© 2020. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2020) 223, jeb220079. doi:10.1242/jeb.220079

RESEARCH ARTICLE

Wolbachia-infected ant colonies have increased reproductive investment and an accelerated life cycle

Rohini Singh* and Timothy A. Linksvayer

ABSTRACT

Wolbachia is a widespread genus of maternally transmitted endosymbiotic bacteria that often manipulates the reproductive strategy and life history of its hosts to favor its own transmission. *Wolbachia*-mediated phenotypic effects are well characterized in solitary hosts, but effects in social hosts are unclear. The invasive pharaoh ant, *Monomorium pharaonis*, shows natural variation in *Wolbachia* infection between colonies and can be readily bred under laboratory conditions. We previously showed that *Wolbachia*-infected pharaoh ant colonies had more queen-biased sex ratios than uninfected colonies, which is expected to favor the spread of maternally transmitted *Wolbachia*. Here, we further characterize the effects of *Wolbachia* on the short- and longer-term reproductive and life history traits of pharaoh ant colonies. First, we characterized the reproductive differences between naturally infected and uninfected colonies at three discrete time points and found that infected colonies had higher reproductive investment (i.e. infected colonies produced more new queens), particularly when existing colony queens were 3 months old. Next, we compared the long-term growth and reproduction dynamics of infected and uninfected colonies across their whole life cycle. Infected colonies had increased colony-level growth and early colony reproduction, resulting in a shorter colony life cycle, when compared with uninfected colonies.

KEY WORDS: Endosymbiotic bacteria, Life history strategy, Ant colony-level fitness, Ant colony life cycle

INTRODUCTION

Wolbachia, a maternally inherited genus of endosymbiotic bacteria, is considered to be the most prevalent endosymbiotic bacteria in arthropods (Sazama et al., 2017; Sazama et al., 2019; Weinert et al., 2015). Infection has a range of effects on host reproduction, including reproductive incompatibility between infected males and uninfected females, reproductive incompatibility between mates infected with different strains of *Wolbachia*, female-biased sex ratios in offspring of infected females, killing of infected males (Engelstädter and Hurst, 2009; Landmann, 2019; Zug and Hammerstein, 2014) and increased fecundity of infected females (Fast et al., 2011; Fry et al., 2004; Weeks et al., 2007). These manipulations of host reproduction by *Wolbachia* are expected to facilitate its own spread in the host populations, even when the manipulation is costly to the host (Bakovic et al., 2018; Jansen et al., 2008; Jiggins, 2017; Kriesner and Hoffmann, 2018; Kriesner et al., 2013; Schuler et al., 2016; Turelli et al., 2018).

Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA.

*Author for correspondence (srohini@sas.upenn.edu)

R.S., 0000-0003-0360-374X; T.A.L., 0000-0001-7034-1546

Received 10 December 2019; Accepted 26 March 2020

Effects of *Wolbachia* on host reproduction vary across host species, ranging from beneficial to detrimental (Engelstädter and Hurst, 2009; Landmann, 2019; Zug and Hammerstein, 2014). For example, *Wolbachia* influences the pheromone profile of infected fruit flies, which in turn affects mating success (Pontier and Schweisguth, 2015) and gamete compatibility (Schneider et al., 2019). In *Drosophila paulistorum*, *Wolbachia* is required for the production of male sexual pheromones for successful mating (Schneider et al., 2019), However, in the case of *Drosophila simulans*, *Wolbachia* regulates the pheromonal communication between male and female pupae during metamorphosis, which affects gametic compatibility between infected and uninfected adult mates (Pontier and Schweisguth, 2015). These examples also illustrate that *Wolbachia* can affect traits that influence social interactions in solitary species, suggesting that *Wolbachia* could also affect various individual- and group-level traits of highly social hosts such as ants.

Wolbachia is estimated to infect 34% of ant species (Russell, 2012), localizing in the germline and various somatic tissues of the worker and queen ants (Andersen et al., 2012; Frost et al., 2014; Ramalho et al., 2018; Sapountzis et al., 2015; Zhukova et al., 2017). Across ant species, *Wolbachia* infection is correlated with colony reproductive strategy, with higher incidence in colonies with dependent colony foundation, i.e. when new colonies are established by a group consisting of single or multiple mated queens and some workers, compared with independent colony foundation, where single queens establish new colonies (Russell, 2012; Treanor and Hughes, 2019; Wenseleers et al., 1998). Interestingly, invasive populations of the Argentine ant (*Linepithema humile*) and the fire ant (*Solenopsis invicta*) show a marked population-wide reduction of infection compared with their native populations (Bouwma et al., 2006; Reuter et al., 2005; Shoemaker et al., 2000; Tsutsui et al., 2003). Furthermore, in the ghost ant (*Tapinoma melanocephalum*), *Wolbachia* plays a role in vitamin B provisioning (Cheng et al., 2019). However, the specific individual- and colony-level effects of *Wolbachia* infection in ants, especially on the reproduction and growth of ant colonies, remain largely unknown.

The invasive pharaoh ant *Monomorium pharaonis* is one of the most successful and well-studied invasive ants (Wetterer, 2010). Most importantly for the current study, pharaoh ant colonies show natural variation in *Wolbachia* infection status (Pontieri et al., 2017; Schmidt, 2010). We previously showed that *Wolbachia*-infected pharaoh ant colonies produce fewer males and have a queen-biased sex ratio (relative number of new queens versus males produced by a colony) when artificially selected for higher caste ratio (relative number of new queens versus workers) across three generations (Pontieri et al., 2017). Since queens are the only reproductive caste in pharaoh ant colonies, such a queen-biased investment is expected to increase the transmission and prevalence of maternally inherited *Wolbachia*. This also suggests that *Wolbachia* may manipulate

Journal of Experimental Biology (2020) 223, jeb220079. doi:10.1242/jeb.220079

colony reproduction and life cycle to increase its own transmission from one generation to the next.

In the current study, we provide a detailed characterization of differences in the reproduction, life cycle, and life history of pharaoh ant colonies that show natural variation in *Wolbachia* infection in the absence of artificial selection. The pharaoh ant colony life cycle begins with intra-colony matings between newly produced males and queens, followed by the production of only sterile workers, and ends with the spontaneous production of new queens and males when the existing queens senesce after approximately 4 months (Fowler et al., 1993). Henceforth, we define this spontaneous production of new queens and males as colony reproduction and we use the counts of queen and male pupae as a proxy to measure colony reproduction. We predict *Wolbachia*-infected colonies to have an increased investment in queens, as workers are obligately sterile and *Wolbachia* is maternally transmitted. Such a queen-biased investment is expected to affect the colony-level productivity and life cycle dynamics. We designed two separate assays to compare (1) the colony-level reproductive investment at discrete time points (i.e. queen ages), and (2) the long-term colony life cycle dynamics in the absence of disturbance (Fig. 1).

