

Collapse of obligate endosymbiosis in selfed progeny of the pea aphid,
Acyrtosiphon pisum

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Abstract

The relationship between inbreeding depression and microbial endosymbiosis in animals has been largely overlooked, despite their widespread occurrence. Aphids host the intracellular symbiotic bacterium *Buchnera aphidicola*, which provides them with nutrition and is vertically transmitted to offspring. Experimental elimination of *Buchnera* results in host sterility. We previously observed such sterility in the selfed progeny of the pea aphid, *Acyrtosiphon pisum*. In the present study, we tested our hypothesis that selfing disrupts aphid–*Buchnera* endosymbiosis and leads to sterile fundatrices in *A. pisum*. Initially, we performed crossing experiments involving nine strains, revealing widespread sterility in *A. pisum*. Subsequently, we selected two strains for more detailed analyses, including quantification of relative *Buchnera* genome copy numbers and fluorescent *in situ* hybridization of *Buchnera*. Fundatrices resulting from selfing in one strain exhibited reduced fecundity and diminished *Buchnera* abundance compared with those resulting from outcrosses. These findings represent the first example of inbreeding depression impacting endosymbiotic abundance in animal–microbe symbiosis. Furthermore, fecundity was positively correlated with *Buchnera* abundance in selfed progenies. Overall, these results suggest that inbreeding negatively affects insects through endosymbiosis collapse.

Keywords: aphid, *Buchnera aphidicola*, fecundity, inbreeding depression, symbiont titer

1 Introduction

Inbreeding often leads to deleterious effects on offspring fitness (Darwin 1876). This phenomenon, known as inbreeding depression, has been observed across various life-history traits (e.g., growth rate, survival until maturity and fecundity) and morphological traits (e.g., adult body size) in numerous animal and plant species (Charlesworth and Charlesworth 1987; Keller and Waller 2002). Inbreeding depression primarily arises from increased homozygosity for deleterious recessive mutations, which persist at low frequencies in populations (Charlesworth and Willis 2009). Many animals and plants host symbiotic microbes crucial for their metabolism and fitness (Gilbert et al. 2012; McFall-Ngai et al. 2013). If a host gene necessary for symbiosis formation or maintenance possesses deleterious recessive mutations, inbreeding may increase homozygosity for such mutations, potentially leading to the collapse of endosymbiosis. However, no studies have investigated whether inbreeding has a deleterious effect on endosymbiosis.

One of the best-studied symbiotic relationships is that between aphids (Hemiptera: Aphididae) and the bacterium *Buchnera aphidicola* (Baumann 2005; Shigenobu and Wilson 2011). *Buchnera* resides within specialized host cells and organs called bacteriocytes and bacteriomes (Braendle et al. 2003), providing essential amino acids and vitamins, lacking in the phloem sap diet, to host aphids (Douglas 1998). *Buchnera* are vertically transmitted from maternal bacteriocytes to embryos (Koga et al. 2012; Miura et al. 2003), with the *Buchnera* population increasing exponentially throughout postembryonic development (Simonet et al. 2016). The abundance of *Buchnera* is associated with host fitness (Chong and Moran 2016) and varies with host genotype (Vogel and Moran 2011), nutritional status (Zhang et al. 2016), and rearing

temperature (Lu et al. 2014). Moreover, host aphids become sterile upon *Buchnera* deprivation via antibiotic treatment or heat exposure (Ishikawa and Yamaji 1985; Ohtaka and Ishikawa 1991), underscoring the evolved stability of vertical transmission and *Buchnera* maintenance over 200 million years of aphid–*Buchnera* symbiosis (Baumann et al. 1995).

Aphids exhibit complex annual life cycles comprising several parthenogenetic generations and one sexual generation (Moran 1992; Simon et al. 2002). Viviparous females reproduce parthenogenetically during spring and summer, whereas oviparous females and males emerge in autumn, leading to sexually produced eggs that overwinter and hatch as parthenogenetic viviparous females called fundatrices in spring (Fig. 1A). Consequently, *Buchnera* abundance is stably heritable during parthenogenetic generations but varies during sexual reproduction (Chong and Moran 2016; Vogel and Moran 2011). Furthermore, inbreeding depression is expected to be high in the cyclically parthenogenetic life cycle due to the accumulation of recessive mutations across parthenogenetic generations (Muirhead and Lande 1997). Indeed, inbreeding depression has been documented in aphids across several traits, including hatching, survival to adulthood, morphological traits and fundatrix fecundity (Akimoto 2006; Dedryver et al. 1998; Helden and Dixon 1997; Huang and Caillaud 2012; Newton and Dixon 1988). Previously, we selfed the ApL strain of the pea aphid, *Acyrtosiphon pisum* (Harris), observing that many fundatrices became nearly or completely sterile (Matsuda et al. 2020). These fundatrices resembled aposymbiotic aphids induced by antibiotic treatment or heat exposure, both exhibiting sterility (Ishikawa and Yamaji 1985; Ohtaka and Ishikawa 1991). This similarity led us to hypothesize that fundatrix sterility is associated with decreased *Buchnera* abundance. It has been shown that

Buchnera titer (the number of *Buchnera* genome copies per aphid genome copy) negatively correlates with reproductive rate among several *A. pisum* strains originating from outcrosses, suggesting that excessively high *Buchnera* titer imposes a cost on host aphids, resulting in low fitness. However, aphids originating from selfing have not been examined for *Buchnera* abundance, possibly owing to their low hatching and survival rates (Chong and Moran 2016).

In the present study, we initially established the widespread occurrence of fundatrix sterility among *A. pisum* strains from various host plants and screened fertile and sterile crosses from selfings and outcrosses of *A. pisum* strains. Subsequently, we compared the lifetime fecundity and *Buchnera* abundance of fundatrices among the screened crosses. Finally, based on the correlation between fecundity and *Buchnera* abundance, we discussed the potential involvement of symbiosis collapse in the sterility of selfed aphid progenies.

