Diet as a major driver of endosymbiont proliferation in cereal weevils

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Highlights

- in the cereal weevil *Sitophilus oryzae*, carbohydrate intake triggers an exponential endosymbiont proliferation in young adults, before a host-controlled endosymbiont clearance;

- the host is incapable of adjusting energy allocation to the endosymbionts, even to the detriment of its own survival;

- on a balanced diet, endosymbiont proliferation is dispensable for host fitness (cuticle tanning and fecundity).

Summary

Nutritional symbiosis between insects and intracellular bacteria (endosymbionts) are a major force of adaptation, allowing animals to colonize nutrient-poor ecological niches[1–6]. Many beetles feeding on tyrosine-poor substrates rely on a surplus of aromatic amino acids produced by bacterial endosymbionts[7–9] that synthesize them autotrophically[10–13]. This surplus of aromatic amino acids is crucial for the biosynthesis of a thick exoskeleton, the cuticle, which is made of a matrix of chitin with proteins and pigments built from the tyrosine-derived 3,4-dihydroxyphenylalanine (DOPA[14]), providing an important defensive barrier against biotic and abiotic stress[10,15]. Other endosymbiont-related advantages for beetles include a faster development[4,16] and improved fecundity[17,18]. The association between the cereal weevil *Sitophilus oryzae* and *Sodalis pierantonius* endosymbiont[19] represents a unique case study: in young adult weevils, endosymbionts undergo a massive proliferation concomitant with the cuticle tanning, then they are fully

eliminated[20]. While endosymbiont clearance is a host-controlled process[20], the mechanism triggering endosymbiont proliferation remains poorly understood. Here, we show that endosymbiont proliferation relies on host carbohydrate intake. Remarkably, insect fecundity was preserved, and the cuticle tanning achieved, even when endosymbiont proliferation was experimentally blocked, except in the context of a severely unbalanced diet. Moreover, a high endosymbiont load coupled with nutrient shortage dramatically impacts host survival, revealing the high energy cost of proliferating endosymbionts and the incapacity of the host to adjust energy allocation.

Results

Endosymbiont proliferation and endosymbiont-dependent cuticle tanning precede adult emergence

Females of *S. oryzae* lay eggs inside cereal grains, where the progeny develops up to early adulthood (**Fig. 1A**). After metamorphosis, gut endosymbionts are located in specialized cells, the bacteriocytes, at the apexes of gut ceaca[21], where they proliferate exponentially before a complete host-controlled clearance[20]. When adult weevils exit the grain by piercing a hole with their rostrum (*i.e.* emergence), the endosymbiont load is close to its maximum, while the host cuticle tanning process, measured as a reduction in thorax redness[11,22], is about to be completed (**Fig. S1**).

To tackle the mechanisms behind endosymbiont proliferation, we have established a semi-artificial rearing protocol allowing timing and sampling of adults before and after grain emergence. Pupae manually extracted from grains were maintained on plates while daily monitoring their development (**Fig. 1A**). Adult stages were defined as follows: adults at stage 1 are orange-colored individuals unable to walk; adults at stage 2 (six to 12 hours after stage 1) are darker in color but still unable to walk; adults at stage 3 (12-24 hours after stage 2) are brown and mobile; all subsequent developmental stages are daily increments (**Fig. 1A**). Flow cytometry quantification of endosymbiont load showed that endosymbiont proliferation started at stage 4, reaching its maximum at stage 6, while clearance was completed at stage 15 (**Fig. 1B**). In parallel, we observed the progressive darkening of the cuticle, which reached its maximum a few days after the higher endosymbiotic load (stage 9, **Fig. 1C**), in agreement with weevils naturally reared on wheat grains (**Fig. S1**). From a comparison of both color and endosymbiont dynamics of plate-reared insects (**Fig. 1B-C**) and insects naturally emerged from grains (**Fig. S1**) we identified stage 5.5 as the moment of emergence.

We confirmed that endosymbionts are crucial for insect fitness, as cuticle tanning was slower in aposymbiotic weevils (*i.e.* animals artificially depleted of endosymbionts[4]), never reaching symbiotic levels (**Fig. S1B** insects naturally emerged from grains; **Fig. 1C** plate-reared insects), even though adult survival was comparable between symbiotic and aposymbiotic insects in laboratory conditions (**Fig. 1D**). Furthermore, symbiotic insects laid and developed inside whole wheat flour kernels supplemented with

antibiotics lacked endosymbiont proliferation (**Fig. S2A**), and not only showed a lighter cuticle (**Fig. S2B**), but also a delay in development (**Fig. S2C**) and lower emergence rates (**Fig. S2D**), thus resembling aposymbiotic animals[4,20].

Endosymbiont proliferation is carbohydrate-dependent and detrimental for host survival when coupled with nutrient shortage

Keeping pupae in whole wheat flour supplemented with E133 blue dye resulted in finding the blue dye in the gut of adult weevils from stage 3, meaning that adult weevils start eating one day before endosymbiont proliferation (**Fig. 1A**). In agreement with this finding, insects kept on plates without food from the pupal stage to adult stage 3, then moved to wheat grains, presented a similar endosymbiont dynamics as insects kept in whole wheat flour from the pupal stage to adult stage 3 (**Fig. 1B**), and no difference in insect survival was observed (**Fig. 1D**). In contrast, feeding adult weevils only from stage 4 or stage 5 onwards caused a delay in the endosymbiont proliferation of two and three days, respectively (**Fig. 2A**), while the overall dynamic profile was unaltered. This suggests that nutrient provision is crucial to sustain endosymbiont proliferation.

We also observed a one-day delay in cuticle tanning of weevils fed from stage 4, and a three-day delay in weevils fed from stage 5 (**Fig. 2B**). Furthermore, the two-day delay in feeding caused a significant decrease in insect survival (**Fig. 2C),** while no effect on fecundity (measured using the number of emerging descendants as a proxy) was observed (**Fig. S3A**). The decrease in survival can be attributed to the additional cost of harboring the endosymbionts, as no variation in survival rate was observed for starved aposymbiotic weevils (**Fig. 2D**). Furthermore, when starvation was applied to symbiotic adults taken 15 days after emergence (when the gut endosymbiont population was already cleared, **Fig. S1A**), their survival was slightly higher than aposymbiotic animals (**Fig. S4**).