MATERIALS AND METHODS

Source of infected and uninfected colonies

We sought to construct replicate experimental colonies that had known *Wolbachia* infection status (i.e. were either infected or uninfected) but were genetically homogeneous. Briefly, as part of a long-term research program, we have systematically intercrossed eight pharaoh ant [*Monomorium pharaonis* (Linnaeus 1758)] lineages, originally collected from locations around the world, for nine generations, in order to create a population of genetically heterogeneous lab colonies, henceforth called heterogeneous stock colonies (Fig. S1A; Pontieri et al., 2017; Schmidt, 2010; Walsh et al., 2019 preprint). Two out of the eight initial lineages were infected with *Wolbachia* (Schmidt, 2010), and based on the known pedigree of colonies in our lab population, we also putatively know the *Wolbachia* infection status of these colonies because *Wolbachia* is maternally inherited (Fig. S1A). We empirically verified the expected infection status of heterogeneous stock colonies in the lab by screening five individual workers per colony using a previously described PCR-based method (Baldo et al., 2006). Systematic intercrossing for nine generations is expected to result in a population of colonies where genetic background is relatively uncoupled from *Wolbachia* infection status (Fig. S1B; permutation test, P=0.46). That is, infected colonies, which have maternal parentage from one or both of the two infected lineages are expected to possess a similar genetic makeup as uninfected colonies, which have paternal parentage from the two infected lineages but only have maternal parentage from the six uninfected lineages (Fig. S1B, Table S1; Pontieri et al., 2017; Schmidt, 2010; Schmidt et al., 2010).

In order to create two sources of known infection status that were relatively genetically homogeneous, we combined 15 of these heterogeneous stock colonies that were infected with *Wolbachia*, and separately combined 14 colonies that were uninfected (note that *M. pharaonis* colonies readily merge after a period of transient aggression that lasts less than 1 day; Pontieri, 2014). We subsequently used these two sources to create replicate experimental colonies of known infection status (see assay 1 and assay 2 below).

In order to synchronize the age of queens in these source colonies, we induced the production of new queens and males, i.e. colony reproduction, by removing all the existing queens (Edwards, 1987, 1991; Schmidt et al., 2011; Warner et al., 2016, 2018). Workers in such queenless colonies are expected to rear new adult queens and males from the existing pool of eggs.

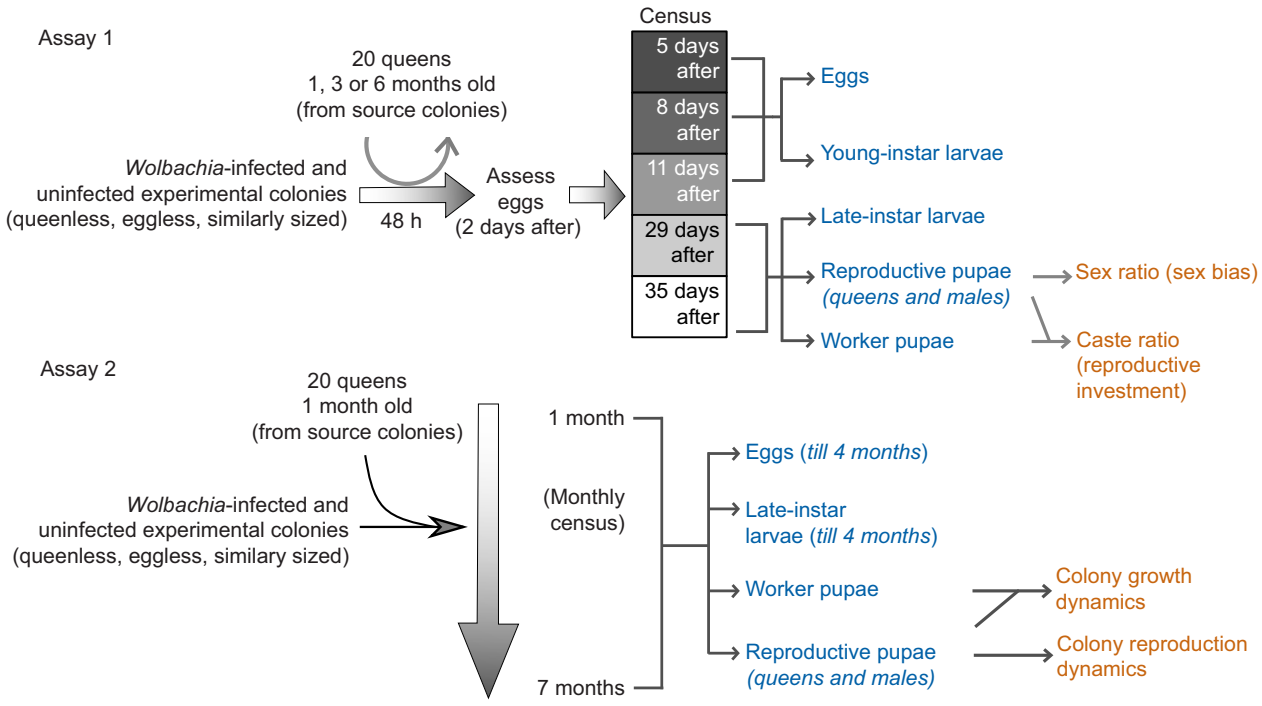

Fig. 1. Schematic description of assays used for measuring the effects of *Wolbachia* infection status on productivity, reproduction and life cycle of pharaoh ant colonies. Assay 1 (top) assessed colony-level reproductive investment at discrete queen ages and assay 2 (bottom) followed colony life cycle dynamics over time. We counted ants at different developmental stages (blue) at various times (arrows on the left of the development stages) to compute colony-level traits (orange) from various combinations of these census values (arrows on the right of the development stages).

Journal of Experimental Biology

Journal of Experimental Biology (2020) 223, jeb220079. doi:10.1242/jeb.220079

We periodically examined these source colonies and removed any new spontaneously produced reproductive larvae/pupae over the course of our experiments to ensure that all queens in these source colonies were of the same age. All colonies were maintained in environmental growth chambers at 27±1°C, 50% RH and 12 h light:12 h dark cycle and were fed *ad libitum* synthetic agar diet (sugar:protein=3:1; Dussutour and Simpson, 2008) and dried mealworms (*Tenebrio molitor*) twice a week.

Quantifying differences in colony growth and reproduction dynamics

We compared productivity and life cycle differences between *Wolbachia*-infected and uninfected pharaoh ant colonies using two assays. In assay 1, we compared the differences in reproductive investment at three discrete time points. In assay 2, we compared the differences in colony productivity and colony life cycle dynamics of the pharaoh ant.