2 Materials and methods

2.1 Insects

We used nine *A. pisum* strains for crossing experiments (Table S1), originally collected from alfalfa *Medicago sativa*, red clover *Trifolium pratense*, *Vicia cracca*, or *Melilotus* spp., in Hokkaido, Japan. Aphids were reared in plastic cups (diameter: 96 mm; depth: 119 mm) containing vetch seedlings (*Vicia faba*) grown on wet Akadama soil (Tachikawa Heiwa Nouen, Kanuma, Japan) under a 16:8 h light:dark (LD) cycle at 16°C ± 1°C, unless otherwise specified. Oviparous females and males were induced by maintaining viviparous females under a 8:16 h LD cycle at 15°C ± 1°C (Ishikawa et al. 2012).

2.2 Crossing and fecundity testing

Initially, we screened fertile and sterile crosses from selfings and outcrosses of the nine *A. pisum* strains in the laboratory (Fig. 1B). Tens of oviparous females and males were placed together in a plastic case with vetch seedlings and allowed to mate. After a few days, oviposited eggs were collected and incubated at 15°C for approximately 14 days. Dark-colored eggs were considered fertilized (Miura et al. 2003), whereas light-colored eggs were deemed unfertilized and discarded. Fertilized eggs were incubated for approximately 2 months (41–68 days) at 4°C ± 1°C to terminate embryonic diapause and subsequently subjected to a 16:8 h LD cycle at 16°C to induce hatching (Shingleton et al. 2003). If no hatching occurred during the first two weeks for each egg batch, observations were discontinued. Fundatrices were continuously reared under the same conditions and assessed for fertility or sterility upon reaching full maturity. Hatching, survival to adulthood, and the percentage of fertile fundatrices were recorded for each cross. Based on the experimental crosses, the ApL and 09003A strains were selected for further analyses as parental strains.

To assess the effect of selfing and outcrossing on fecundity in greater detail, lifetime fecundity was compared among fundatrices from crosses within and between the ApL and 09003A strains. Adult fundatrices were individually placed in a plastic case (100 × 65 × 28 mm) with a vetch seedling and allowed to produce nymphs under a 16:8 h LD cycle at 16°C. Fundatrices were transferred to new vetch seedlings weekly to prevent overcrowding. Progenies were counted for each fundatrix until its death. Pairwise comparisons of lifetime fecundity among different crosses were conducted using the Brunner–Munzel test via the “nparcomp” package in R version 4.1.2 (R Core

Team 2021).

2.3 DNA extraction and quantitative polymerase chain reaction

To evaluate the effect of selfing and outcrossing on *Buchnera* abundance, the *Buchnera* genome copy number per individual was determined for fundatrices from crosses within and between the ApL and 09003A strains. Adult fundatrices were flash-frozen in liquid nitrogen within three days of emergence and stored at -80°C until DNA extraction. Total DNA was extracted from whole bodies using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) and stored at -20°C until use. Quantitative polymerase chain reaction (qPCR) assays were performed using KOD SYBR qPCR Mix (TOYOBO, Osaka, Japan) and the LightCycler 96 system (Roche, Basel, Switzerland), following the manufacturers' instructions. The single-copy gene *dnaK* of *B. aphidicola* was amplified using the same volume of template DNA to calculate the relative *Buchnera* genome copy number for each aphid individual. The forward and reverse primers used for qPCR were 5'-GTT GGT GGT CAA ACT AGA ATG CCT-3' and 5'-ACT CCT CCC TGT ACT GCA GC-3', respectively. Additionally, qPCR assays were performed for newly emerged adult viviparous females of the two parental strains in the same manner to assess interclonal variation in relative *Buchnera* genome copy number. Pairwise comparisons of the relative *Buchnera* genome copy number among different crosses were performed using the Brunner–Munzel test.

2.4 Fluorescence *in situ* hybridization

To elucidate the abundance and localization of *Buchnera* in fundatrices resulting from selfing of the ApL strain, we performed fluorescence *in situ* hybridization (FISH),

following the method outlined by Koga et al. (2009). Newly emerged adult fundatrices were fixed overnight in Carnoy's solution (ethanol:chloroform:acetic acid = 6:3:1), followed by decolorization using a 6% H₂O₂ alcoholic solution for 7–20 days at room temperature (RT). After hydration in PBSTx (0.1% TritonX-100 in 1 × PBS), hybridization was performed overnight at RT in a solution comprising 20 mM Tris-HCl (pH 8.0), 0.9 M NaCl, 0.01% sodium dodecyl sulfate and 30% formamide, supplemented with 100 μM fluorescent-labelled oligonucleotide DNA probe: Cy5-ApisP2a (5'-Cy5-CCT CTT TTG GGT AGA TCC-3') targeting the 16S rRNA of *B. aphidicola* (Koga et al. 2003). Host insect tissues were stained with 4',6-diamidino-2-phenylindole (DAPI), and stained samples were mounted using VECTASHIELD (Vector Laboratories, Burlingame, USA) before observation under an FV1000 confocal microscope (Olympus, Tokyo, Japan). Similarly, FISH images of newly emerged adult 09003A♀ × ApL♂ fundatrices were obtained for comparison purposes.

2.5 Correlation between fecundity and *Buchnera* abundance

The relationship between fecundity and *Buchnera* abundance was investigated in fundatrices resulting from selfing of the ApL strain. Fundatrices were allowed to produce nymphs for 15 days following adult emergence under a 16:8 h LD cycle at 16°C, and the number of progenies during the first 15 days of adult life was recorded for each fundatrix. Following progeny counting, these fundatrices were flash-frozen in liquid nitrogen on day 16, with DNA extraction and qPCR assays subsequently performed as described earlier. Pearson correlation coefficients were calculated to assess the correlation between 15-day fecundity and relative *Buchnera* genome copy number. Notably, the fundatrices used for qPCR were selected arbitrarily to cover a

wide fecundity range.