In a severely unbalanced diet, consisting only of starch (*i.e.* carbohydrates), endosymbiont dynamics was similar to the control condition (weevils fed with wheat from stage 3, **Fig. 2E**), and the cuticle tanning was completed faster (**Fig. 2F**), probably thanks to the higher friability of starch grains, which might be easier to break down and digest than wheat grains. A small endosymbiont gut population persisted until stage 27 (**Fig. S5**), suggesting that the host-controlled clearance[20] can be delayed to extend endosymbiont presence in an extremely poor environment. Starch diet did not reduce but rather increased symbiotic insect survival in the first 40 days of adulthood (**Fig. 2G)**, although it completely abolished insect reproduction (**Fig. S3B**) and led to 100% mortality of aposymbiotic weevils (**Fig. 2H**), as previously described[20].

DOPA accumulation was previously suggested as a putative molecular signal for endosymbiont clearance[20]. Here, the semi-artificial rearing system allowed showing that DOPA increase was concomitant with the

endosymbiont clearance rather than anticipating it (**Fig. S6A**), suggesting that DOPA is likely a transient molecule for mobilizing nitrogen-rich compounds freed by endosymbiont clearance. Indeed, DOPA increase was delayed by 2-3 days in weevils fed from stage 5 (**Fig. S6A**), coinciding with achieved cuticle tanning and the onset of endosymbiont clearance. In starch-fed weevils, DOPA levels resembled those of aposymbiotic weevils (**Fig. S6B**), suggesting that molecules enriched in aromatic amino acids are less abundant and/or recycled more efficiently than in grain-fed weevils to cope with the shortage of amino acids.

Overall, carbohydrate intake appeared necessary and sufficient to trigger endosymbiont proliferation. Furthermore, higher mortality of young starved symbiotic insects revealed the host incapacity of adjusting the energy allocated to endosymbiont maintenance.

Endosymbiont proliferation is dispensable for cuticle tanning and fecundity when coupled with a balanced diet

Since the majority of beetles relying on endosymbionts for cuticle tanning do not show an exponential rise in endosymbiont load[10–12,23], we asked whether a smaller endosymbiotic population would ensure cuticle tanning also in cereal weevils. To do so, we starved weevils at stage 4 and stage 5, after having fed them at stage 3. This arrested the endosymbiont proliferation, and triggered endosymbiont decrease from stages 5 to 8 (**Fig. 3A)**. Remarkably, a second proliferation phase was observed after weevils were fed again, likely driven by carbohydrate intake, with a maximum load at stage 11 and a complete clearance between stage 15 and 20 (**Fig. 3A**). In both exponential phases, the endosymbiont load reached half the height of the control condition (weevils fed with wheat from stage 3), suggesting that the total endosymbiont load might be genetically controlled by the host. The first phase did not result in increased cuticle tanning, with stage 7 weevils still resembling control stage 4 weevils (**Fig. 3B**). In contrast, a few days after the second proliferation phase, cuticle tanning was fully achieved (stage 12 **Fig. 3B**). Consistently with the hypothesis of a high metabolic cost associated with endosymbiont proliferation, symbiotic weevils starved between stage 4 and stage 5 displayed 30% mortality, while no increase in mortality was observed for aposymbiotic weevils equally stressed (**Fig. 3C-D).** Consistently with the hypothesis that DOPA accumulation represents a transient mobilization of nitrogen-rich storage molecules, two DOPA peaks were observed in concomitance with the two endosymbiont clearance phases (**Fig. S6C**).

We therefore hypothesized that a lower endosymbiont load would be sufficient for cuticle tanning. To test this, while avoiding additional stress on the host, we fed adult weevils with whole wheat flour kernels supplemented with a cocktail of antibiotics[4,24]. While control weevils (fed with whole wheat flour kernels from stage 3) displayed a traditional rise and clearance of endosymbionts, the antibiotic supplementation triggered only a mild rise of endosymbionts at stage 4, followed by a complete clearance (**Fig. 3E**). With antibiotic supplementation, we did not observe differences in cuticle tanning (**Fig. 3F**), fecundity (**Fig. S3A**)

or survival in symbiotic or aposymbiotic weevils (**Fig. 3G-H**), while DOPA accumulation was slightly reduced, likely due to the lower endosymbiont load (**Fig. S6D**). The same endosymbiont dynamics was observed for weevils fed on starch kernels supplemented with antibiotics, except for the fact that, as already noted (**Fig 2E**), a small endosymbiotic population was retained longer (**Fig. 3E**). Remarkably, the antibiotic treatment combined with a severely unbalanced diet (starch only) reduced insect survival of starch-fed symbiotic animals (**Fig. 3G**), and their cuticle tanning was severely impaired: although faster (*i.e.* completed at stage 5) the process soon stopped at levels comparable to aposymbiotic weevils (**Fig. 3F**).

While these findings attested that endosymbiont proliferation is highly energy-consuming and carbohydrate-dependent, they also revealed that, in the absence of a severely unbalanced diet, endosymbiont proliferation in young weevils is not required to ensure two of the major advantages known of this endosymbiosis, *i.e.* improved fecundity and cuticle tanning.

Discussion

By altering or delaying the endosymbiont proliferation in young adult weevils, we observed that a lower endosymbiont load still guaranteed normal cuticle tanning and fecundity – the two main known symbiotic advantages for *S. oryzae* – unless coupled with severely unbalanced diet (**Fig. 4**). This is unlikely to occur in nature, since endosymbiont proliferation occurs when weevils are still located inside cereal grains. Although we cannot exclude other fitness advantages for the host (*e.g.* stronger protection from parasites[13,25,26]), we have also shown that endosymbiont proliferation relies on energy availability in the form of carbohydrates and that the host seems to be incapable of controlling energy allocation to endosymbionts (**Fig. 4**). Therefore, while endosymbiont clearance is a host-dependent process[20], endosymbiont proliferation seems to escape host control.