Assay 1: Reproductive investment of colonies at discrete time points

In assay 1, we measured the total number of new queens and males produced by 10 replicate infected and seven replicate uninfected colonies across three discrete time points (i.e. when queens were 1, 3 and 6 months old) that span the reproductive lifespan of the queens. We created similarly sized replicate experimental colonies of known infection status with no queens and with approximately 500 workers and 500 brood (eggs, larvae and pupae). These queenless experimental colonies were kept for 10 days during which all eggs transitioned to older developmental stages since pharaoh ant workers are obligately sterile and cannot lay eggs (Fig. 1; Hölldobler and Wilson, 1990). Once these queenless experimental colonies were eggless, we added 20 age-matched queens from source colonies to these experimental colonies for only 48 h (Fig. 1). We added known-aged infected queens from infected source colonies only to infected experimental colonies and known-aged uninfected queens from uninfected source colonies to uninfected experimental colonies. After 48 h, we transferred these queens back to their respective source colonies and we counted the number of eggs laid by these queens (Fig. 1). These experimental queenless colonies now contained eggs from age-matched queens and were kept until eggs developed into new worker, male or queen pupae (approximately 35 days). We counted the number of new worker, male and queen pupae produced 29 and 35 days after adding age-matched queens to the experimental colonies. We added these two counts to calculate the total number of worker, male and queen pupae produced by each replicate colony. We used these total counts to compute the relative investment in new queens versus workers, i.e. colony caste ratio (Pontieri et al., 2017). We also computed the relative investment in new queens versus males, i.e. colony sex ratio (Pontieri et al., 2017). Note that we used a blind design, where counters were blind to the infection status of colonies for data collection.

Assay 2: Colony growth, reproduction, and life cycle dynamics

In assay 2, we tracked 14 infected and 12 uninfected experimental colonies for 7 months in order to compare the (1) colony productivity, both workers and reproductives, and (2) colony life cycle dynamics of naturally infected and uninfected colonies across the colony life cycle.

We created similarly sized queenless and eggless experimental colonies, with approximately 500 workers and 500 brood (eggs, larvae and pupae) in the same manner as described for assay 1. Once eggless, we added 20 1-month-old infected queens from the infected source colonies to each infected experimental colony and 20 1-month-old uninfected queens from uninfected source colonies to each uninfected replicate experimental colony (Fig. 1). We counted the colonies after 48 h of adding queens to quantify the initial colony composition, and we did not manipulate the colonies any further. The queens aged naturally in these colonies and we surveyed the colony composition across the whole colony life cycle on a monthly basis. Specifically, for the first 4 months we counted each developmental stage, from eggs to pupae and reproductive adults (Fig. 1). After 4 months, the colonies were sizable and it was difficult to get accurate counts of younger developmental stages. Hence, after 4 months we restricted the counts to new male and queen pupae and adults, and worker pupae (Fig. 1). At each time point, we calculated net colony productivity as the total number of pupae (workers, queens and males) present at the time of census (Fig. 1). We did not compute caste and sex ratio for these colonies in assay 2 as they grew to very different sizes and variation in colony size is known to affect colony caste ratio (Schmidt et al., 2011). We used a blind design for data acquisition, where we were blind to the colony infection status at the time of census.

We also assessed differences in worker body mass between infected and uninfected colonies over a period in assay 2. We collected 15 early stage worker pupae from each replicate colony after 2, 3, 4 and 6 months from the beginning of the assay. We identified early stage worker pupae as those with white bodies and pigmented eyes (Linksvayer, 2006). We dried these pupae at 55°C for 20 h before storing them at −20°C till the time of weighing them on Sartorius microbalance (MSU3.6P-000-DM) in milligrams up to three decimal places. We used a blind design for data collection.

Statistical analysis

We used R version 3.5.2 (https://www.r-project.org/), with lme4 (Bates et al., 2015), pscl (Zeileis et al., 2008), MASS (Venables and Ripley, 2002), and car packages (Fox and Weisberg, 2011) for data analysis, and ggplot2 (Wickham, 2009) for plotting graphs. We built generalized linear mixed effect models (GLMM; Bolker et al., 2009) to assess the overall effects of predictor variables (*Wolbachia* infection and queen age) on response variables (fitness traits such as total number of queens, sex ratio, and caste ratio), with source colonies as a random factor. We performed a *post hoc* TukeyHSD test on GLMM for pairwise comparison of response variables across queen age or time. To assess the effect of *Wolbachia*×queen age (assay 1) or *Wolbachia*×time (assay 2) interaction on colony-level phenotypic traits, we used generalized linear models (GLMs; Bolker et al., 2009) with *Wolbachia* infection, queen age/time and *Wolbachia*×queen age/time interaction as fixed factors. To compare infected and uninfected colonies at specific time points, we used GLMs. For count data, we constructed GLMMs with Poisson and GLMs with negative binomial or quasi-Poisson error distributions. For caste and sex ratio, we constructed GLMMs assuming binomial and GLMs assuming quasi-binomial error distributions. Since larger colonies tend to invest relatively more in new workers versus new queens in terms of caste ratio when compared to smaller colonies (Schmidt et al., 2011), we included log-transformed colony productivity (i.e. total number of new workers, queens and males produced, as a measure of colony size) as a fixed factor when assessing caste and sex ratio differences in assay 1. In assay 2, experimental colonies produced new males only between 4 and 7 months after starting the assay. We compared the differences in production of male pupae between infected and uninfected colonies

Journal of Experimental Biology (2020) 223, jeb220079. doi:10.1242/jeb.220079

during this period. For assessing differences in dry weight of worker pupae collected in assay 2, we used linear mixed effects models (LMMs; Galecki and Burzykowski, 2013) with mean dry mass per colony as the response variable, *Wolbachia*×time interaction as a fixed factor, log-transformed colony productivity as a fixed factor and colony ID as a random factor. For age-specific effects of *Wolbachia* infection, we constructed LMM with mean dry mass per colony at a specific time point as the response variable, *Wolbachia* as a fixed factor, log-transformed colony productivity as a fixed factor and colony ID as a random factor. We computed the statistical significance of each component of the LMM model via ANOVA from the car package (Fox and Weisberg, 2011). Datasets for assay 1, assay 2, and genetic relatedness are shown in Tables S1–S3. R scripts and output from statistical models are available on Dryad. See the Data Availability section for more details.

Analysis of genetic relatedness amongst colonies

We compared the genetic relatedness among the heterogeneous stock lab colonies that were used to create source colonies in the current study. We used genetic relatedness values from a published dataset from our lab (Walsh et al., 2019 preprint). We used a permutation test in R using lmPerm (https://CRAN.R-project.org/package=lmPerm) and coin package (Hothorn et al., 2006) to assess if colonies within a *Wolbachia* infection group were more or less related than colonies with different *Wolbachia* infection status. Please refer to Table S3 for the genetic relatedness values and Dryad (Singh and Linksvayer, 2020) for the R script used for this analysis.

RESULTS

Assay 1: *Wolbachia*-infected colonies had higher queen production and reproductive investment

Overall in assay 1, *Wolbachia*-infected colonies produced more queen pupae (GLMM; LRT=8.62, P=0.003; Fig. 2A) and had queen-biased caste ratios (GLMM; LRT=5.95, P=0.014; Fig. 2C) and sex ratios (GLMM; LRT=4.65, P=0.041; Fig. 2D). In particular, *Wolbachia*-infected experimental colonies with 3-month-old queens produced more new queens (GLM: F=5.63, P=0.031; Fig. 2A) but a similar number of males (GLMM; LRT=0.03, P=0.84; Fig. 2B), resulting in a queen-biased caste ratio (GLM: F=9.01, P=0.009; Fig. 2C) in these colonies.

