# Sexual selection reveals a cost of pathogen resistance undetected in life history assays

## Supplementary Material

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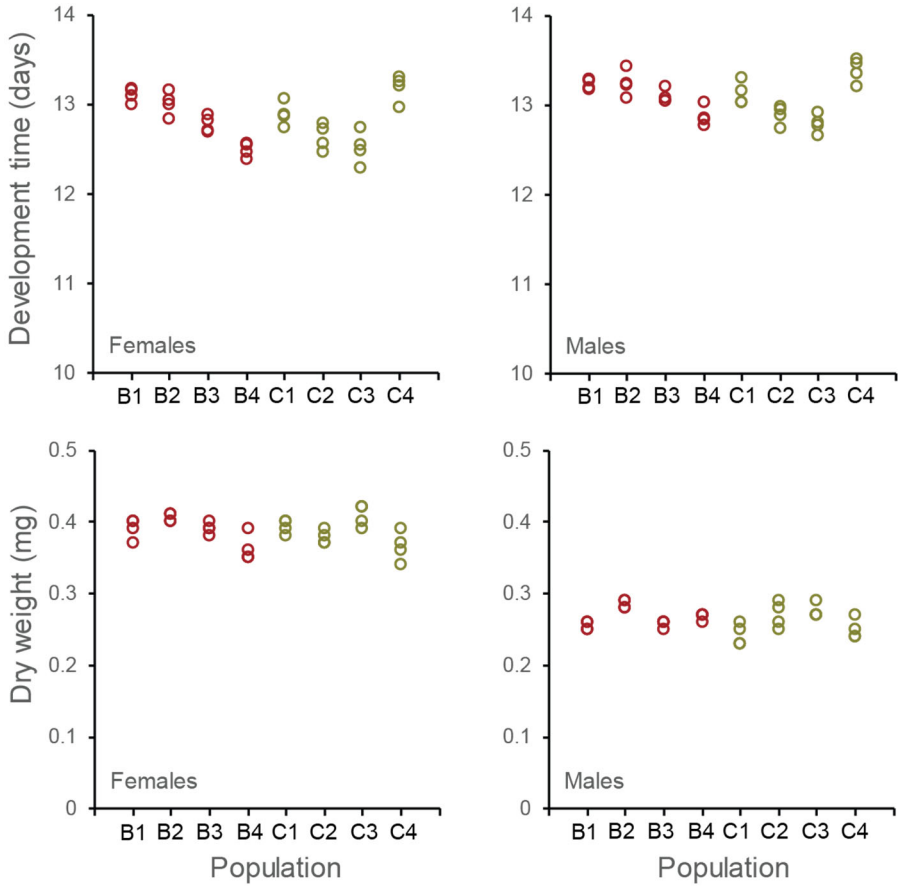
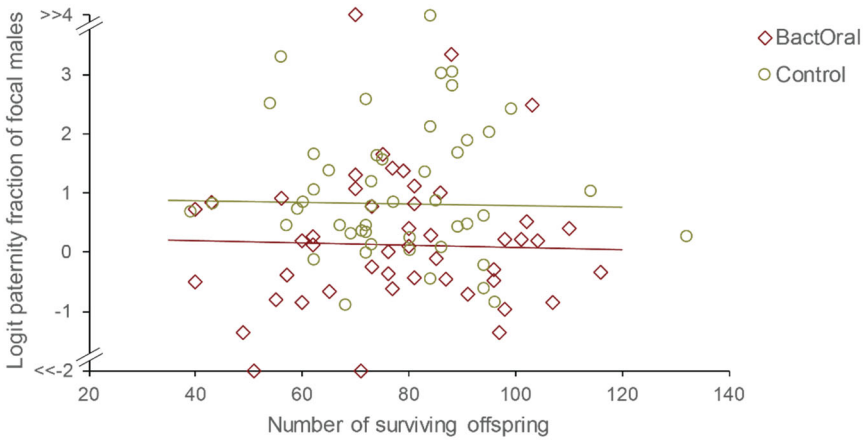
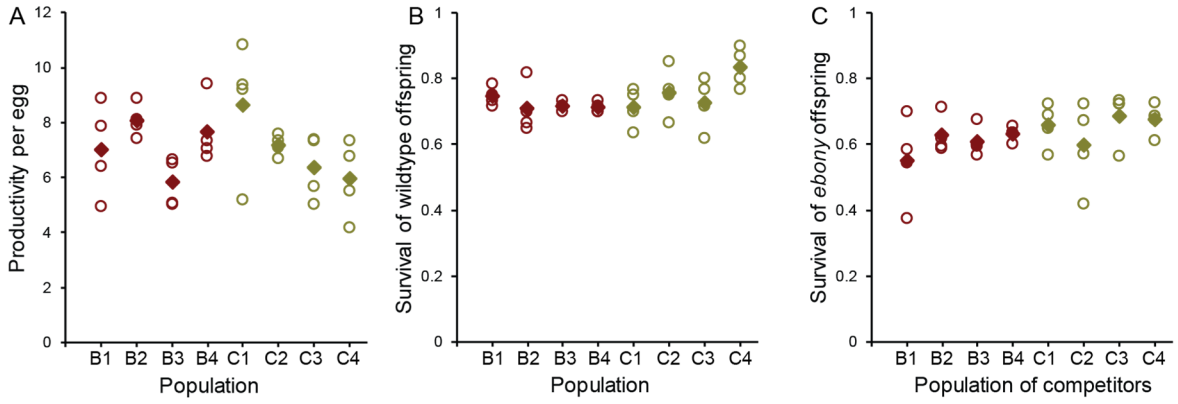
## Supplementary Figures