Symbiotic interactions are constantly evolving, in a continuum ranging from parasitism to mutualism, depending on changes in the interacting species and in their environment[27–31]. In insects, endosymbiont acquisition generally starts with domestication of parasites or commensals[32]. Host control over endosymbionts is often observed, as endosymbionts gradually lose the ability of autonomous life through genome shrinkage[33,34] and point mutations[35], sometimes retaining only the metabolic pathways that confer fitness advantage for the host[13]. Endosymbiont loss and/or replacement generally occur in concomitance with excessive genome shrinkage limiting host advantages[36–39].

Other examples of host-controlled endosymbiont clearance during the host life cycle have been observed[40]. In contrast, hints of endosymbiont control over the host are rare and usually observed in facultative endosymbioses. For instance, recent findings have shown that free-living, facultative symbiotic algae are still able to colonize and proliferate in some species of cnidaria even when impaired in photosynthesis – the

main host fitness advantage[41]. This raises the possibility of species-specific events of parasitic-like behavior of algal endosymbionts in contexts of nutrient shortage, similar to what we have observed for *S. pierantonius*.

How can the obligate endosymbiotic nature of *S. pierantonius* be reconciled with its transient parasitic-like behavior? As in the algal-cnidaria case, this might be the product of a compatible host-bacterium combination. An alternative, complementary explanation is that the endosymbiont still retains vestigial characteristics of its previous pathogenic nature, as the acquisition of *S. pierantonius* is recent (~ 28 million years ago)[19]. *S. pierantonius* retains other elements typical of free-living bacteria, such as a complex genome[19] containing genes of a type 3 secretion system and a flagellum. These genes are all upregulated during insect metamorphosis, when the endosymbionts migrate to colonize the cells of the newly-formed gut caeca[21], similarly to a pathogen infection. Interestingly, the sister species *Sitophilus zeamais* presents a similar endosymbiont dynamics, while the related species *Sitophilus granarius* shows lower and more constant endosymbiont levels in young adults[20]. Comparison of different *Sitophilus* species and their endosymbionts in various stress conditions, together with artificial endosymbiont replacement and genetic modification strategies, would provide an ideal model for probing the mechanisms and constraints of endosymbiont domestication[42,43].

Figure legends

Figure 1. Endosymbiont proliferation and endosymbiont-dependent cuticle tanning precede adult emergence. A) Schematic representation of the natural insect development on grain and the semi-artificial rearing system, after manual extraction of the pupae from the grains. Each pupa was kept on a well of a 10x10 well-plate (with whole wheat flour or without – control condition) until reaching adult stage 3, then kept on wheat grains. Insects were observed daily to monitor the development on the basis of cuticle color, and ability to move and feed. Stage 9 aposymbiotic weevils are also shown for comparison. **B)** Endosymbiont dynamics of weevils reared on plates from the pupal stage with or without (control condition) whole flour supplementation until stage 3, then fed on wheat. **C)** Cuticle tanning progress, measured as a decrease in thorax redness, for plate-reared symbiotic and aposymbiotic weevils. **D)** Survival curves of aposymbiotic and symbiotic weevils reared as in **B)** and **C)**. Endosymbiont dynamics and cuticle comparisons were made by two-way ANOVA followed by Turkey's multiple comparison test. Survival curves were analyzed with the Kaplan-Meier method followed by a Log-rank test. Shaded regions represent 95% CI. *: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$. Error bars represent standard error of the mean. Orange bars in **B)** and **C)** depict the day food was provided to control and aposymbiotic insects.

Figure 2. Endosymbiont proliferation is carbohydrate-dependent and detrimental for host survival when coupled with nutrient shortage. Gut endosymbiont dynamics **(A)** and cuticle tanning progress **(B)** for

symbiotic weevils fed with wheat grains from stage 3 onwards (control condition, as in **1B and 1C**, respectively), from stage 4, or from stage 5. Colored arrows indicate the stage when food was administered. **C)** Symbiotic and **D)** aposymbiotic survival curves of weevils fed from stage 4 or 5 in comparison to control. Gut endosymbiont dynamics **(E)** and cuticle tanning progress **(F)** for weevils fed with starch grains from day 3 onwards, in comparison to control (as in **1B** and **1C**, respectively). **G)** Symbiotic and **H)** aposymbiotic survival curves of starch-fed weevils in comparison to control (as in **1D**). Endosymbiont dynamics and cuticle color comparisons were made by two-way ANOVA followed by Turkey's multiple comparison test. Survival curves were analyzed with the Kaplan-Meier method followed by Log-rank test. Shaded regions represent 95% CI. *: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$. Error bars represent standard error of the mean.

Figure 3. Endosymbiont proliferation is dispensable for cuticle tanning and fecundity when coupled with a balanced diet. Gut endosymbiont dynamics **(A)** and cuticle tanning progress **(B)** for weevils fed with wheat at stage 3, then starved between stages 4 and 5, in comparison control (as in **1B** and **1C**, respectively). Colored arrows indicate the stage when food was administered, and the gray area indicates starvation period. **C)** Symbiotic and **D)** aposymbiotic survival curves of fed, then starved weevils in comparison to control (as in **1D**). Gut endosymbiont dynamics **(E)** and cuticle tanning progress **(F)** for weevils fed with wheat kernels supplemented or not (control condition) with antibiotics, or starch kernels supplemented or not with antibiotics from stage 3 onwards. **G)** Symbiotic and **H)** aposymbiotic survival curves of weevils fed with wheat kernels supplemented or not (control condition) with antibiotics, or starch kernels supplemented or not with antibiotics from stage 3 onwards. Endosymbiont dynamics and cuticle color comparisons were made by two-way ANOVA followed by Turkey's multiple comparison test. Survival curves were analyzed with the Kaplan-Meier method followed by Log-rank test. Shaded regions represent 95% CI. *: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$. Error bars represent standard error of the mean.

Figure 4: Cost/Benefits and main controlling partners at various stages of the *S. oryzae/S. pierantonius* symbiosis. The host-independent endosymbiont rise in young adult weevils can be detrimental, advantageous or neutral for host development and fecundity, depending on the diet. Endosymbiont rise is followed by host-controlled bacterial clearance that leads to energy recycle.