In addition to *Wolbachia* infection, queen age also affected colony-level traits. The total number of eggs present in the experimental colonies after 48 h increased with queen age (GLMM; F=1421.15, P<0.001; Fig. S2A). The total number of new queens produced from these eggs was also dependent on maternal age (GLMM: LRT=419, P<0.001), specifically, colonies with 3-month-old queens produced the highest number of new queens (GLM: z<18, P<0.001; Fig. S2B). Furthermore, all colonies with older queens produced more males (GLMM: LRT=224.48, P<0.001; Fig. S2C) and workers (GLMM: LRT=1767.97, P<0.001; Fig. S2D). Specifically, experimental colonies with 6-month-old queens had male-biased sex ratios (GLMM: LRT=130.35,

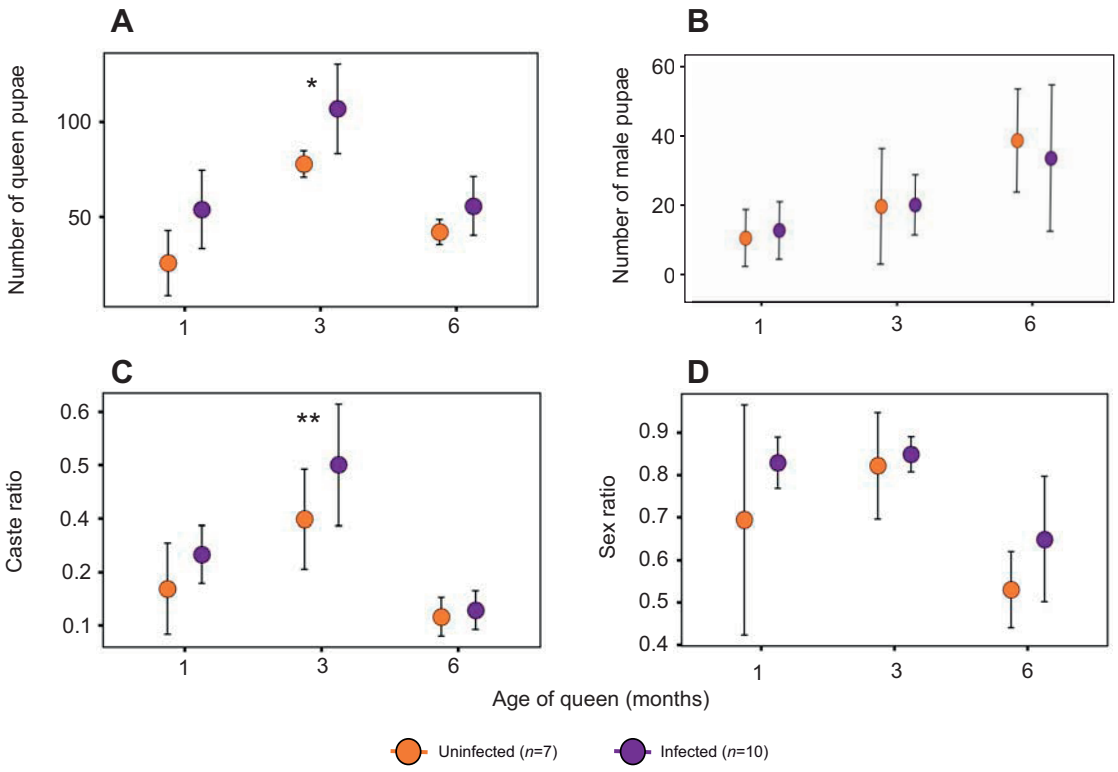

Fig. 2. *Wolbachia* increases reproductive investment of pharaoh ant colonies, depending on queen age. (A) Infected colonies produced more queen pupae when queens used for the assay were 3 months old. (B) No differences in the total number of male pupae produced by infected and uninfected colonies. (C) Infected colonies have increased queen-biased caste ratio when queens used for the assay were 3 months old. (D) *Wolbachia*-infected colonies show a non-significant trend towards queen-biased sex ratio. Filled circles represent the mean trait value and error bars represent the 95% confidence interval of the mean. *Wolbachia*-related differences are represented as *P<0.05 and **P<0.01, and were estimated by age-specific GLMs. The number (n) of replicate colonies in the assay is shown at the bottom.

Journal of Experimental Biology (2020) 223, jeb220079. doi:10.1242/jeb.220079

P<0.001; Fig. S2E) and worker-biased caste ratios (GLMM: LRT=579.27, P<0.001; Fig. S2E).

Assay 2: *Wolbachia*-infected colonies have increased colony-level growth, early colony reproduction and faster colony life cycle

Across the colony lifespan, *Wolbachia*-infected colonies overall produced more new workers (GLMM: LRT=6.7, P=0.009; Fig. 3A), had a non-significant trend towards more new queens (GLMM: LRT=3.46, P=0.062; Fig. 3B) and produced a similar number of males (GLMM: LRT=1.76, P=0.18; Fig. 3C) relative to uninfected colonies. Interestingly, *Wolbachia*-infected colonies spontaneously produced new queens and males earlier than uninfected colonies (Fig. 3B,C). At specific time points, infected colonies had more total number of queens 4 months (GLM: F=13.25, P=0.001) and 5 months (GLM: F=12.44, P=0.001; Fig. 3B) after starting the assay, relative to uninfected colonies at the same points. Similarly, infected colonies produced more males 4 months (GLM: LRT=7.81, P=0.02) and 5 months (GLM: LRT=9.03, P=0.01; Fig. 3C) after starting the assay, relative to uninfected colonies at the same time points. This is in contrast to uninfected colonies that seem to spontaneously produce new queens and males after ∼6 months (Fig. 3B,C). Furthermore, *Wolbachia*-infected colonies had increased worker productivity 2 months (GLM: F=8.76, P=0.007), 6 months (GLM: F=6.4, P=0.019) and 7 months (GLM: F=6.38, P=0.019) after starting the assay relative to uninfected colonies at the same time point. Interestingly, infected and uninfected colonies produced a similar number of eggs (GLMM: LRT=0.4, P=0.51; Fig. S3A), although infected colonies had more late-instar larvae relative to uninfected colonies 2 months after starting the assay (GLM: F=4.85, P=0.039; Fig. S3B). The dry mass of *Wolbachia*-infected worker pupae was also dependent on time (LMM: χ^2=17.76, P<0.001; Fig. S3C) and infected worker pupae were heavier 2 months after starting the assay (LMM: F=8.72, P=0.007; Fig. S3C). While colony productivity was not a major predictor of worker pupae dry weight differences across all time points (LMM: χ^2=1.21, P=0.27), it was however, a major predictor of differences in dry weight 6 months after starting the assay (LMM: F=5.91, P=0.02).