3 Results

3.1 Hatching, survival and fertility

We performed 74 crosses, obtaining fertilized eggs in 71 crosses from the nine *A. pisum* strains (Fig. 1 and Table S2). Three crosses involving KNb males produced only unfertilized eggs, indicating male sterility. Hatching success was significantly higher in outcrossed eggs than in selfed eggs (Brunner–Munzel test, $P = 0.015$). Conversely, survival to adulthood and the percentage of fertile fundatrices did not differ significantly between selfed and outcrossed progenies (Brunner–Munzel test, both $P > 0.05$). The 09003A strain exhibited relatively high hatching, survival and fertility scores when selfed or outcrossed with the ApL strain, prompting further analysis of fundatrix fecundity between these two strains.

We compared lifetime fecundity among fundatrices from crosses within and between the ApL and 09003A strains (Fig. 2). ApL♀ × ApL♂ fundatrices produced significantly fewer progenies compared with fundatrices from the other three crosses (Brunner–Munzel test with Bonferroni adjustment, $P < 0.05$). Notably, over 80% of fundatrices from the ApL♀ × ApL♂ cross were sterile. In contrast, the fecundity of 09003♀ × 09003♂ was significantly lower than that of ApL♀ × 09003♂ but equivalent to that of 09003A♀ × ApL♂.

3.2 *Buchnera* abundance

To investigate the variation in *Buchnera* abundance among fundatrices, we performed qPCR analyses, quantifying the relative *Buchnera* genome copy number per individual

(Fig. 3). The median relative *Buchnera* genome copy number varied by up to 100-fold among the crosses. Specifically, ApL♀ × ApL♂ fundatrices exhibited significantly lower relative *Buchnera* genome copy numbers compared with fundatrices from the other crosses (Brunner–Munzel test with Bonferroni adjustment, $P < 0.05$). In contrast, no significant difference in relative *Buchnera* genome copy number was observed between the two parental strains (Brunner–Munzel test, $P > 0.05$).

We also performed whole-body FISH, observing differences in *Buchnera* density between outcrossed and selfed progenies (Figs. 4 and S1). In adult 09003A♀ × ApL♂ fundatrices, bacteriocytes adhered to each other in the aphids' abdomens, exhibiting *Buchnera* signals therein. Conversely, in adult ApL♀ × ApL♂ fundatrices, individuals displayed smaller bacteriocytes (diameter $< 50 \mu\text{m}$) and/or faint *Buchnera* signals. These results indicate that aphid–*Buchnera* symbiosis collapsed in most adult ApL♀ × ApL♂ fundatrices.

3.3 Correlation between fecundity and *Buchnera* abundance

To determine whether fundatrix sterility correlated with decreased *Buchnera* abundance, we quantified both the 15-day fecundity and relative *Buchnera* genome copy number in the same individual (Fig. 5). In total, 5 fertile and 18 sterile fundatrices were collected from the cross within ApL strain, with the number of progenies ranging from 0 to 57 over 15 days. Based on qPCR assays, relative *Buchnera* genome copy numbers were lower in the 18 sterile fundatrices than in their 5 fertile counterparts. Additionally, a significant positive correlation was observed between fecundity and relative *Buchnera* genome copy number, suggesting that fundatrices were fertile when their relative *Buchnera* genome copy numbers exceeded a certain threshold, with symbiosis collapse

likely contributing to fundatrix sterility.

4 Discussion

4.1 Effect of selfing and outcrossing on survival and reproduction

Our crossing experiment revealed considerable variations in hatching success, nymphal survival and fertile fundatrix percentage among *A. pisum* crosses (Fig. 1). Hatching success was lower following selfing than following outcrossing, although for some crosses, the 4°C treatment duration may have been too brief to terminate embryonic diapause. Additionally, the lifetime fecundity of fundatrices was lower when the ApL strain was selfed compared to when it was outcrossed (Fig. 2). These findings highlight the existence of inbreeding depression in selfings of certain *A. pisum* strains, consistent with observations in other aphid species (Dedryver et al. 1998; Helden and Dixon 1997; Huang and Caillaud 2012). However, high hatching failure, nymphal mortality, or adult sterility were observed not only in some selfings but also in some outcrosses.

Acyrtosiphon pisum populations originating from different host plants may be genetically divergent (Peccoud et al. 2008; Via 1991); thus, the high mortality or sterility observed in some outcrossed progenies may result from genetic incompatibilities between parental strains (Table S1). Further studies such as genome scans into the genetic divergence among the aphid strains are warranted.

4.2 Effect of selfing and outcrossing on endosymbiosis

Our qPCR results revealed that *Buchnera* abundance in selfed progenies of the ApL strain was lower than that in outcrossed progenies and parental strains (Fig. 3). This finding was supported by FISH images showing low *Buchnera* density in many selfed

progenies (Fig. 4). To the best of our knowledge, this is the first experimental study to quantitatively demonstrate inbreeding depression in endosymbiont abundance in animal–microbe symbioses. However, *Buchnera* abundance in the 09003A strain’s selfed progenies was comparable to that in outcrossed progenies (Fig. 3), suggesting that the parental ApL strain possesses one or more deleterious recessive alleles related to fecundity and/or symbiotic regulation in its offspring, unlike the 09003A strain. Such variation in the strength of inbreeding depression may be attributed to the duration of parthenogenesis. Although aphid parthenogenetic clones are believed to be genetically identical, they accumulate mutations at a low rate in the laboratory (estimated at 2.7×10^{-10} per haploid genome per parthenogenetic generation) (Fazalova and Nevado 2020) and occasionally exhibit phenotypic mutations, such as altered body color (Li and Akimoto 2022). Compared with the 09003A strain, the ApL strain has been maintained parthenogenetically in the laboratory over more years (Table S1), suggesting that it may harbor a greater accumulation of deleterious mutations.

