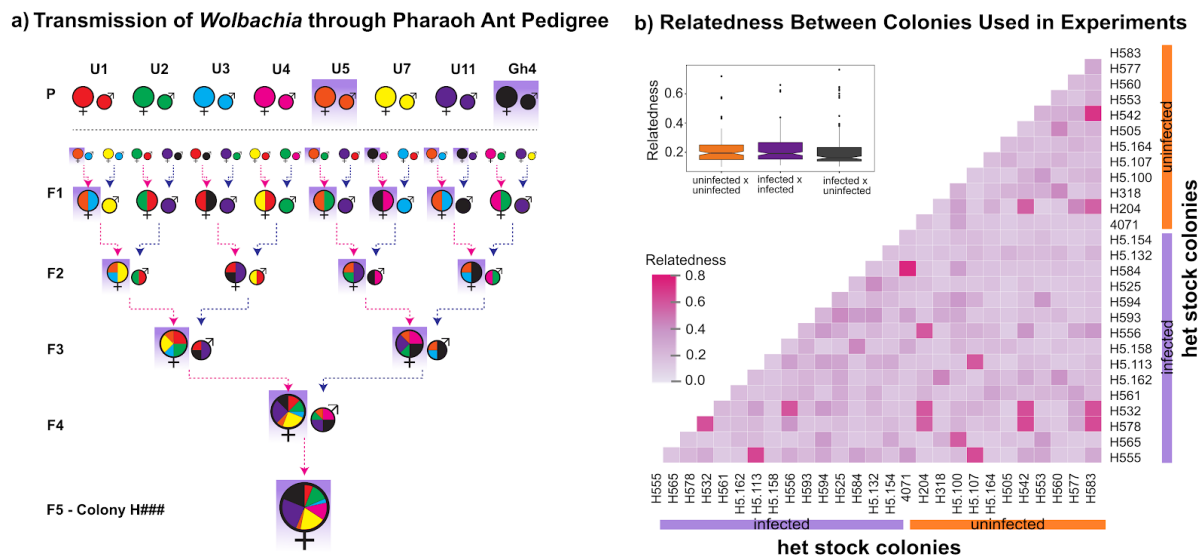
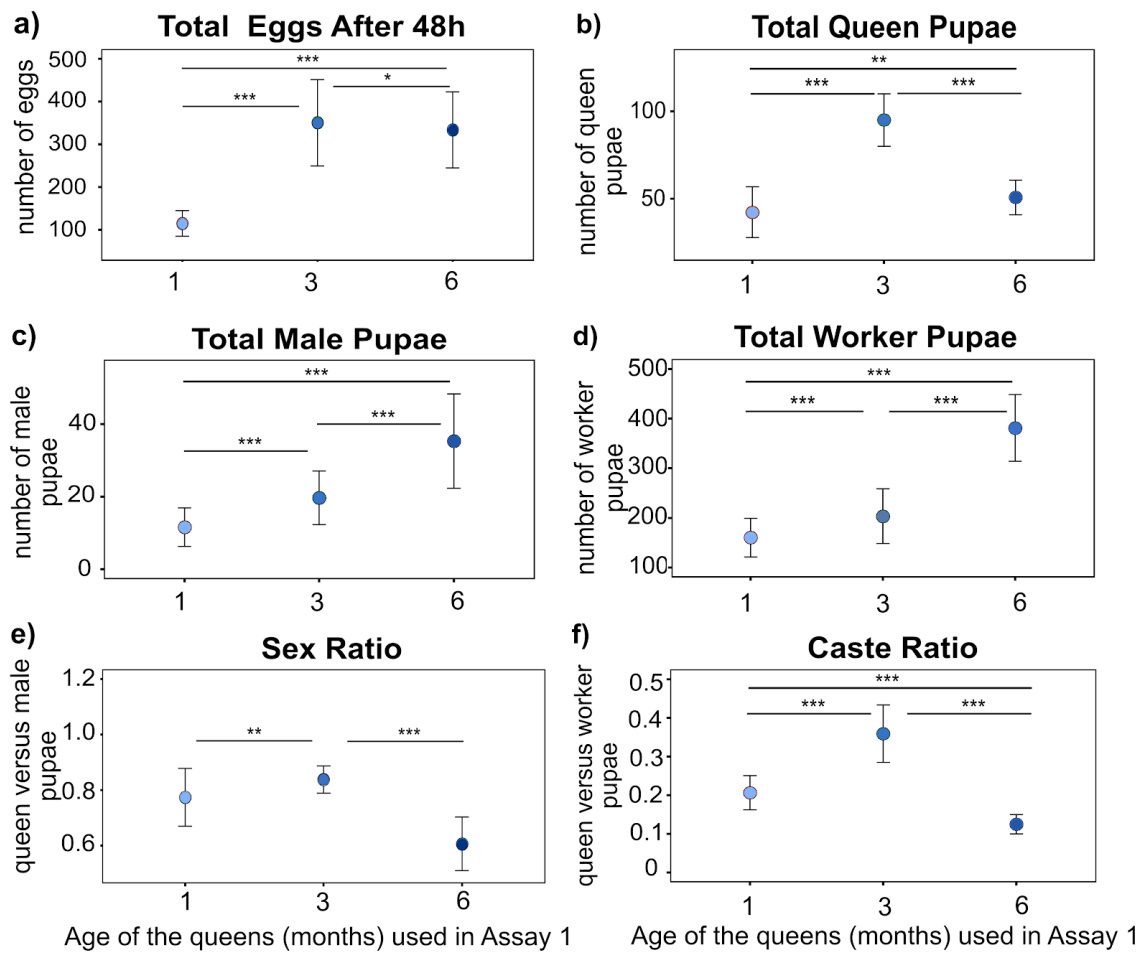