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Authors contribution

Conceptualization: EDA, AH, RR; Methodology: EDA, VL, SP, IR, FB, AV; Writing: EDA, ED, AH, RR; Visualization: EDA, RR; Supervision: PDS, ED, AH, RR; Funding acquisition: RR.

Declaration of interest

The authors declare no conflict of interest.

STAR Methods

Insect rearing and growth conditions

Symbiotic and aposymbiotic *S. oryzae* insects were fed with organic wheat grains sterilized at -80 °C. Insects were kept in plastic boxes in a stove at 27 °C and 70% relative humidity, in the dark.

For antibiotic supplementation experiments, wheat flour kernels were prepared using commercial whole wheat flour (Francine, France) or starch (Stijfsel Remy, Belgium), with the addition of 0.1% (v/v) of chlortetracycline (Sigma-Aldrich) and 0.5% (v/v) penicillin G (Sigma-Aldrich). To prepare the kernels, flour/starch and, when needed, antibiotics were mixed with water (q.s.) to make a smooth dough. The dough was spread on a plastic surface and dried overnight at room temperature, then cut in little round pieces (kernels) and stored at 4°C before use.

For analysis of insect development on antibiotic-supplemented whole wheat flour kernels, two-week-old symbiotic and aposymbiotic adult weevils (n= 50) were fed for 24 hours with 20 whole wheat flour kernels (supplemented or not with antibiotics), then insects were removed and the kernels were kept in the incubator and observed daily to monitor: the day of progeny emergence, the number of emergents, the endosymbiont load at emergence as well as the thorax cuticle color 12 days after emergence.

To monitor the moment adult weevils start eating after metamorphosis, pupae were manually extracted from grains and kept in plate wells with whole flour supplemented with E133 dye (100 µl of dye for 3.5 g flour). The E133 dye was first mixed with the flour with water (q.s.), let dry at room temperature overnight and then grinded. Guts of insects corresponding to various developmental stages (from stage 1 to stage 9) were dissected and observed with light microscopy.

Endosymbiont quantification by flow cytometry

The protocol for endosymbiont quantification was modified from Login et al., 2011[44]. Briefly, a minimum of three pools (per condition and developmental stage) of four midguts each were dissected in TA buffer (25

mM KCl, 10 mM $MgCl_2$, 250 mM sucrose and 35 mM Tris/HCl, pH 7.5). The samples were manually grinded in 100 µl TA buffer up to homogenization and centrifuged at 0.5 rpm for 2 minutes to sediment impurities. The supernatant was diluted in 400 µl TA buffer, then filtered with a 40 µm Flowmi filter (SP Scienceware) and centrifuged at 10 000 rpm for five minutes. The supernatant was discarded and the pellet was kept at 4°C in 4% paraformaldehyde (PFA, Electron Microscopy Science) before analysis.

Before quantification, pellets were centrifuged at 11 000 rpm for 20 minutes at 4°C, the PFA supernatant was discarded and samples were resuspended in 700 µl ultrapure water and 0.08% of SYTO9 dye (Invitrogen). additional water dilutions were made if the bacterial concentration was above the detection limit of the instrument.

Quantification was performed with BD Accuri C6 Plus cytometer (flow: 14 µl/min for 1 minute, cutoff at 6000). Normalization was obtained by subtracting the values obtained from guts of aposymbiotic weevils. All measurements performed for endosymbiont load analyses are independent from measurements of cuticle color, insect survival, fecundity and DOPA quantifications.

Analysis of cuticle color

The cuticle darkening process was monitored at various insect stages and conditions by using the Natsumushi software v. 1.10[11,22] on pictures taken with an Olympus XC50 camera attached to a Leica MZFLIII binocular and the CellF software (Olympus Soft Imaging System) under the same lightning conditions. Quantification was performed as illustrated in Anbutsu et al., 2017[11] using the thorax region, because of its color uniformity. Briefly, pixels with brightness over top 10% or below bottom 10% were excluded from analysis. Then RGB values for all (= n) pixels were measured and averaged by Σ (R – mean [R, G, B])/n to obtain the proxy redness thorax mean value. Eight to 15 individuals were measured per condition and stage. All measurements performed for cuticle color analyses are independent from measurements of endosymbiont load, insect survival, fecundity and DOPA quantifications.

Survival measurements

For plate-reared weevils, insects were isolated at pupal stage on plate wells and assigned to a specific diet (n = 100 insects per diet condition, of mixed sexes, unsexed individuals) starting at adult stage 3, with the exception of whole flour reared weevils of Fig. 1B and Fig. 1D, which were reared on whole wheat flour from the pupal stage up to adult stage 3. Dead weevils were counted daily between stage 4 and stage 10, then weekly up to stage 40.

For weevils naturally emerging from grains, insects were isolated at emergence, kept for two weeks and then starved for two days. Dead weevils were counted daily from the 16th to the 22nd day after emergence, then once per week up to the 44th day after emergence.

All measurements performed for cuticle insect survival are independent from measurements of endosymbiont load, color analyses, fecundity and DOPA quantifications.

Fecundity

We used the number of emerging descendants as a proxy to measure insect fecundity of plate-reared symbiotic and aposymbiotic weevils, as well as weevils fed from stage 5, starch-fed weevils and antibiotic-fed weevils. Fifteen couples of randomly-paired male/female weevils per condition were established at stage 3. Then, weevils were subjected to the specific diet condition up to stage 8. At this point, antibiotic-fed weevils were shifted to the control diet (wheat grains). From stage 8 up to stage 45, the diet was changed every 3, 4 or 5 days (20 kernels each time). All wheat or starch grains were kept for two months to allow the emergence and counting of the progeny.

All measurements performed for fecundity analyses are independent from measurements of endosymbiont load, cuticle color, insect survival and DOPA quantifications.

DOPA measurements

Measurements of free DOPA were performed on pools of frozen weevils (each pool made of three weevils) and the analysis was performed on three to five replicates per condition and stage. The whole weevil body was used for the analysis. Measurements were performed as in Vigneron et al., 2014[20], using norvaline as an internal standard and a reverse phase HPLC method with a C18 column (Zorbax Eclipse-AAA 3.5 um, 150 x 4.6 mm, Agilent Technologies).