Inbreeding depression is considered critical for the fitness of both aphids and *Buchnera* in some *A. pisum* strains and may have driven the evolution of inbreeding avoidance (Charlesworth and Charlesworth 1987). Oviparous females of *A. pisum* avoid fertilization by genetically related males, with cryptic female choice potentially playing a role in the process (Huang and Caillaud 2012). Such inbreeding avoidance mechanisms could reduce the likelihood of sterility and endosymbiosis collapse due to inbreeding in natural populations. Additionally, dispersal via flight may contribute to selfing avoidance. *Acyrtosiphon pisum* males exhibit genetically determined wing polymorphism (winged or wingless), with the ApL and 09003A strains producing only winged males (Table S1). Interestingly, the Tpra strain, which produces both winged

and wingless males, showed the highest scores for hatching success, nymphal survival, and fertile fundatrix percentage among all the selfings, albeit with a relatively small sample size (Fig. 1). These results suggest that strains homozygous for the winged allele have accumulated deleterious recessive mutations, whereas strains with the wingless allele have experienced more frequent inbreeding and purged such mutations.

4.3 Relationship between symbiont abundance and host fecundity

In addition to the lower lifetime fecundity and *Buchnera* abundance observed in selfings of the ApL strain compared with other crosses, we found a positive correlation between *Buchnera* abundance and host fecundity during the first 15 days in selfed progenies (Fig. 5). This trend contrasts with the findings of Chong and Moran (2016), who found that the *Buchnera* titer was negatively correlated with host lifetime fecundity, possibly due to metabolic costs associated with maintaining symbionts. Two alternative hypotheses may explain the positive correlation between *Buchnera* abundance and aphid fecundity: (i) selfing primarily impaired host physiological performance, indirectly suppressing *Buchnera* abundance, or (ii) selfing primarily disrupted *Buchnera* regulation mechanisms, leading to endosymbiosis collapse and subsequent suppression of host reproduction. The second hypothesis appears more plausible, given that elimination of *Buchnera* results in host sterility (Ishikawa and Yamaji 1985; Ohtaka and Ishikawa 1991). Nonetheless, further investigation is required to validate this hypothesis.

4.4 Possible mechanisms underlying endosymbiosis collapse

Although the collapse of endosymbiosis observed in this study is thought to be

genetically induced, its mechanistic basis remains unclear. Environmental stresses, such as high temperatures, are known to reduce symbiont abundance in various animal–microbe symbiotic systems (Weis 2008; Wernegreen 2012). However, to our knowledge, there has been no report on genetically induced loss of obligate symbionts. It is possible that the population growth of *Buchnera* and/or bacteriocyte development may be disrupted in fundatrices from the ApL♀ × ApL♂ cross. *Buchnera* has lost several genes necessary for the regulation of cell division and growth, with the host aphid now playing a crucial role in regulating the bacteria’s population growth (Shigenobu et al. 2000). Aphids express several cysteine-rich peptides with antimicrobial activities, known as BCRs, in their bacteriocytes to control *Buchnera* growth (Shigenobu and Stern 2013; Uchi et al. 2019). Additionally, aphids activate the lysosomal system responsible for *Buchnera* degradation, depending on host age and wing morph (Nishikori et al. 2009; Simonet et al. 2018). Defects in these regulatory mechanisms may result in decreased *Buchnera* abundance. Furthermore, bacteriocyte differentiation is controlled by gene expression exclusively from the host aphid (Braendle et al. 2003). For example, several transcription factors, including those encoded by homeobox genes, are involved in bacteriocyte development in insects, including aphids (Braendle et al. 2003; Li et al. 2022; Matsuura et al. 2015; Rafiqi et al. 2020). The degenerated bacteriocytes observed in our study may have reflected a developmental disorder in these cells. It is also possible that the amount of vertically transmitted *Buchnera* is reduced in the ApL♀ × ApL♂ cross compared with other crosses. *Buchnera* cells are exocytosed from the maternal bacteriocyte to the extracellular space and endocytosed by the posterior syncytial cytoplasm of the blastula during early embryogenesis (Koga et al. 2012). Further molecular developmental

studies are warranted to identify which step is causing the endosymbiosis collapse.

Author contributions

NM and SS designed the research. NM and MS performed experiments. NM and SS wrote the manuscript. All authors read and approved the manuscript.

Competing interests

The authors declare that they have no competing interests.

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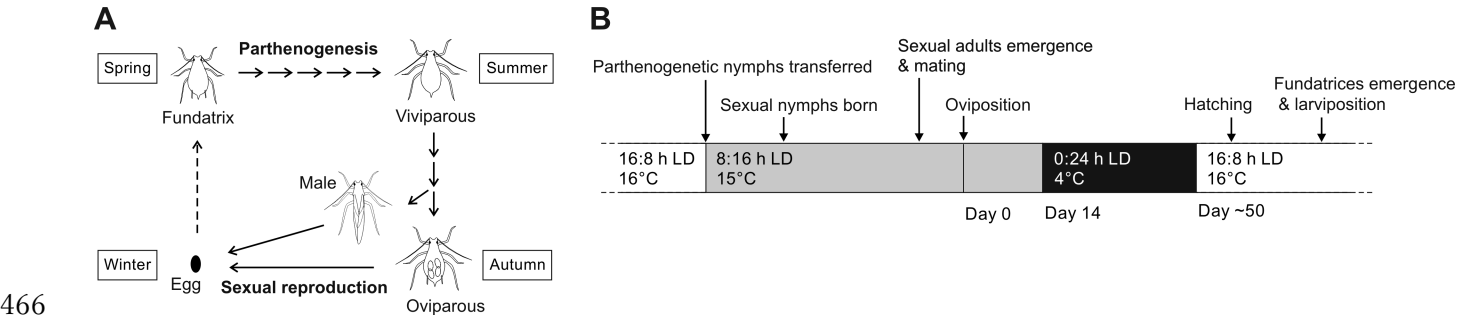
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465 **Figures**



467 **Fig. 1** Schematic diagram on (A) the life cycle of *Acyrthosiphon pisum* and (B) experimental
468 procedures.