Supplementary Figure S1. Egg-to-adult developmental time and dry body weight of females and males from the BactOral and Control populations (the symbols correspond to replicate means). Least-square means ± SE for BactOral versus Control populations: developmental time females 12.84 ± 0.14 versus 12.80 ± 0.14 days, males 13.11 ± 0.14 versus 13.05 ± 0.14 days; dry weight females 0.387 ± 0.007 versus 0.386 ± 0.007 mg, males 0.266 ± 0.007 versus 0.259 ± 0.007 mg.




Supplementary Figure S2. The relationship between the proportion of offspring sired by males from the BactOral and Control populations in the competitive paternity assay and the total number of offspring emerging from a given vial. The regression lines were fitted in a generalized mixed model with binomial distribution and logit link, allowing for heterogeneity of slopes. Replicate populations are pooled for the graph but included as a random effect in the model.




Supplementary Figure S3. Alternative measures of success of BactOral and Control populations in the productivity and larval competition assays. (A) The total number of adult flies in the second divided by the initial number of eggs in the first generation (the product of variables in Fig 3A and 3B). (B) Egg-to-adult survival of the BactOral and Control larvae in the larval competition assay. (C) Egg-to-adult survival of the *ebony* larvae when competing with BactOral versus Control larvae.

# Appendix:
# Estimation of the loss of heterozygosity during experimental evolution

The expected loss of heterozygosity due to genetic drift is described by the recurrence equation

$$H_{t+1} = \left(1 - \frac{1}{2N_e}\right) H_t$$

where $H_t$ is the heterozygosity at time $t$ and $N_e$ is the effective population size (Crow and Kimura 1970). During selection for pathogen resistance, 620 individuals of each BactOral population were infected with *P. entomophila* and the survivors were allowed to breed to produce the next generation. Thus, the number of breeding adults $N$ differed between generations as the function of pathogen-induced mortality. I used Web Plot Digitizer (https://automeris.io/WebPlotDigitizer/) to extract the proportion of survivors $S_t$ in each generation from Figure 1A in Martins et al. (2013). For the few generations, for which the data were missing in the figure, the proportion of survivors were estimated by linear interpolation between the preceding and the following generation. The number of breeding adults in each generation was then estimated by multiplying $S_t$ by the number of individuals subject to infection, i.e., 620. Finally, based on a recent study of genomic variation in laboratory populations (Hardy et al. 2018), I assumed that the effective population size in a given generation is half of the number of breeding adults (i.e., $2N_e = N$). With these assumptions, the fraction of the initial heterozygosity remaining in the BactOral populations after the 24 generations of selection can be estimated as

$$\frac{H_{24}}{H_0} = \prod_{t=0}^{23} \left(1 - \frac{1}{620\, S_t}\right) = 0.949$$

(note that first generation of selection in Figure 1A in Martins et al. 2013 is denoted as generation 0). The analogous estimate for the Control population was obtained using the same equation and assuming that all 620 individuals survive to breed (i.e., $S_t = 1$), yielding $H_{24}/H_0 = 0.962$. Given that the BactOral and Control populations were initially established from the same base population, they would be expected to have the same initial heterozygosity $H_0$. Therefore, the ratio of the final heterozygosities $H_{24}$(BactOral)/$H_{24}$(Control) is estimated as 0.949/0.962 = 0.986.

If one assumes more conservatively that the effective population size is a quarter rather than a half of the number of breeding adults, the estimates of the fraction of heterozygosity retained $H_{24}/H_0$ are 0.901 and 0.925 for BactOral and Control populations, respectively, and their ratio is 0.974.

The actual heterozygosity could be estimated and compared directly from genome-wide data on sequence polymorphism. Such data are not available for the BactOral and Control populations. However, sequence-based heterozygosity estimates are available for a set of populations selected for 34 generations for resistance to *Drosophila* C virus (VirSys populations) and the corresponding control (ContSys) populations evolving under a sham treatment (Martins et al. 2014). These populations were established from the same base population as the BactOral and Control populations and selected in the same laboratory using the same protocol except for the type of infection and sham treatment. They thus offer an opportunity to verify the reliability of the above approach to estimate the loss of heterozygosity based on demographic data by comparing them to the sequence-based estimates. I therefore applied the same approach as described above to the VirSys and ContSys populations, extracting the survival data for the VirSys population from Figure 1A in Martins et al. (2014) and assuming *Ne* equal to half of the number of survivors. The fraction of heterozygosity retained after 34 generations predicted by this approach was 0.915 for VirSys and 0.947 for ContSys populations, with their ratio equal to 0.97. The corresponding estimates from the genomic data can be obtained from values reported in Supplementary Figure S2B in Martins et al. (2014) vary somewhat among chromosomes, but averaged across the five main chromosomal arms they are 0.932 and 0.954, respectively, with their ratio being 0.98. Thus, the modeling approach described above performs well and, if anything, might slightly underestimate the amount of heterozygosity retained.