All measurements performed for DOPA analyses are independent from measurements of endosymbiont load, cuticle color, insect survival and fecundity.

Supplemental information

Figure S1. Endosymbiont dynamics and cuticle tanning in grain-reared weevils. Gut endosymbiont dynamics **(A)** and cuticle tanning progress **(B)** for weevils which developed in wheat grains and emerged spontaneously as adults. Results confirm and extend previous findings[20]. Red bar: emergence (E) from grain. All subsequent time points (E+X) represent days after emergence. Cuticle color comparisons between symbiotic and aposymbiotic weevils were made by two-way ANOVA followed by Turkey's multiple comparison test. *: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$. Error bars represent standard error of the mean.

Figure S2: Symbiotic weevils on whole wheat flour kernels supplemented with antibiotics resemble aposymbiotic weevils. A group of 100 symbiotic or aposymbiotic weevils were left for 24 hours with 30 whole wheat flour kernels supplemented or not with antibiotics. The weevils were then removed and the kernels kept for monitoring: **A)** endosymbiont load at emergence, **B)** thorax redness at 12 days after emergence , **C)** day of progeny emergence from grain and **D)** egg laying rate for symbiotic insects laid in whole wheat flour kernels (See STAR Methods) supplemented (red) or not (mustard) with antibiotics, compared to aposymbiotic insects fed in the same way. Comparisons were performed by Kruskar-Wallis test, or t-test for experiments with only two populations. *: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$. Error bars represent standard error of the mean.

Figure S3: Comparison of insect fecundity under various stress treatments. A) Couples of one male-one female eight-day old weevils fed with wheat grains from stage 3 onwards (control condition), fed from stage 5, antibiotic-treated, or aposymbiotic weevils were reared on twenty wheat grains. The batch of grains was changed every 3 to 5 days, until weevils reached 45 days of age. The effect of each condition on fecundity was calculated with a mixed-effect model with Geisser-Greenhouse correction followed by a Turkey's multiple comparison test (with fixed batch variable). *: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$. Error bars represent standard error of the mean. **B)** Ovaries of stage 24 control weevils or weevils fed on starch from stage 3 onwards. The starch-fed weevils did not correctly develop the ovaries, and no eggs/progeny were retrieved from the starch kernels.

Figure S4: Effect of starvation on adult weevils after endosymbiont clearance. Symbiotic and aposymbiotic weevils at 15 days after emergence were either **(A)** kept on wheat grains or **(B)** starved for two days (E+16-E+17) before being fed again with wheat grains. Grey region represents the starvation period. Survival comparison between symbiotic and aposymbiotic weevils until E+44 was performed with the Kaplan-Meier method followed by Log-rank test. Shaded regions represent 95% CI. *: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$. Error bars represent standard error of the mean.

Figure S5: Extended endosymbiont survival in condition of severe unbalanced diet. Prolonged endosymbiont dynamic profile of starch-fed weevils from stage 3 onwards compared to control weevils (fed with wheat grains from stage 3 onwards). Note the retention of a small endosymbiont population until stage 25.

Figure S6: In symbiotic weevils, free DOPA levels increase during endosymbiont clearance except in conditions of severely unbalanced diet. Free DOPA levels in the whole insect bodies were measured for weevils subjected to various stress treatments. **A)** control weevils (fed with wheat grains from stage 3) and weevils fed from stage 5; **B)** control weevils (as in **A**), aposymbiotic weevils and weevils fed on starch from stage 3; **C)** fed, then starved weevils and control weevils (as in **A**); **D)** weevils fed on wheat or starch kernels

supplemented or not with antibiotics. Here, the wheat kernel diet from stage 3 represents the control condition. Comparisons were performed by two-way ANOVA followed by Turkey's multiple comparison test. *: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$. Error bars represent standard error of the mean.