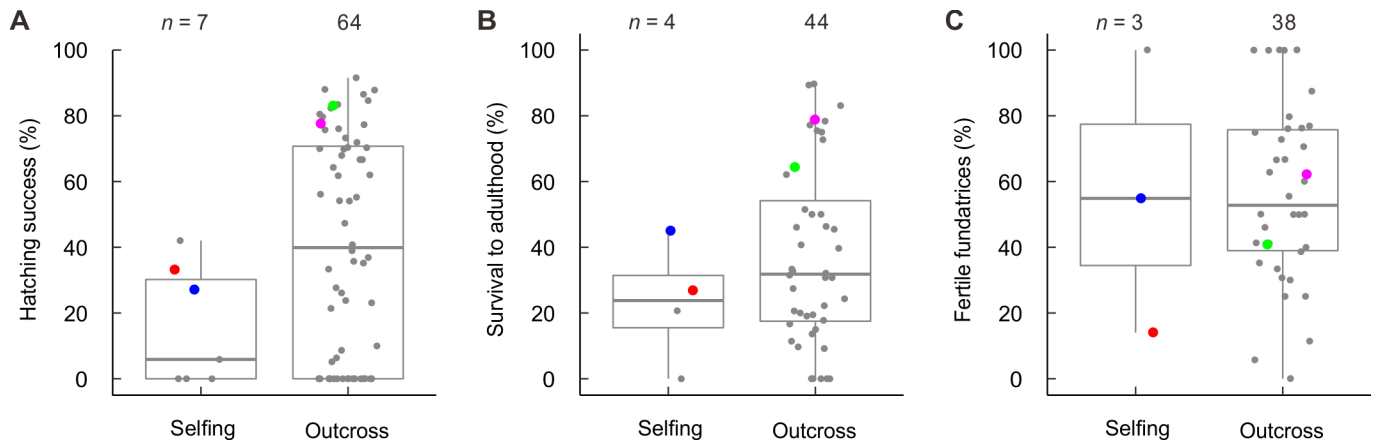


Fig. 2 Experimental crossing results in *Acyrthosiphon pisum*. (A) Hatching success (percentage of fertilized eggs hatching into live fundatrices), (B) nymphal survival (percentage of fundatrices surviving to adulthood) and (C) percentage of fundatrices producing live offspring. Red: ApL♀ × ApL♂; blue: 09003A♀ × 09003A♂; magenta: ApL♀ × 09003A♂; green: 09003A♀ × ApL♂; gray: other crosses. For more detailed data, refer to Table S2.

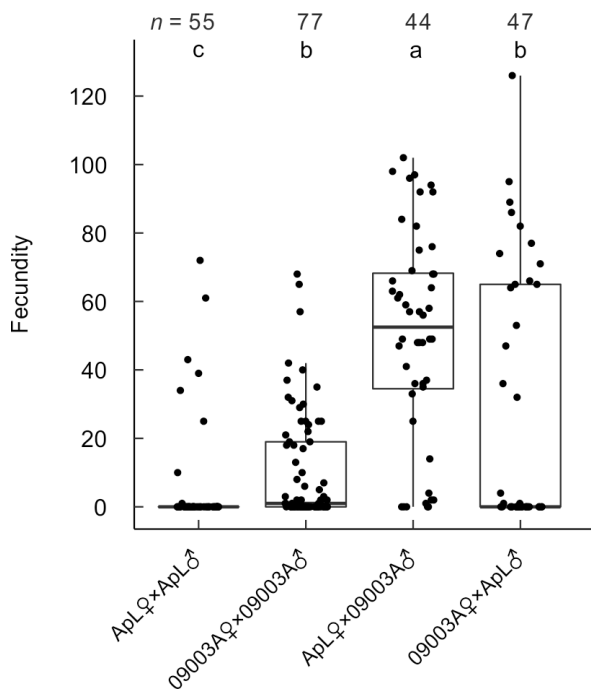


Fig. 3 Comparison of lifetime fecundity among fundatrices from different crosses in *Acyrthosiphon pisum*. Different letters indicate significant differences (Brunner–Munzel test with Bonferroni adjustment, $P < 0.05$, $n = 40–77$).

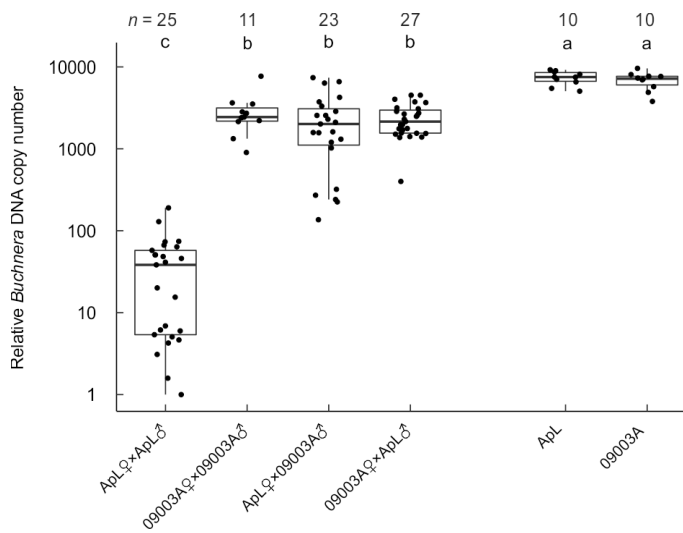


Fig. 4 Comparison of *Buchnera* abundance among fundatrices from different crosses in *Acyrthosiphon pisum*. Relative *Buchnera* genome copy number per individual was measured using qPCR targeting the *Buchnera dnaK* gene. The lowest value was set at 1. Different letters indicate significant differences (Brunner–Munzel test with Bonferroni adjustment, $P < 0.05$, $n = 10–27$).