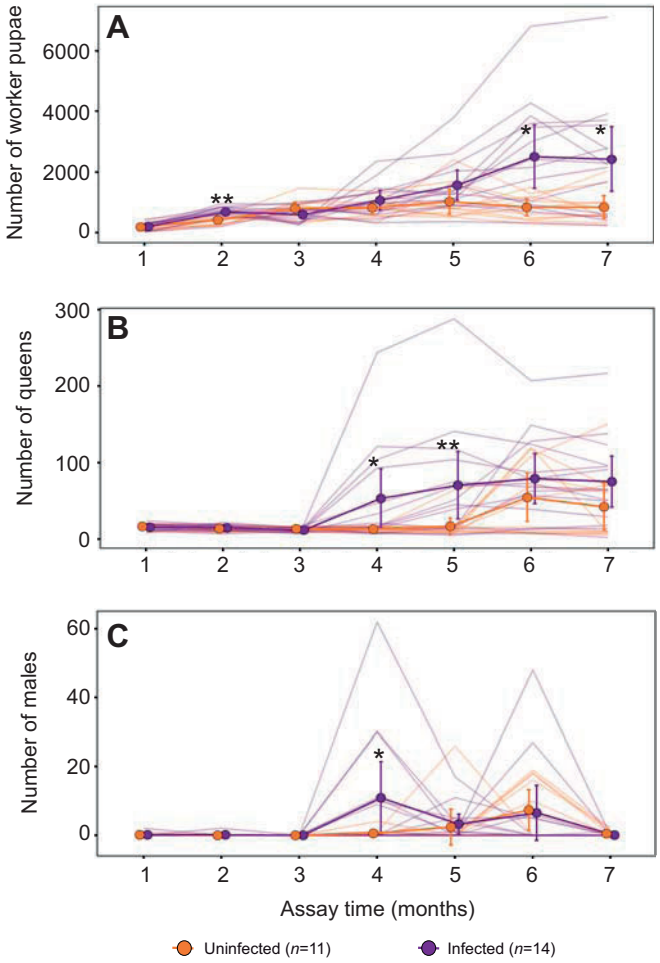

Fig. 3. Infected colonies show increased growth and early onset of reproduction. (A) Infected colonies produced more worker pupae 2 months after starting the assay. (B) Infected colonies had an early spontaneous production of new queens (adults and pupae). (C) Infected colonies had an early spontaneous production of new males (adults and pupae). Filled circles represent the mean trait value and error bar represents the 95% confidence interval of the mean. Light-colored lines represent individual colony-level values. *Wolbachia*-driven differences are represented as *P<0.05 and **P<0.01, and were estimated by age-specific GLM. The number (n) of replicate colonies in the assay is shown at the bottom of the figure.

DISCUSSION

In the current study, we provide a detailed characterization of differences in productivity, reproductive investment and life cycle dynamics of pharaoh ant colonies that had similar genotypes but differed in *Wolbachia* infection status. *Wolbachia*-infected pharaoh ant colonies have a reproductive (Fig. 2 and Fig. 3B,C) and growth (Fig. 3A) advantage that is dependent on the age of the queens (assay 1) and time or stage of the colony life cycle (assay 2). Furthermore, infected colonies spontaneously produced new reproductives (i.e. new queens and males) earlier than uninfected colonies (Fig. 3B,C). Usually, the presence of reproductively fecund queens in pharaoh ant colonies suppresses the production of new queens and males (Edwards, 1987, 1991; Fowler et al., 1993; Warner et al., 2018). Hence the spontaneous production of new reproductives suggests that *Wolbachia*-infected queens may experience early reproductive senescence compared with uninfected queens. While we did not directly quantify queen mortality, a steady increase in worker and queen numbers over a period (Fig. 3A,B) suggest that new queens were being added even when some of the old queens were still alive in the colonies (Fig. 3B). These results point to accelerated colony life cycle dynamics, and possibly an alternative life history strategy for *Wolbachia*-infected queens.

Increased growth and an accelerated life cycle of *Wolbachia*-infected pharaoh ant colonies is expected to increase colony size and the frequency of colony reproduction (i.e. decrease the generation time) relative to uninfected colonies, which should be favorable in expanding populations. Invasive species such as pharaoh ants likely find themselves in conditions where such rapid population expansion is favored, e.g. following invasion into a new habitat. New pharaoh ant colonies are established when some of the existing queens and workers ‘bud’ off from the sufficiently large parent colony and occupy new nest sites (Buczkowski and Bennett, 2009; Fowler et al., 1993). *Wolbachia*-infected colonies may possibly have a higher frequency of such colony-founding events, which may increase their invasiveness. Moreover, rapid expansion of *Wolbachia*-infected pharaoh ant colonies may also result in increased prevalence of *Wolbachia*. Infection can sweep through a

host population if there is a growth advantage to the host or manipulation of host reproduction by *Wolbachia* (Jansen et al., 2008; Kriesner and Hoffmann, 2018). Future experiments mapping the incidence of *Wolbachia* in the invasive population of pharaoh ants across the globe will be insightful.

The probability of infection sweeping through pharaoh ant populations and a concomitant increase in the invasiveness of *Wolbachia*-infected populations, can be expected to depend on multiple factors such as environmental conditions, and frequency and type of inter-colony and intra-colony interactions. For example, *Wolbachia* density in hosts is sensitive to ambient temperatures and it decreases with either increase or decrease in temperatures (Bordenstein and Bordenstein, 2011; Hurst et al., 2000). Thus, it is possible that fluctuating environmental temperatures may affect *Wolbachia* density in ant hosts and hence limit the subsequent phenotypic effects and potential fitness advantages of infected pharaoh ant colonies. Furthermore, competition between colonies for nest space, food and other resources may also limit the propagation of infected pharaoh ant colonies. Ant colony growth and reproduction is socially regulated, i.e. different members of the colony regulate colony growth and reproduction (Aron et al., 2001; Clark et al., 2006; Penick and Liebig, 2012; Schmickl and Karsai, 2018; Warner et al., 2016), including regulation of caste development in colonies by workers (Warner et al., 2018), regulation of queen development by workers (Clark et al., 2006; Penick and Liebig, 2012) and the importance of late-instar larvae for the production of new queens and males (Warner et al., 2016). Hence, interactions within and between colonies, possibly in response to the environment or amongst nest mates of differing infection status, may also affect the spread of *Wolbachia.* In the wild, rapidly expanding invasive and *Wolbachia*-infected pharaoh ant colonies will likely come in contact with both infected and uninfected colonies. Pharaoh ant colonies show transient inter-colony aggression, and colonies in the laboratory readily merge despite being highly genetically differentiated (Pontieri, 2014). However, it is uncertain how frequently and readily colonies merge in the wild (Schmidt et al., 2010). Future studies simulating such scenarios with both *Wolbachia*-infected and -uninfected individuals within the same colony will further elucidate the dynamics of *Wolbachia* sweeping through colonies and populations.

In a previous study, where we artificially selected for differences in colony caste ratio (i.e. increased or decreased investment in new queens relative to workers) in replicate populations across three generations, we found that *Wolbachia*-infected colonies had queen-biased sex ratios, specifically due to decreased male production (Pontieri et al., 2017). In the current study, we similarly observed that infected colonies invested relatively more in new queens (i.e. we observed increased queen production, queen-biased caste ratios and queen-biased sex ratios), but infected colonies did not produce fewer males. Thus, both studies point to female-biased sex allocation differences associated with *Wolbachia* infection that are expected to favor the spread of *Wolbachia*, and the specific differences between our current and previous studies could have resulted because of small differences in genetic sources used or in environmental conditions (e.g. differences in nutrition, temperature, or humidity) between the two studies.