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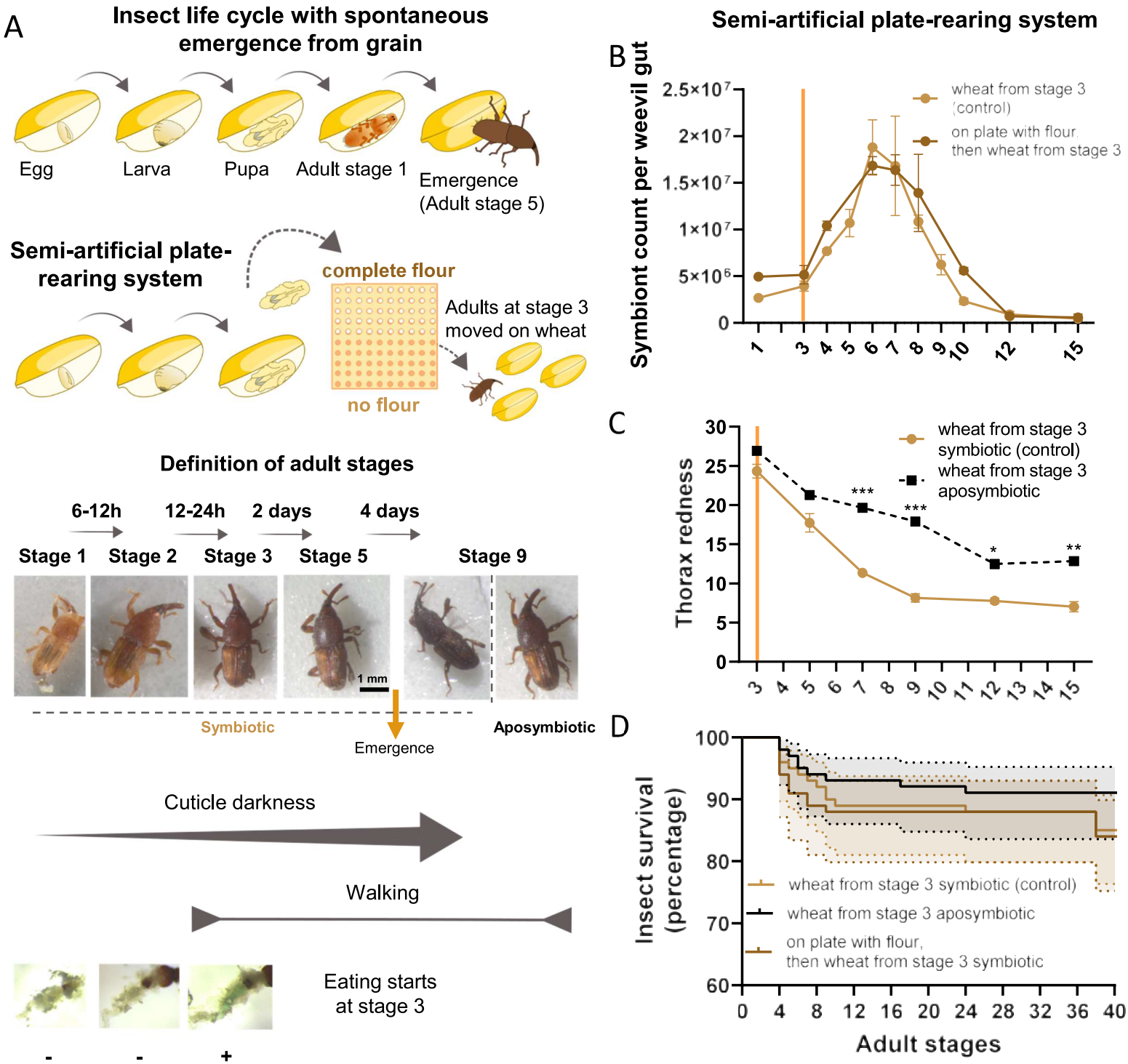
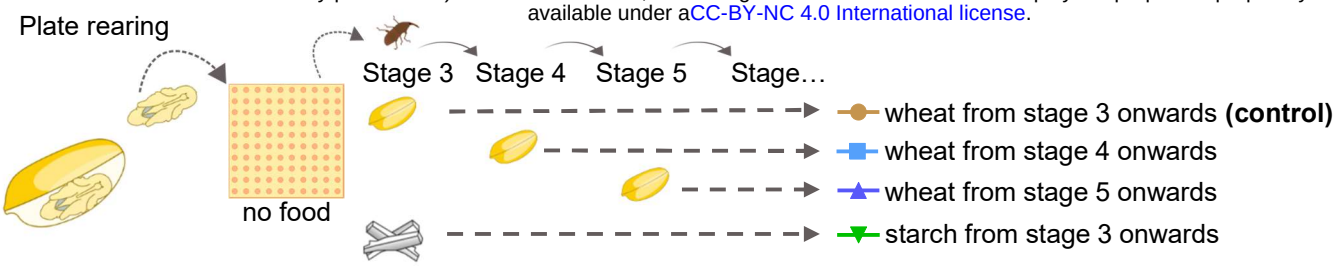

A
Insect life cycle with spontaneous emergence from grain
Egg
Larva
Pupa
Adult stage 1
Emergence (Adult stage 5)
Semi-artificial plate-rearing system
complete flour
Adults at stage 3 moved on wheat
no flour
Definition of adult stages
6-12h
12-24h
2 days
4 days
Stage 1
Stage 2
Stage 3
Stage 5
Stage 9
1 mm
Symbiotic
Aposymbiotic
Emergence
Cuticle darkness
Walking
Eating starts at stage 3
- - +
B
Semi-artificial plate-rearing system
Symbiont count per weevil gut
2.5×10⁷
2×10⁷
1.5×10⁷
1×10⁷
5×10⁶
0
wheat from stage 3 (control)
on plate with flour, then wheat from stage 3
1 3 4 5 6 7 8 9 10 12 15
C
Thorax redness
30 25 20 15 10 5 0
wheat from stage 3 symbiotic (control)
wheat from stage 3 aposymbiotic
***
***
*
**
3 4 5 6 7 8 9 10 11 12 13 14 15
D
Insect survival (percentage)
100 90 80 70 60
wheat from stage 3 symbiotic (control)
wheat from stage 3 aposymbiotic
on plate with flour, then wheat from stage 3 symbiotic
0 4 8 12 16 20 24 28 32 36 40
Adult stages