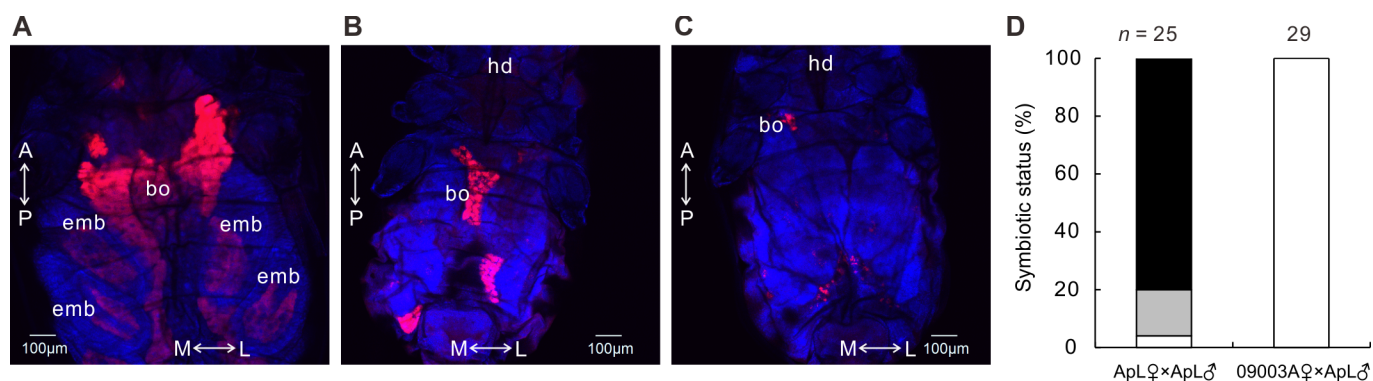


Fig. 5 Comparison of symbiotic status between fundatrices from different crosses in *Acyrthosiphon pisum*. (A–C) Ventral view of adult fundatrices from the 09003♀ × ApL♂ (A) and ApL♀ × ApL♂ (B, C) crosses. Blue (DAPI) and red (Cy5) signals show nuclei and *Buchnera*, respectively. (D) Fundatrix classification based on symbiotic status. White, gray, and black bars indicate fundatrices with clear *Buchnera* signals in large bacteriocytes (diameter > 50 μm), those with clear *Buchnera* signals in small bacteriocytes (diameter < 50 μm), and those with faint *Buchnera* signals, respectively. Abbreviations; A-P, anterior-posterior axis; bo, bacteriome; emb, embryo; hd, head; M-L, medial-lateral axis.

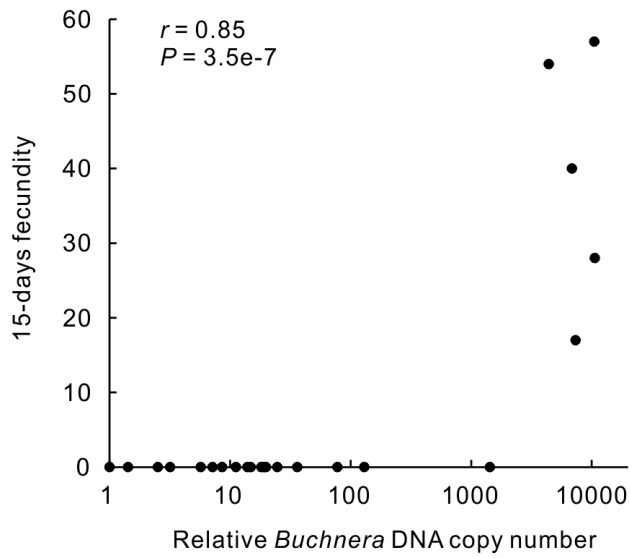


Fig. 6 Relationship between *Buchnera* abundance and fecundity in fundatrices of *Acyrtosiphon pisum*. Relative *Buchnera* genome copy number per individual was measured using qPCR targeting the *Buchnera dnaK* gene. The Pearson correlation coefficient (r) and P -value are shown ($n = 25$).

Supplementary information

Table S1 The *Acyrtosiphon pisum* strains used in this study.

Clone name	Collection year	Host plant (origin)	Male wing phenotype
ApL	2005	<i>Medicago sativa</i>	Winged
E	2005	<i>Vicia cracca</i>	Winged
07C-1	2007	<i>Vicia cracca</i>	Winged
IwaG	2011	<i>Vicia cracca</i>	Winged
IwaA	2017	<i>Vicia cracca</i>	Winged
Tpra	2015	<i>Trifolium pratense</i>	Mixed
08AP2	2008	<i>Medicago sativa</i>	Wingless
09003A	2009 ^a	<i>Melilotus</i> spp.	Winged
KNb	2017	<i>Medicago sativa</i>	Winged

^aThe clone was born from a sexual cross in 2009.

Table S2 Hatching, survival and fertility rate of experimental sexual crosses.