The differences between *Wolbachia*-infected and -uninfected colonies that we observed, while similar to the phenotypic effects of *Wolbachia* infection in solitary species, are expected to arise partly from mechanisms fairly unique to social organisms. For example, infected pharaoh ant colonies produced more pupae (Fig. 3A) but a similar number of eggs (Fig. S3a) compared with uninfected colonies. This suggests that infected colonies have a higher egg-to-pupa survival. This could be attributed to either individual-level differences in the quality of the eggs laid by the queens or the collective differences in foraging and nursing behaviors of infected workers, or both. These differences could also possibly be due to the beneficial nutritional provisioning by *Wolbachia*, as *Wolbachia* has been shown to be a nutritional mutualist in other insects (Brownlie et al., 2009; Hosokawa et al., 2010; Nikoh et al., 2014), including the ghost ant, *Tapinoma melanocephalum* (Cheng et al., 2019). Future studies investigating possible nutritional symbiosis between *Wolbachia* and pharaoh ant queens and its implication on the viability of brood and adults will be insightful.

In summary, we show novel productivity and life history differences between pharaoh ant colonies showing natural differences in *Wolbachia* infection. *Wolbachia*-infected queens and colonies had an accelerated life cycle that may be favored as an alternative life history strategy. Such effects may be beneficial for the rapid expansion of invasive pharaoh ant colonies and for the increased spread of *Wolbachia* in populations. Our results also underscore the importance of queen age when comparing colony fitness and life cycle dynamics. Overall, our research shows that the pharaoh ant *Monomorium pharaonis* is a tractable, highly social system for studying the effects of *Wolbachia* across generations. Future studies are necessary to tease apart the specific mechanisms by which *Wolbachia* manipulates individual- and colony-level traits. These include directly studying the lifespan of *Wolbachia*-infected and -uninfected queens as well as comparing physiological correlates of aging and reproductive senescence (Corona et al., 2007; Keller and Jemielity, 2006; Negroni et al., 2019).

Acknowledgements
We thank Isaias Jacinto (University of Pennsylvania), Naima Okami (Brown University) and Matthew Dougherty (University of Pennsylvania) for assisting with Assay 1 and Assay 2 at various time points. We are grateful to Chao Tong, Michael Warner and Justin Walsh for their feedback at various steps of the current work and for their comments on multiple drafts of this manuscript. We are extremely grateful to Dr. Jacob Russell (Drexel University) for critical inputs at different stages of this work.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: R.S., T.A.L.; Methodology: R.S.; Validation: R.S.; Formal analysis: R.S.; Investigation: R.S.; Resources: T.A.L.; Writing - original draft: R.S.; Writing - review & editing: R.S., T.A.L.; Visualization: R.S.; Supervision: T.A.L.; Project administration: R.S.; Funding acquisition: T.A.L.

Funding
This work was funded by grants to T.A.L. from the National Science Foundation (IOS-1452520) and the University of Pennsylvania Research Foundation.

Data availability
The R scripts used for analysis in the article and the output from statistical models can be accessed from the Dryad Digital Repository (Singh and Linksvayer, 2020): dryad.tht76hdw5.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.220079.supplemental

References
Andersen, S. B., Boye, M., Nash, D. R. and Boomsma, J. J. (2012). Dynamic *Wolbachia* prevalence in *Acromyrmex* leaf-cutting ants: potential for a nutritional symbiosis. *J. Evol. Biol.* **25**, 1340-1350. doi:10.1111/j.1420-9101.2012.02521.x

Aron, S., Keller, L. and Passera, L. (2001). Role of resource availability on sex, caste and reproductive allocation ratios in the Argentine ant, *Linepithema humile*: resource availability in the Argentine ant, *Linepithema humile*. *J. Anim. Ecol.* **70**, 831-839. doi:10.1046/j.0021-8790.2001.00545.x

Bakovic, V., Schebeck, M., Telschow, A., Stauffer, C. and Schuler, H. (2018). Spatial spread of *Wolbachia* in *Rhagoletis cerasi* populations. *Biol. Lett* **14**. doi:10.1098/rsbl.2018.0161

Baldo, L., Dunning Hotopp, J. C., Jolley, K. A., Bordenstein, S. R., Biber, S. A., Choudhury, R. R., Hayashi, C., Maiden, M. C. J., Tettelin, H. and Werren, J. H. (2006). Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. *Appl. Environ. Microbiol.* **72**, 7098-7110. doi:10.1128/AEM.00731-06

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using *lme4*. *J. Stat. Softw.* **67**, 1-48. doi:10.18637/jss.v067.i01

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H. and White, J.-S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* **24**, 127-135. doi:10.1016/j.tree.2008.10.008

Bordenstein, S. R. and Bordenstein, S. R. (2011). Temperature affects the tripartite interactions between bacteriophage WO, *Wolbachia*, and cytoplasmic incompatibility. *PLoS ONE* **6**, e29106. doi:10.1371/journal.pone.0029106

Bouwma, A. M., Ahrens, M. E., DeHeer, C. J. and DeWayne Shoemaker, D. (2006). Distribution and prevalence of *Wolbachia* in introduced populations of the fire ant *Solenopsis invicta*. *Insect Mol. Biol.* **15**, 89-93. doi:10.1111/j.1365-2583.2006.00614.x

Brownlie, J. C., Cass, B. N., Riegler, M., Witsenburg, J. J., Iturbe-Ormaetxe, I., McGraw, E. A. and O'Neill, S. L. (2009). Evidence for metabolic provisioning by a common invertebrate endosymbiont, *Wolbachia pipientis*, during periods of nutritional stress. *PLoS Pathog.* **5**, e1000368. doi:10.1371/journal.ppat.1000368

Buczkowski, G. and Bennett, G. (2009). Colony budding and its effects on food allocation in the highly polygynous ant, *Monomorium pharaonis*. *Ethology* **115**, 1091-1099. doi:10.1111/j.1439-0310.2009.01698.x

Cheng, D., Chen, S., Huang, Y., Pierce, N. E., Riegler, M., Yang, F., Zeng, L., Lu, Y., Liang, G. and Xu, Y. (2019). Symbiotic microbiota may reflect host adaptation by resident to invasive ant species. *PLoS Pathog.* **15**, e1007942. doi:10.1371/journal.ppat.1007942

Clark, R. M., Anderson, K. E., Gadau, J. and Fewell, J. H. (2006). Behavioral regulation of genetic caste determination in a *Pogonomyrmex* population with dependent lineages. *Ecology* **87**, 2201-2206. doi:10.1890/0012-9658(2006)87[2201:BROGCD]2.0.CO;2

Corona, M., Velarde, R. A., Remolina, S., Moran-Lauter, A., Wang, Y., Hughes, K. A. and Robinson, G. E. (2007). Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. *Proc. Natl. Acad. Sci. USA* **104**, 7128-7133. doi:10.1073/pnas.0701909104