Fig. 2



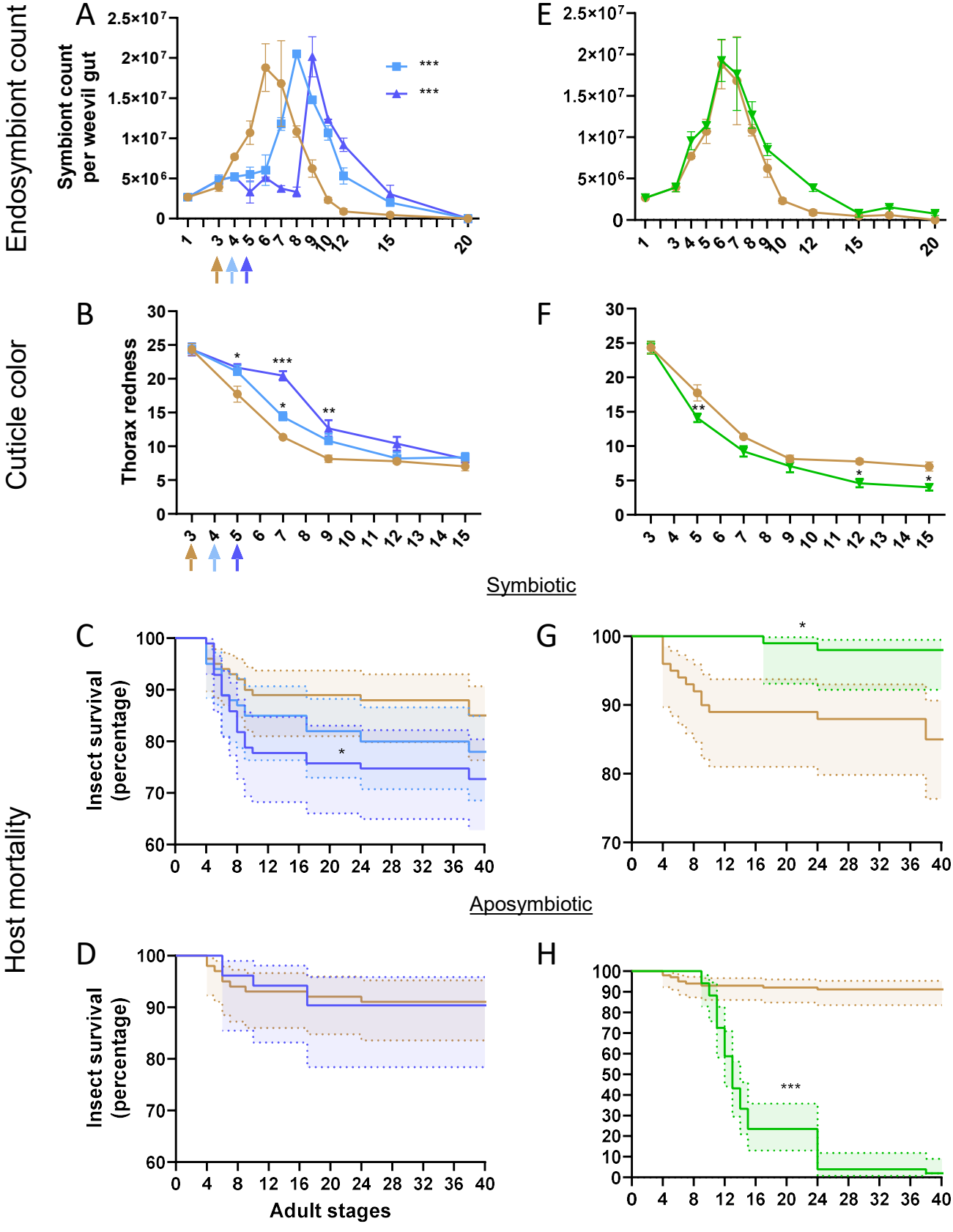
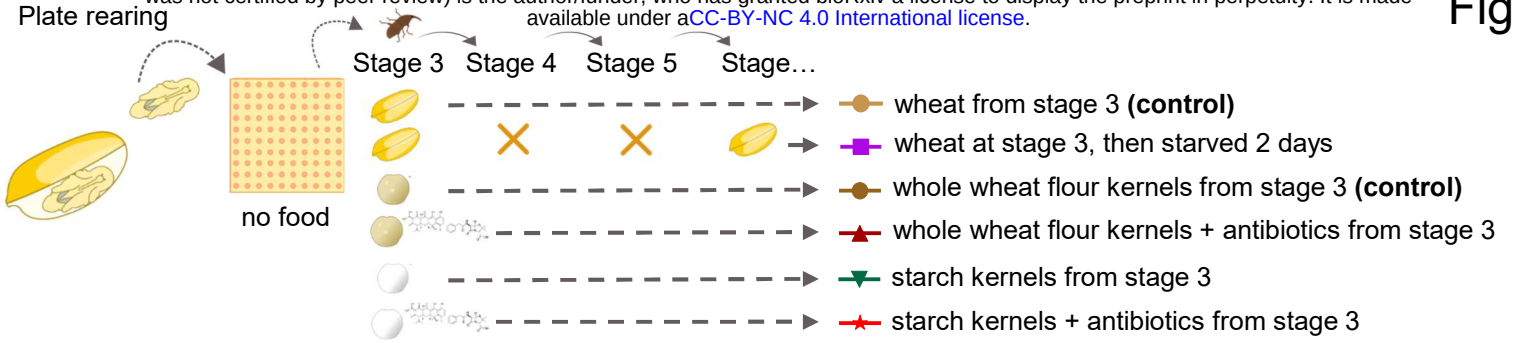
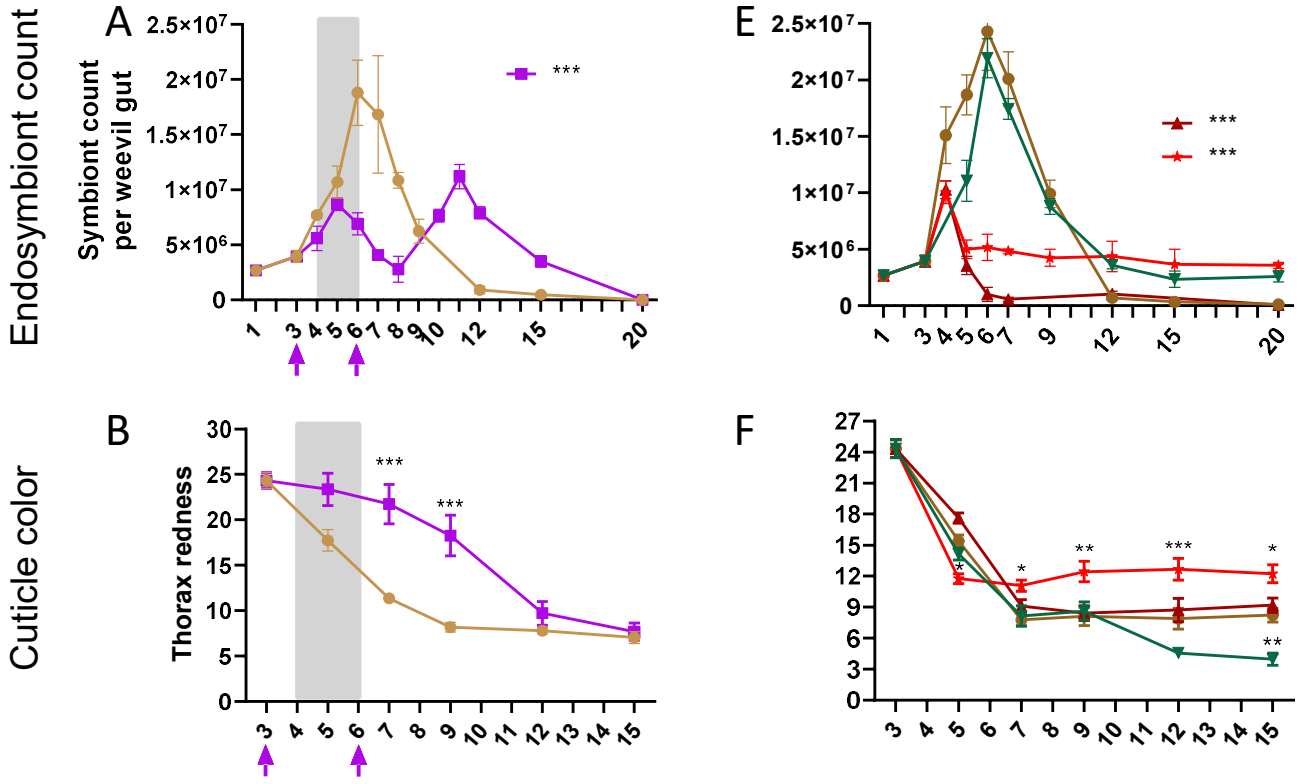
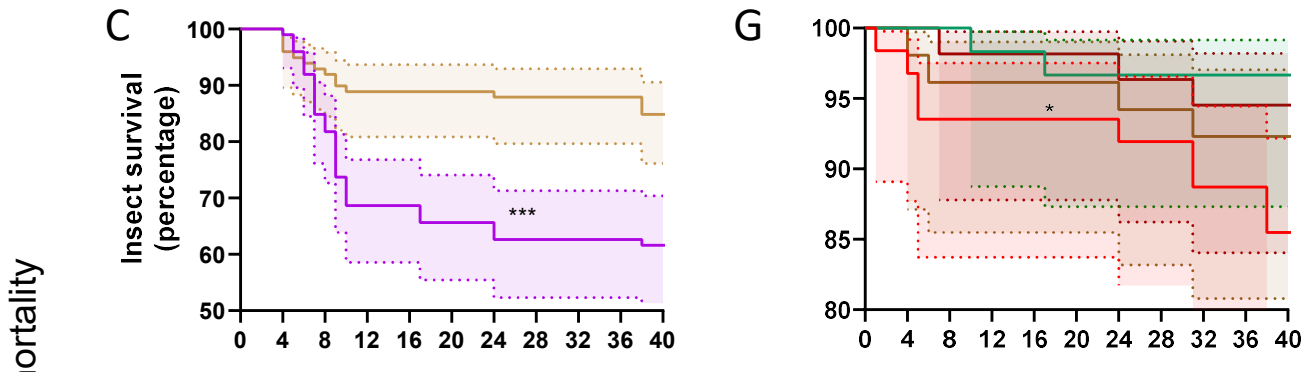