Father	Mother	Eggs	Hatchings (%)	Adults emerged (%)	Fertile adults (%)
ApL	ApL	638	212 (33.3%)	57 (26.9%)	8 (14.0%)
ApL	E	95	87 (91.6%)	8 (9.2%)	2 (25.0%)
ApL	07C-1	139	78 (56.1%)	13 (16.7%)	4 (30.8%)
ApL	IwaG	195	45 (23.1%)	15 (33.3%)	6 (40.0%)
ApL	IwaA	24	13 (54.2%)	4 (30.8%)	4 (100.0%)
ApL	Tpra	84	31 (36.9%)	3 (9.7%)	2 (66.7%)
ApL	08AP2	57	19 (33.3%)	0 (0.0%)	0 (—)
ApL	09003A	142	118 (83.1%)	76 (64.4%)	31 (40.8%)
ApL	KNb	364	128 (35.2%)	59 (46.1%)	45 (76.3%)
E	ApL	217	165 (76.0%)	148 (89.7%)	89 (60.1%)
E	E	84	0 (0.0%)	0 (—)	0 (—)
E	07C-1	39	2 (5.1%)	0 (—)	0 (—)
E	IwaG	90	0 (0.0%)	0 (—)	0 (—)
E	IwaA	84	0 (0.0%)	0 (—)	0 (—)
E	Tpra	148	0 (0.0%)	0 (—)	0 (—)
E	08AP2	40	28 (70.0%)	9 (32.1%)	5 (55.6%)
E	09003A	49	0 (0.0%)	0 (—)	0 (—)
E	KNb	100	62 (62.0%)	17 (27.4%)	12 (70.6%)
07C-1	ApL	193	161 (83.4%)	100 (62.1%)	46 (46.0%)
07C-1	E	62	0 (0.0%)	0 (—)	0 (—)
07C-1	07C-1	34	2 (5.9%)	0 (0.0%)	0 (—)
07C-1	IwaG	113	0 (0.0%)	0 (—)	0 (—)
07C-1	IwaA	21	5 (23.8)	1 (20.0%)	1 (100.0%)
07C-1	Tpra	28	0 (0.0%)	0 (—)	0 (—)
07C-1	08AP2	6	4 (66.7%)	3 (75.0%)	3 (100.0%)
07C-1	09003A	187	40 (21.4)	6 (15.0%)	2 (33.3%)
07C-1	KNb	74	35 (47.3)	4 (11.4%)	3 (75.0%)
IwaG	ApL	67	37 (55.2)	29 (78.4%)	12 (41.4%)
IwaG	E	31	0 (0.0)	0 (—)	0 (—)
IwaG	07C-1	30	0 (0.0)	0 (—)	0 (—)
IwaG	IwaG	20	0 (0.0%)	0 (—)	0 (—)
IwaG	IwaA	43	0 (0.0%)	0 (—)	0 (—)
IwaG	Tpra	80	0 (0.0%)	0 (—)	0 (—)
IwaG	08AP2	91	77 (84.6%)	64 (83.1%)	51 (79.7%)
IwaG	09003A	42	15 (35.7%)	0 (—)	0 (—)
IwaG	KNb	61	33 (54.1%)	17 (51.5%)	7 (41.2%)
IwaA	ApL	184	162 (88.0%)	51 (31.5%)	18 (35.3%)

