Supplemental Data

A Bacterium Targets Maternally Inherited Centrosomes to Kill Males in *Nasonia*

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Supplemental Experimental Procedures

Host Lines

An *Arsenophonus*-infected *N. vitripennis* line, *f-line*, was established from a single infected female collected from a site owned by Cornell University that is located near Harford, New York. The *Arsenophonus* infection was then transferred to the standard laboratory lines *LabII* (*Wolbachia*-infected) and *AsymC* (*Wolbachia*-uninfected) by co-parasitization of the same blowfly host pupae with mated *f-line* females and laboratory line females. This results in horizontal transfer of the bacterium to the previously uninfected line [1]. Infected virgin F1 females were collected and mated to males from their own respective line to establish the *Arsenophonus*-infected laboratory lines used in this study. These lines therefore have the same genetic background as the uninfected standard laboratory lines. The infection status of these lines was confirmed with PCR using *Arsenphonus*- and *Wolbachia*-specific primers for the 16S rDNA gene.

Embryo Collection and Fixation

For collection of unfertilized embryos, virgin *N. vitripennis* females were fed 50% honey in water for 2-3 days and allowed to oviposit into blowfly host pupae for either 2 or 5 hr, depending on the desired developmental stage. Embryos were fixed in 100% methanol and rehydrated in 1xPTX before staining. To visualize maternal centrosomes during the

earliest stages, embryos were lanced following fixation in methanol to allow permeation of anti-α-Tubulin antibodies. Ovaries were dissected and fixed as described [33].

Immuno-staining

Primary antibodies were incubated overnight at 4°C at the following dilutions: mouse anti-α-Tubulin (Sigma) at 1/500 and rabbit anti-Lamin (gift from P. A. Fisher) at 1/100. After three washes in 1x PBTA, tissues were incubated at room temperature for 1.5 hr with Cy3 or Cy5-coupled anti-mouse or anti-rabbit antibodies (1/300) (Invitrogen). Host DNA and *Wolbachia* were stained by incubating tissues briefly in Oligreen (1/1000, Molecular Probes) before mounting or with DAPI in Vectashield mounting medium (Vector Labs). All images were obtained on a Leica DM IRB confocal microscope (courtesy of the microscopy facility of the Department of Molecular Biology and Genetics at Cornell University) and processed with Adobe Photoshop version 7.0.

Supplemental Reference

1. Verheyen, E., and Cooley, L. (1994). Looking at oogenesis. Methods Cell Biol. *44*, 545–561.