlling, and transmitting *Buchnera*. On the *Buchnera* side, surface molecules that may interact with host systems include outer-membrane porins and the outer components of the flagellar apparatus. *Buchnera* lacks a flagellum but retains a large set of genes that underlie the flagellar secretion system, which is abundantly expressed on the symbiont cell surface (39). Furthermore, some of the flagellar proteins show unusual evolutionary acceleration in *Buchnera* (40, 41), potentially reflecting coevolution with host control mechanisms.

Buchnera cells are highly polyploid, and can vary in ploidy across aphid life stages (42, 43). A recent study (28) highlighted the cellular dynamics of *Buchnera* across the *A. pisum* life cycle using *Buchnera* cell counts rather than *Buchnera* genomic copy number, as in our experiments. It is likely that cell numbers and genomic copy numbers are strongly correlated; and it is not clear which is a better indicator of functional capacity of symbionts within a host individual. In any case, our measurement of symbiont titer reveals substantial variation in regulation of the symbiosis.

With the establishment of a maternally inherited obligate symbiosis, partner lineages enter into an irreversible coevolutionary relationship where symbionts provide significant benefits to hosts, which coadapt to maintain the interaction (12, 15). This relationship is distinct from those of hosts and their pathogens, which can also exhibit within-host evolution (44); for obligate, heritable symbiotic partners, hosts must maintain a viable symbiosis even if it involves a fitness cost. This study shows that intraspecific variation in hosts affects the regulation of obligate symbiosis and sheds light on its potential long-term evolutionary consequences.

Materials and Methods

Aphid Clones and Sexual Crosses. All *A. pisum* clones used in this study were reared on seedlings of the broad bean *Vicia faba* in laboratory growth chambers under long-day (16-h of light: 8-h of dark) conditions at 20 °C. These clones represent *A. pisum* females sampled from alfalfa or broad bean in the United States between 1998 and 2014 and maintained as laboratory clones since collection. For this study, a single female from each clone was used to establish clonal lineages and maintained under constant conditions. Of the four North American *A. pisum* clones used in this study, LSR1 and 5AY share nearly identical *Buchnera* (16 single-nucleotide differences and no frameshifts in coding genes in a 640-kb genome) and are divergent from *Buchnera* found in AustinC and TucsonC clones, which have similar *Buchnera* (12 single-nucleotide differences, no frameshifts) We refer to *Buchnera* from AustinC and TucsonC as *Buchnera* haplotype A, and to *Buchnera* from 5AY and LSR1 as *Buchnera* haplotype B. These groups differ by 1,428 single-nucleotide differences, including many that affect amino acid residues, as well as 455 insertions/deletions, of which some disrupt protein-coding sequences. Clone AustinC was cured of secondary symbionts using antibiotic treatments (45). TucsonC was cured of secondary symbionts in a previous study (34). Both LSR1 and 5AY lack secondary symbionts. Thus, our sampling of clones eliminates potential effects that the presence of secondary symbionts may have on obligate symbiont titer.

To examine genetic variation affecting symbiont titer and aphid host fitness, we crossed four *A. pisum* clones to generate full-sibling and half-sibling progeny clones. We used standard methods to induce sexual female and male forms by exposing clones to a gradual reduction in daylight over 6 wk to reach short-day conditions (10-h of light:14-h of dark); conditions under which most *A. pisum* clones are known to produce both male and sexual females (oviparae) (46). Crosses were set up on caged plants, and the resulting eggs were "overwintered," and subsequently monitored for hatching fundatrices to establish new clonal lines caged on individual host plants (23, 27, 46). For all experiments, progeny clones and parental clones were divided into three separate sublines and allowed to reproduce for at least three generations before data collection, to control for maternal and environmental effects.

Real-Time qPCR. Real-time qPCR was used to estimate symbiont titer, the number of *Buchnera* genome copies compared with the number of aphid genome copies using single-copy genes from both *Buchnera* and the aphid nuclear genome. A single individual was measured for each subline within a clone. For each clone, nymphs were allowed to develop for 6 d to reach their fourth instar and then collected for titer measurements. Each individual was collected in a tube, frozen in liquid nitrogen, crushed with a pestle, and the resulting homogenate was treated using the Qiagen DNEasy kit to isolate DNA for qPCR.

Aphid genome copy number was estimated by measuring copy number of the single-copy gene encoding elongation factor-1 alpha (*ef1*α) using the primers ApEF1a 107F and ApEF1a 246R, yielding a product of 139 bp (27). *Buchnera* genome copy number was estimated by measuring copy number of the single-copy symbiont gene encoding a heat-shock protein (*groES*) using primers designed in this study: *groES* 18F (5′–CATGATCGTGTGCTTGTTAAG–3′) and *groES* 98R (5′– CTGTTCCTCGAGTCGATTTCC–3′), yielding a product of 80 bp. We prepared standards ranging from10^2 to 10^8 copies for each gene to generate a standard curve, which was used to estimate absolute copy number. All qPCR samples were prepared using BioRad iTaq SYBR Green master mix and performed on an Eppendorf Realplex Mastercycler. All reactions used the same "touchdown" cycling conditions, which included: 95 °C for 2 min, [95 °C for 10 s, 65(−1) °C for 15 s, 72 °C for 20 s] × 5 cycles, and (95 °C for 10 s, 60 °C for 15 s, 72 °C for 20 s) × 35 cycles. For each sample, the number of copies of *ef1*α and *groES* were determined using the Realplex Mastercycler software based on comparisons with serial dilutions of the qPCR standards. Reactions were performed in triplicate for each sample, and the average was used to represent that subline.

Measurement of Aphid Fitness. Average fitness was measured for sublines of new progeny clones and for sublines of the original parental clones. Adults of each clone were placed on plants overnight at 20 °C and removed, resulting in even-aged nymphs on each plant. Aphids were monitored daily, and dates of molting to adulthood and weights of new adults were recorded. Newly eclosed adults were placed individually on new plants, and the date of first reproduction and total number of nymphs produced during the following 6 d was recorded. Fecundity for each subline was estimated as the total number of progeny produced in the first 6 d following first reproduction. This measure accounts for a majority of the offspring an adult *A. pisum* will produce in a generation, as daily production declines a few days after first reproduction (47, 48). In field populations, aphids undergo high mortality, further decreasing the contribution of fecundity of older females. Reproductive rate was calculated as the total number of offspring produced in the first 6 d divided by number of days from birth to first reproduction plus 1 d. All fitness parameters were measured twice for each subline.

Statistical Analyses. All statistical analyses were performed using R 3.2.3, with a statistical significance threshold of $P < 0.05$. *Buchnera* titer was estimated by the relative number of *Buchnera* gene-copies divided by the number of the single-copy host gene. Significance of *Buchnera* titer variation among progeny clones and parental clones was tested using ANOVA. Aphid fitness measurements were analyzed using Kruskal–Wallis tests. Correlations between *Buchnera* titer and host fitness parameters were tested using Spearman's rank correlation coefficient.

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