Fig. 3

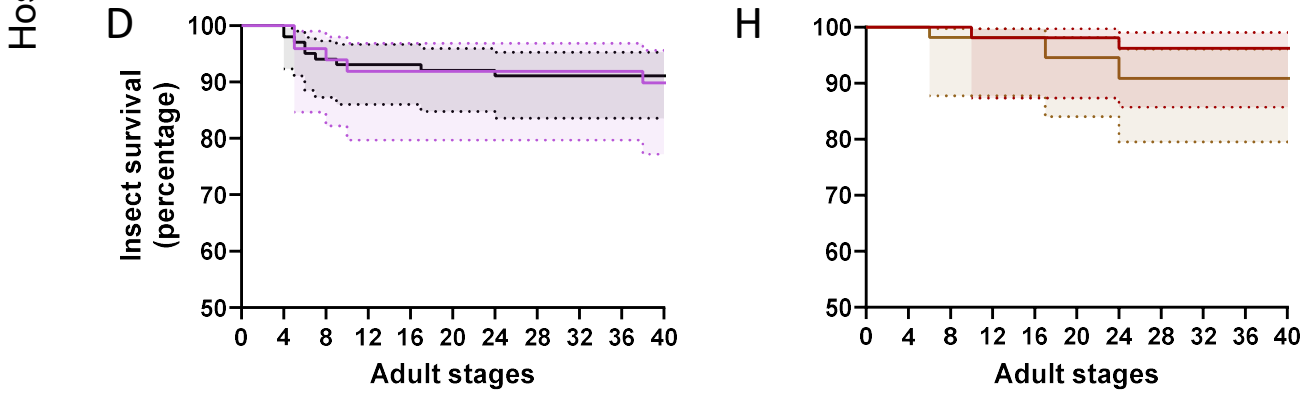




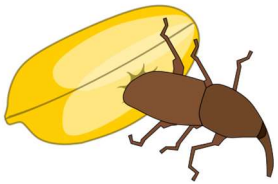
Symbiotic



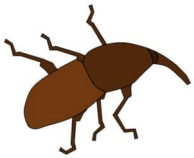
Aposymbiotic



Host developmental stage



Young adult at emergence

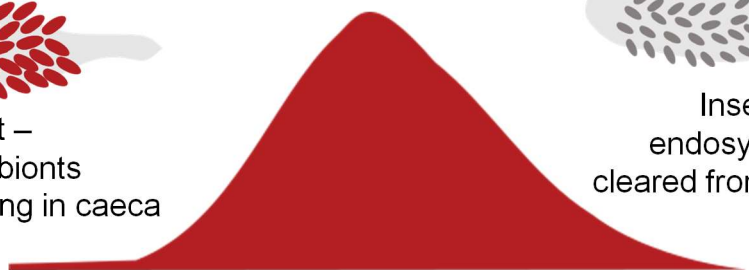


Fully-developed adult

Bacterial load

Insect gut – endosymbionts proliferating in caeca

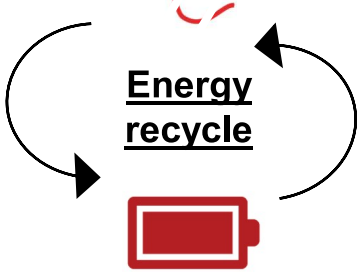
Insect gut – endosymbionts cleared from caeca



Effects on host fitness

Sugar-dependent proliferation in insect caeca

Host-controlled clearance



Balanced diet

no effect on survival, cuticle tanning or fecundity

Starvation

Higher mortality

Severely unbalanced diet

Partial compensation for cuticle tanning and survival