Evolutionarily conserved *Wolbachia*-encoded factors control pattern of stem-cell niche tropism in *Drosophila* ovaries and favor infection

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Wolbachia **are intracellular bacteria that infect invertebrates at pandemic levels, including insect vectors of devastating infectious diseases. Although *Wolbachia* are providing novel strategies for the control of several human pathogens, the processes underlying *Wolbachia*'s successful propagation within and across species remain elusive. *Wolbachia* are mainly vertically transmitted; however, there is also evidence of extensive horizontal transmission. Here, we provide several lines of evidence supporting *Wolbachia*'s targeting of ovarian stem cell niches—referred to as "niche tropism"—as a previously overlooked strategy for *Wolbachia* thriving in nature. Niche tropism is pervasive in *Wolbachia* infecting the *Drosophila* genus, and different patterns of niche tropism are evolutionarily conserved. Phylogenetic analysis, confirmed by hybrid introgression and transinfection experiments, demonstrates that bacterial factors are the major determinants of differential patterns of niche tropism. Furthermore, bacterial load is increased in germ-line cells passing through infected niches, supporting previous suggestions of a contribution of *Wolbachia* from stem-cell niches toward vertical transmission. These results support the role of stem-cell niches as a key component for the spreading of *Wolbachia* in the *Drosophila* genus and provide mechanistic insights into this unique tissue tropism.**

endosymbiont | maternal transmission | microbial tissue tropism | germline stem cell niche | somatic stem cell niche

The most common maternally transmitted bacteria in invertebrates are alphaproteobacteria belonging to the genus *Wolbachia*, representing the largest pandemic on the planet (reviewed by ref. 1). These *Rickettsia*-like bacteria are estimated to infect a great number of invertebrate species, including insect vectors of infectious diseases and pathogenic filarial worms. Recently, it has been shown that *Wolbachia* strains derived from *Drosophila melanogaster*, when introduced into mosquito vectors, can invade and sustain themselves in mosquito populations (2). Several phenotypes observed in *Drosophila* are also maintained in the mosquito nonnative hosts: reduction of adult lifespan, reproductive manipulation, and resistance against several pathogens, including Dengue, Chikungunya, West Nile Virus, and both chicken and human Plasmodium (3–6).

Because *Wolbachia* are maternally transmitted, their presence in the germ line is essential for their vertical propagation to the next generation. However, *Wolbachia* are often found in several somatic tissues as well, and this distribution varies among different *Wolbachia*–host associations (7–11). The role of these bacteria in somatic cells is not clear.

Wolbachia can also move horizontally within and between species (12–16). The mechanism by which horizontal transmission occurs in nature is poorly understood. Regardless of how *Wolbachia* reach a new host, after the initial infection event, reaching the germ line is an essential requirement for successful transmission to the next generation (1). It has been previously reported in *D. melanogaster* that, upon recent infection through microinjection, *Wolbachia* enter the region of the ovary containing the germarium. Several germaria reside at the anterior tip of each ovary and house all of the stem cells necessary to make an egg (Fig. 1*A*). Within the germarium, the major route for *Wolbachia* to enter the germ line in this artificial infection model is through the somatic stem-cell niche (SSCN; Fig. 1*A*, light blue cells) (17). The SSCN is the microenvironment that harbors the somatic stem cell (Fig. 1*A*, dark blue cells), which in turn generates the somatically derived follicle cells that envelope the germ line and secrete the eggshell. This observation in *D. melanogaster* raised the possibility of tropism for stem-cell niches as a mechanism to facilitate reaching the germ line during horizontal infection.

The same work also showed that *Wolbachia* accumulate at the SSCN in maternally infected flies. Additionally, in another fruit fly, *Drosophila mauritiana*, *Wolbachia* also target the germ-line stem-cell niche (GSCN; Fig. 1*A*, green cells) in long-term maternally infected flies (18). The GSCN is a somatic structure at the anterior tip of the germarium, composed of terminal filament (TF) and cap cells (CC) (Fig. 1*A*; TF, light green; CC, dark green) that support the germ-line stem cells (GSC; Fig. 1*A,* yellow cells). The GSCs are the source of the germ-line cells that develop into the eggs. These observations and subsequent work in other invertebrates (19–21) suggest that stem-cell niche tropism plays a widespread role in germ-line infection during long-term maternal transmission of *Wolbachia*, in addition to the potential role during horizontal transmission.