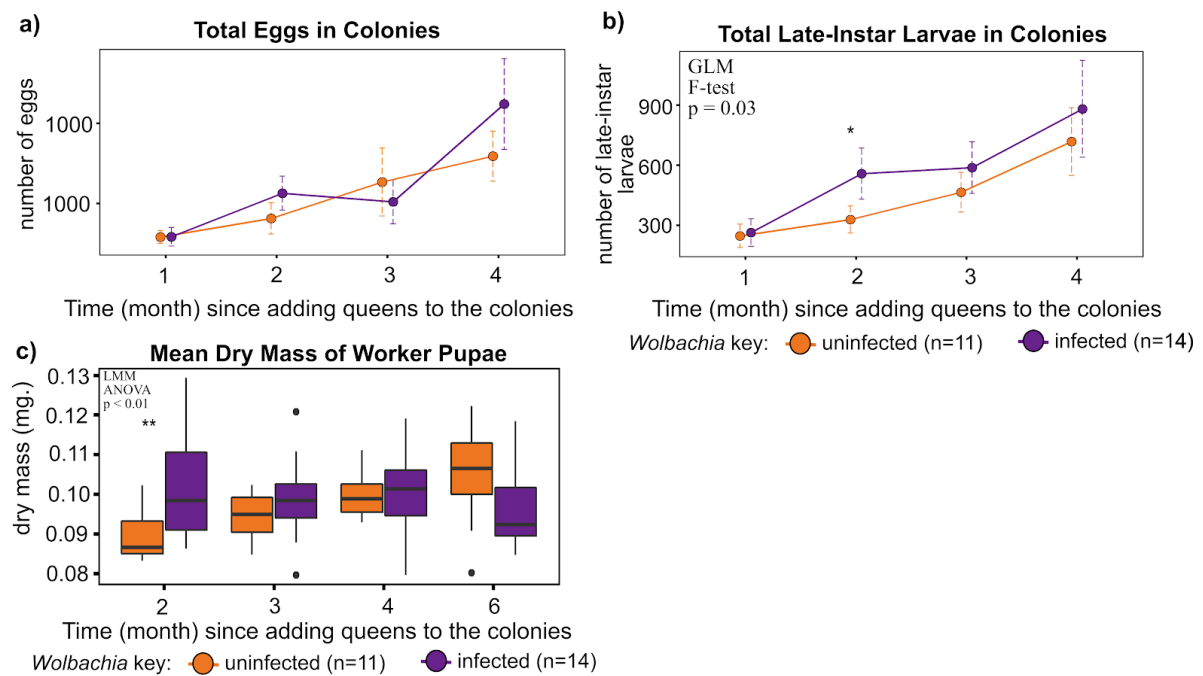
## Supplementary Figures



**Fig. S1. *Wolbachia* transmission through pedigree and relatedness amongst pharaoh ant colonies.** (a) Schematic representation of intercrossing between eight parental lineages and their subsequent daughter colonies for nine generations to create a single colony ‘H###’ (H### representing unique colony ID) in the 5<sup>th</sup> generation. Similarly, crosses were used across nine generations to produce genetically diverse pharaoh ant het stock colonies, some of which have been used as source colonies in the current study (adapted from (Walsh et al., 2019)). *Wolbachia* infected queens (females) are highlighted with purple boxes since only queens transmit infection across generations. (b) Genetic relatedness between heterogeneous stock pharaoh ant colonies used to create source colonies in the current study. These heterogeneous stock lab colonies were created following a similar crossing scheme as represented in (a). X and Y-axis of the matrix represent heterogeneous stock colony ID’s. The inset box plot represents the distribution of raw values across three types of plausible heterogeneous stock colony pairs during crossing.



**Fig. S2: Colony-level fitness traits vary across queen age.** (a) One month-old queens laid the least number of eggs within 48h. (b) Colonies with three months-old queens produced the highest number of queen pupae. (c) Male production increased as the queens became older. (d) Worker production increased as the queens became older. (e) Male biased sex ratio in older queens. (f) Colonies with three months-old queens had the higher queen-biased caste ratio. X- axis represents the discrete queen ages used in Assay 1, Y-axis represents the trait value, filled circles represent the mean trait value and error bar represents the 95% confidence interval. Statistical differences, as estimated by TukeyHSD of GLMM for effect of queen ages, are represented by \*p < 0.05, \*\*p < 0.01 and \*\*\*p < 0.001. 16 colonies were analyzed per time point.



**Fig. S3: Growth dynamics of the early developmental stages in colonies and dry mass of worker pupae.** (a) Infected and uninfected colonies produced a similar number of eggs. (b) Infected colonies had a higher number of late-instar larvae after 2 months of adding queens to experimental colonies. (c) Infected worker pupae were heavier after 2 months of starting Assay 2. X-axis represents the time, in months, since Assay 2 was started, Y-axis represents the trait value. For (a) and (b), filled circles represent the mean trait value and error bar represents the 95% confidence interval. *Wolbachia*-driven difference is represented as  $*p < 0.05$ , and was estimated by age-specific GLM. For (c) Y-axis represents the trait value. *Wolbachia*-driven differences are represented as  $**p < 0.01$ , which was estimated by ANOVA of age-specific LME. *Wolbachia* color key, along with the number of colonies in the assay (n), are at the bottom of the figure panel.

Table S1. Raw data for Assay 1

[Click here to Download Table S1](#)

Table S2. Raw Data for Assay 2

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Table S3. Raw data for colony relatedness

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