Dussutour, A. and Simpson, S. J. (2008). Description of a simple synthetic diet for studying nutritional responses in ants. *Insectes Soc.* **55**, 329-333. doi:10.1007/s00040-008-1008-3

Edwards, J. P. (1987). Caste regulation in the pharaoh's ant *Monomorium pharaonis*: the influence of queens on the production of new sexual forms. *Physiol. Entomol.* **12**, 31-39. doi:10.1111/j.1365-3032.1987.tb00721.x

Edwards, J. P. (1991). Caste regulation in the pharaoh's ant *Monomorium pharaonis*: recognition and cannibalism of sexual brood by workers. *Physiol. Entomol.* **16**, 263-271. doi:10.1111/j.1365-3032.1991.tb00565.x

Engelstädter, J. and Hurst, G. D. D. (2009). The ecology and evolution of microbes that manipulate host reproduction. *Annu. Rev. Ecol. Evol. Syst.* **40**, 127-149. doi:10.1146/annurev.ecolsys.110308.120206

Fast, E. M., Toomey, M. E., Panaram, K., Desjardins, D., Kolaczyk, E. D. and Frydman, H. M. (2011). *Wolbachia* enhance *Drosophila* stem cell proliferation and target the germline stem cell niche. *Science* **334**, 990-992. doi:10.1126/science.1209609

Fowler, H. G., Alves, L. E. and Bueno, O. C. (1993). Reproductive strategies of the exotic Pharaoh's ant, *Monomorium pharaonis* (L.) (Hymenoptera: Formicidae) in Brazil. *Invertebr. Reprod. Dev.* **23**, 235-238. doi:10.1080/07924259.1993.9672320

Fox, J. and Weisberg, S. (2011). *An R Companion to Applied Regression*, 2nd. Thousand Oaks, CA: Sage.

Frost, C. L., Pollock, S. W., Smith, J. E. and Hughes, W. O. H. (2014). *Wolbachia* in the flesh: symbiont intensities in germ-line and somatic tissues challenge the conventional view of *Wolbachia* transmission routes. *PLoS One* **9**, e95122. doi:10.1371/journal.pone.0095122

Fry, A. J., Palmer, M. R. and Rand, D. M. (2004). Variable fitness effects of *Wolbachia* infection in *Drosophila melanogaster*. *Heredity* **93**, 379-389. doi:10.1038/sj.hdy.6800514

Galecki, A. and Burzykowski, T. (2013). *Linear Mixed-Effects Models Using R: A Step-by-Step Approach*. Springer Publishing Company.

Hölldobler, B. and Wilson, E. O. (1990). *The Ants*. Harvard University Press.

Hosokawa, T., Koga, R., Kikuchi, Y., Meng, X.-Y. and Fukatsu, T. (2010). *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proc. Natl. Acad. Sci. USA* **107**, 769-774. doi:10.1073/pnas.0911476107

Hothorn, T., Hornik, K., van de Wiel, M. A. and Zeileis, A. (2006). A lego system for conditional inference. *Am. Stat.* **60**, 257-263. doi:10.1198/000313006X118430

Hurst, G. D., Johnson, A. P., Schulenburg, J. H. and Fuyama, Y. (2000). Male-killing *Wolbachia* in *Drosophila*: a temperature-sensitive trait with a threshold bacterial density. *Genetics* **156**, 699-709.

Jansen, V. A. A., Turelli, M. and Godfray, H. C. J. (2008). Stochastic spread of *Wolbachia*. *Proc. Biol. Sci.* **275**, 2769-2776. doi:10.1098/rspb.2008.0914

Jiggins, F. M. (2017). The spread of *Wolbachia* through mosquito populations. *PLoS Biol.* **15**, e2002780. doi:10.1371/journal.pbio.2002780

Keller, L. and Jemielity, S. (2006). Social insects as a model to study the molecular basis of ageing. *Exp. Gerontol.* **41**, 553-556. doi:10.1016/j.exger.2006.04.002

Kriesner, P. and Hoffmann, A. A. (2018). Rapid spread of a *Wolbachia* infection that does not affect host reproduction in *Drosophila simulans* cage populations. *Evolution* **72**, 1475-1487. doi:10.1111/evo.13506

Kriesner, P., Hoffmann, A. A., Lee, S. F., Turelli, M. and Weeks, A. R. (2013). Rapid sequential spread of two *Wolbachia* variants in *Drosophila simulans*. *PLoS Pathog.* **9**, e1003607. doi:10.1371/journal.ppat.1003607

Landmann, F. (2019). The *Wolbachia* endosymbionts. *Microbiol Spectr* **7**. doi:10.1128/microbiolspec.BAI-0018-2019

Linksvayer, T. A. (2006). Direct, maternal, and sibsocial genetic effects on individual and colony traits in an ant. *Evolution* **60**, 2552-2561. doi:10.1111/j.0014-3820.2006.tb01889.x

Negroni, M. A., Foitzik, S. and Feldmeyer, B. (2019). Long-lived *Temnothorax* ant queens switch from investment in immunity to antioxidant production with age. *Sci. Rep.* **9**, 7270. doi:10.1038/s41598-019-43796-1

Nikoh, N., Hosokawa, T., Moriyama, M., Oshima, K., Hattori, M. and Fukatsu, T. (2014). Evolutionary origin of insect-*Wolbachia* nutritional mutualism. *Proc. Natl. Acad. Sci. USA* **111**, 10257-10262. doi:10.1073/pnas.1409284111

Penick, C. A. and Liebig, J. (2012). Regulation of queen development through worker aggression in a predatory ant. *Behav. Ecol.* **23**, 992-998. doi:10.1093/beheco/ars062

Pontier, S. M. and Schweisguth, F. (2015). A *Wolbachia*-sensitive communication between male and female pupae controls gamete compatibility in *Drosophila*. *Curr. Biol.* **25**, 2339-2348. doi:10.1016/j.cub.2015.07.052

Pontieri, L. (2014). Discrimination behavior in the supercolonial pharaoh ant. PhD Thesis, University of Copenhagen, København, Denmark.

Pontieri, L., Schmidt, A. M., Singh, R., Pedersen, J. S. and Linksvayer, T. A. (2017). Artificial selection on ant female caste ratio uncovers a link between female-biased sex ratios and infection by *Wolbachia* endosymbionts. *J. Evol. Biol.* **30**, 225-234. doi:10.1111/jeb.13012

Ramalho, M. O., Vieira, A. S., Pereira, M. C., Moreau, C. S. and Bueno, O. C. (2018). Transovarian transmission of *Blochmannia* and *Wolbachia* endosymbionts in the neotropical weaver ant *Camponotus textor* (Hymenoptera, Formicidae). *Curr. Microbiol.* **75**, 866-873. doi:10.1007/s00284-018-1459-3

Reuter, M., Pedersen, J. S. and Keller, L. (2005). Loss of *Wolbachia* infection during colonisation in the invasive Argentine ant *Linepithema humile*. *Heredity* **94**, 364-369. doi:10.1038/sj.hdy.6800601

Russell, J. A. (2012). The ants (Hymenoptera: Formicidae) are unique and enigmatic hosts of prevalent *Wolbachia* (Alphaproteobacteria) symbionts. *Myrmecol. News* **16**, 7-23.