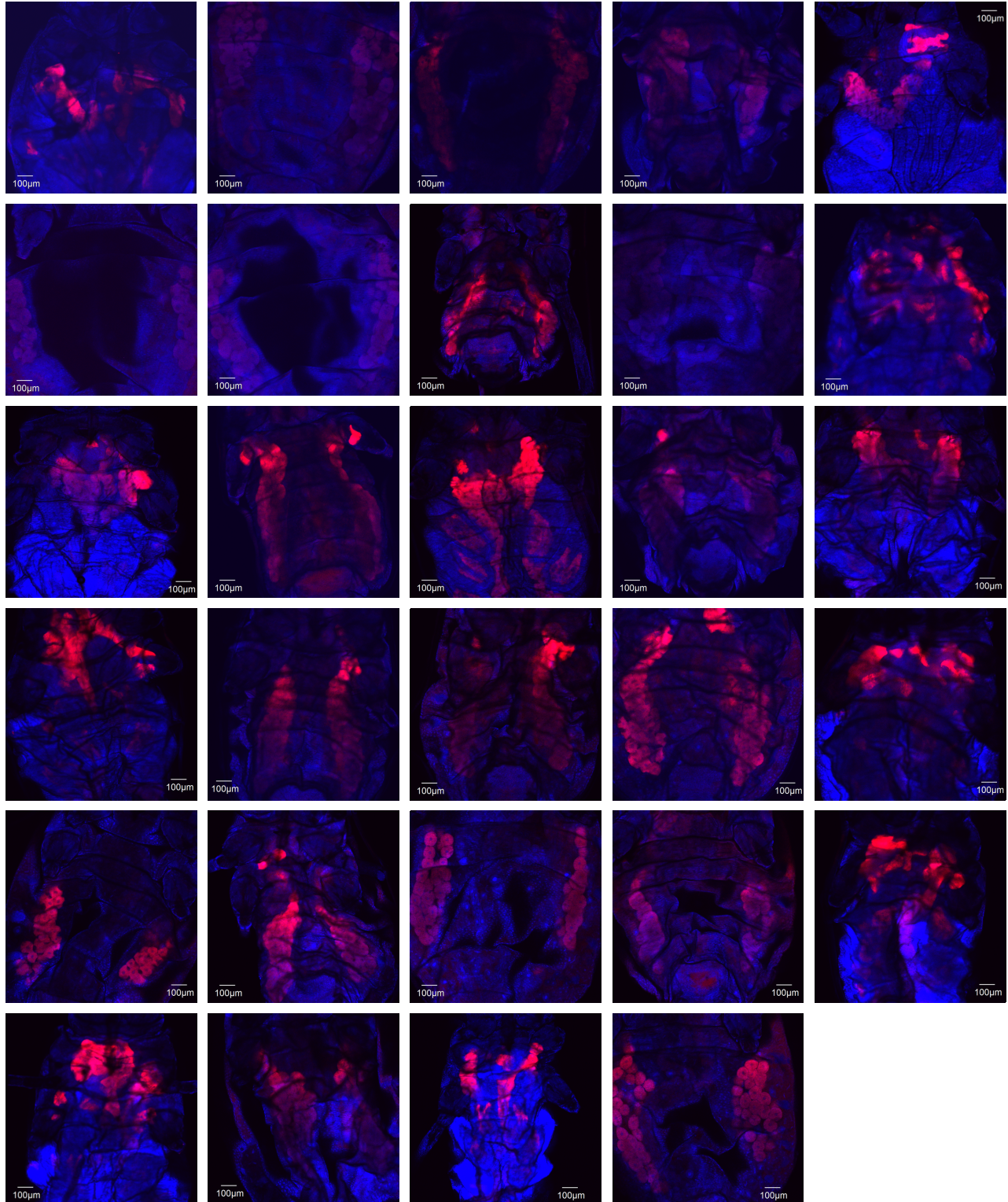
IwaA	E	68	0 (0.0%)	0 (—)	0 (—)
IwaA	07C-1	17	0 (0.0%)	0 (—)	0 (—)
IwaA	IwaG	51	0 (0.0%)	0 (—)	0 (—)
IwaA	IwaA	91	0 (0.0%)	0 (—)	0 (—)
IwaA	Tpra	104	9 (8.7%)	2 (22.2%)	1 (50.0%)
IwaA	08AP2	41	33 (80.5%)	24 (72.7%)	21 (66.7%)
IwaA	09003A	190	74 (38.9%)	18 (24.3%)	9 (50.0%)
IwaA	KNb	33	22 (66.7%)	3 (13.6%)	2 (66.7%)
Tpra	ApL	130	107 (82.3%)	35 (32.7%)	2 (5.7%)
Tpra	E	81	57 (70.4%)	44 (77.2%)	17 (38.6%)
Tpra	07C-1	41	36 (87.8%)	7 (19.4%)	— (—)
Tpra	IwaG	40	4 (10.0%)	0 (0.0%)	0 (—)
Tpra	IwaA	68	42 (61.8%)	8 (19.0%)	8 (100.0%)
Tpra	Tpra	69	29 (42.0%)	6 (20.7%)	6 (100.0%)
Tpra	08AP2	70	53 (75.7%)	40 (75.5%)	12 (30.0%)
Tpra	09003A	89	64 (71.9%)	— (—)	— (—)
Tpra	KNb	64	45 (70.3%)	8 (17.8%)	2 (25.0%)
08AP2	ApL	136	95 (69.9%)	44 (46.3%)	5 (11.4%)
08AP2	E	49	20 (40.8%)	— (—)	— (—)
08AP2	07C-1	59	47 (79.7%)	42 (89.4%)	21 (50.0%)
08AP2	IwaG	47	13 (27.7%)	4 (30.8%)	2 (50.0%)
08AP2	IwaA	1	0 (0.0%)	0 (—)	0 (—)
08AP2	Tpra	53	36 (67.9%)	0 (0.0)	0 (—)
08AP2	09003A	89	77 (86.5%)	35 (45.5%)	22 (62.9%)
08AP2	KNb	42	27 (64.3%)	11 (40.7%)	8 (72.7%)
09003A	ApL	286	222 (77.6%)	175 (78.8%)	109 (62.3%)
09003A	E	167	0 (0.0%)	0 (—)	0 (—)
09003A	07C-1	46	12 (26.1%)	6 (50.0%)	6 (100.0%)
09003A	IwaG	131	0 (0.0%)	0 (—)	0 (—)
09003A	IwaA	59	0 (0.0%)	0 (—)	0 (—)
09003A	Tpra	63	4 (6.3%)	2 (50.0%)	0 (0.0%)
09003A	08AP2	163	126 (77.3%)	50 (39.7%)	38 (76.0%)
09003A	09003A	670	182 (27.2%)	82 (45.1%)	45 (54.9%)
09003A	KNb	86	63 (73.3%)	13 (20.6%)	10 (76.9%)
KNb	ApL	55*	0 (0.0%)	0 (—)	0 (—)
KNb	07C-1	53*	0 (0.0%)	0 (—)	0 (—)
KNb	KNb	47*	0 (0.0%)	0 (—)	0 (—)

* Only unfertilized eggs were oviposited.

Fig. S1 Ventral view of adult fundatrices and the localization of *Buchnera*. Blue (DAPI) and red (Cy5) signals indicate nuclei and *Buchnera*, respectively.

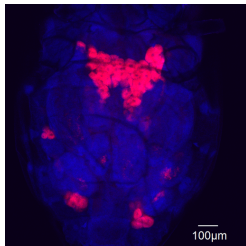
09003A ♀ × ApL♂

Clear *Buchnera* signals and large bacteriocytes

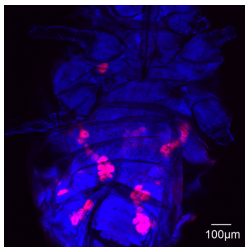
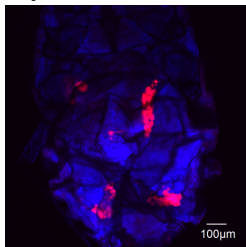
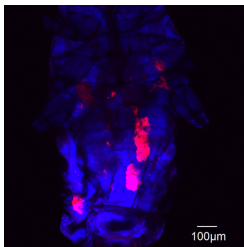
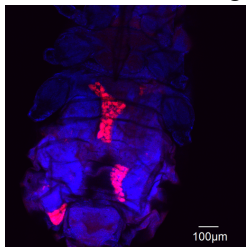


ApL ♀ × ApL ♂

Clear *Buchnera* signals and large bacteriocytes



Clear *Buchnera* signals and small bacteriocytes



Faint *Buchnera* signals