Sapountzis, P., Zhukova, M., Hansen, L. H., Sørensen, S. J., Schiøtt, M. and Boomsma, J. J. (2015). *Acromyrmex* leaf-cutting ants have simple gut microbiota with nitrogen-fixing potential. *Appl. Environ. Microbiol.* 81, 5527-5537. doi:10.1128/AEM.00961-15

Sazama, E. J., Bosch, M. J., Shouldis, C. S., Ouellette, S. P. and Wesner, J. S. (2017). Incidence of *Wolbachia* in aquatic insects. *Ecol. Evol.* **7**, 1165-1169. doi:10.1002/ece3.2742

Sazama, E. J., Ouellette, S. P. and Wesner, J. S. (2019). Bacterial endosymbionts are common among, but not necessarily within, insect species. *Environ. Entomol.* **48**, 127-133. doi:10.1093/ee/nvy188

Schmickl, T. and Karsai, I. (2018). Integral feedback control is at the core of task allocation and resilience of insect societies. *Proc. Natl. Acad. Sci. USA* **115**, 13180-13185. doi:10.1073/pnas.1807684115

Schmidt, A. M. (2010). The invasion biology and sociogenetics of pharaoh ants. PhD Thesis, University of Copenhagen, København, Denmark.

Schmidt, A. M., d'Ettorre, P. and Pedersen, J. S. (2010). Low levels of nestmate discrimination despite high genetic differentiation in the invasive pharaoh ant. *Front. Zool.* **7**, 20. doi:10.1186/1742-9994-7-20

Schmidt, A. M., Linksvayer, T. A., Boomsma, J. J. and Pedersen, J. S. (2011). Queen–worker caste ratio depends on colony size in the pharaoh ant (*Monomorium pharaonis*). *Insectes Soc.* **58**, 139-144. doi:10.1007/s00040-010-0126-x

Schneider, D. I., Ehrman, L., Engl, T., Kaltenpoth, M., Hua-Van, A., Le Rouzic, A. and Miller, W. J. (2019). Symbiont-driven male mating success in the neotropical *Drosophila paulistorum* superspecies. *Behav. Genet.* **49**, 83-98. doi:10.1007/s10519-018-9937-8

Schuler, H., Köppler, K., Daxböck-Horvath, S., Rasool, B., Krumböck, S., Schwarz, D., Hoffmeister, T. S., Schlick-Steiner, B. C., Steiner, F. M., Telschow, A. et al. (2016). The hitchhiker's guide to Europe: the infection dynamics of an ongoing *Wolbachia* invasion and mitochondrial selective sweep in *Rhagoletis cerasi*. *Mol. Ecol.* **25**, 1595-1609. doi:10.1111/mec.13571

Shoemaker, D. D., Ross, K. G., Keller, L., Vargo, E. L. and Werren, J. H. (2000). *Wolbachia* infections in native and introduced populations of fire ants (*Solenopsis* spp.). *Insect Mol. Biol.* 9, 661-673. doi:10.1046/j.1365-2583.2000.00233.x

Singh, R. and Linksvayer, T. (2020). *Wolbachia*-infected ant colonies have increased reproductive investment and an accelerated life cycle. *Dryad Dataset*, https://doi.org/10.5061/dryad.tht76hdw5

Treanor, D. and Hughes, W. O. H. (2019). Limited female dispersal predicts the incidence of *Wolbachia* across ants (Hymenoptera: Formicidae). *J. Evol. Biol.* **32**, 1163-1170. doi:10.1111/jeb.13510

Tsutsui, N. D., Kauppinen, S. N., Oyafuso, A. F. and Grosberg, R. K. (2003). The distribution and evolutionary history of *Wolbachia* infection in native and introduced populations of the invasive argentine ant (*Linepithema humile*): Phylogeography of *Wolbachia* in argentine ants. *Mol. Ecol.* **12**, 3057-3068. doi:10.1046/j.1365-294X.2003.01979.x

Turelli, M., Cooper, B. S., Richardson, K. M., Ginsberg, P. S., Peckenpaugh, B., Antelope, C. X., Kim, K. J., May, M. R., Abrieux, A., Wilson, D. A. et al. (2018). Rapid global spread of wRi-like *Wolbachia* across multiple *Drosophila*. *Curr. Biol.* **28**, 963-971.e8. doi:10.1016/j.cub.2018.02.015

Venables, W. N. and Ripley, B. D. (2002). *Modern Applied Statistics with S*. Springer.

Walsh, J., Pontieri, L., d'Ettorre, P. and Linksvayer, T. A. (2019). Ant cuticular hydrocarbons are heritable and associated with variation in colony productivity. *bioRxiv* 819870. doi:10.1101/819870

Warner, M. R., Kovaka, K. and Linksvayer, T. A. (2016). Late-instar ant worker larvae play a prominent role in colony-level caste regulation. *Insectes Soc.* **63**, 575-583. doi:10.1007/s00040-016-0501-3

Warner, M. R., Lipponen, J. and Linksvayer, T. A. (2018). Pharaoh ant colonies dynamically regulate reproductive allocation based on colony demography. *Behav. Ecol. Sociobiol.* **72**, 31. doi:10.1007/s00265-017-2430-1

Weeks, A. R., Turelli, M., Harcombe, W. R., Reynolds, K. T. and Hoffmann, A. A. (2007). From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. *PLoS Biol.* **5**, e114. doi:10.1371/journal.pbio.0050114

Weinert, L. A., Araujo-Jnr, E. V., Ahmed, M. Z. and Welch, J. J. (2015). The incidence of bacterial endosymbionts in terrestrial arthropods. *Proc. Biol. Sci.* **282**, 20150249. doi:10.1098/rspb.2015.0249

Wenseleers, T., Ito, F., Van Borm, S., Huybrechts, R., Volckaert, F. and Billen, J. (1998). Widespread occurrence of the micro-organism *Wolbachia* in ants. *Proc. Biol. Sci.* **265**, 1447-1452. doi:10.1098/rspb.1998.0456

Wetterer, J. K. (2010). Worldwide spread of the pharaoh ant, *Monomorium pharaonis* (Hymenoptera: Formicidae). *Myrmecol. News* **13**, 115-129.

Wickham, H. (2009). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

Zeileis, A., Kleiber, C. and Jackman, S. (2008). Regression models for count data in R. *J. Stat. Softw.* **27**. doi:10.18637/jss.v027.i08

Zhukova, M., Sapountzis, P., Schiøtt, M. and Boomsma, J. J. (2017). Diversity and transmission of gut bacteria in *Atta* and *Acromyrmex* leaf-cutting ants during development. *Front. Microbiol.* **8**, 1942. doi:10.3389/fmicb.2017.01942

Zug, R. and Hammerstein, R. (2014). Bad guys turned nice? A critical assessment of *Wolbachia* mutualisms in arthropod hosts. *Biol. Rev.* **90**, 89-111. doi:10.1111/brv.12098