Here, using cell biological, phylogenetic, genetic, and transinfection tools, we provide evidence that stem-cell niche tropism is an evolutionarily conserved mechanism for *Wolbachia* hereditary and nonhereditary transmission. We show that this tropism is a widespread occurrence across the *Drosophila* genus. Phylogenetic analyses reveal selective pressures promoting strong conservation of the same pattern of niche tropism among closely related *Wolbachia* strains. Furthermore, quantification of bacterial densities across different regions of the germarium shows an increase of *Wolbachia* loads in the germ line during or immediately after interaction with infected stem-cell niches. Finally, through hybrid crosses and transinfection experiments, we show that *Wolbachia*-encoded factors, rather than the host genetic background, are the major determinants of different patterns of stem-cell niche tropism.

Results

Wolbachia **Tropism to the Somatic Stem-Cell Niche Is Pervasive Across the *Drosophila* Genus in All Species Tested.** To determine whether niche targeting is an evolutionarily conserved occurrence across the *Drosophila* genus, we conducted a survey of 11 different *Wolbachia* strains that naturally infect nine different *Drosophila* species

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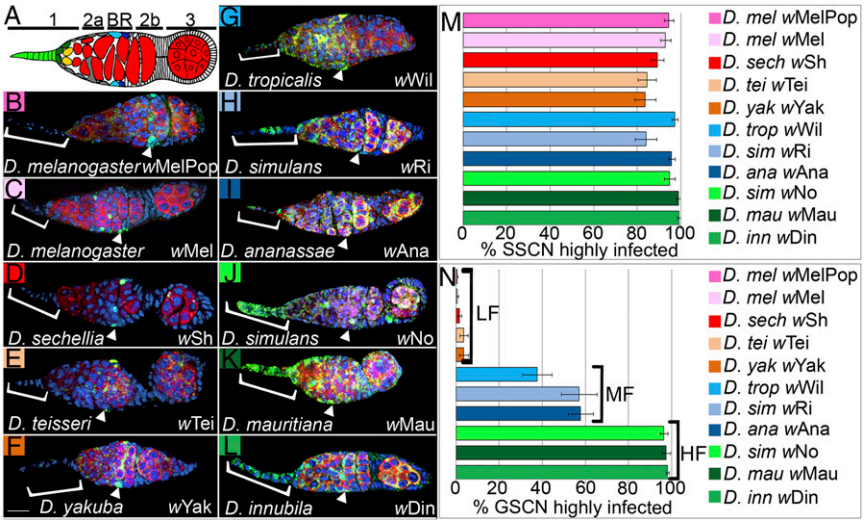

Fig. 1. *Wolbachia* tropism for stem-cell niches is present across the *Drosophila* genus, with specific patterns of distribution. (*A*) Representative diagram of a *Drosophila* germarium with the regions and cell types indicated: GSCN, in green [formed by TF cells (light green) and CCs (dark green)]; GSC in yellow; escort cells in gray; SSCN in light blue; SSC in dark blue; and germ line in red. (*B–L*) *Wolbachia* distribution in germaria of different *Drosophila* species. DNA is in blue, germ-line marker (Vasa) is in red, and *Wolbachia* is in green. *Wolbachia* highly infect the SSCN in all species and also infect the GSCN in several species (*G–L*). (Scale bar: 10 μm.) (*M*) Frequency of SSCN tropism. (*N*) Frequency of GSCN tropism. Brackets indicate groups with statistically similar frequencies. Groups are statistically significantly different from each other. $N \sim 100$ germaria each. For details see *SI Appendix*, Table S2. Error bars represent SEM. *D. ana*, *D. ananassae*; *D. inn*, *D. innubila*; *D. mau*, *D. mauritiana*; *D. mel*, *D. melanogaster*; *D. sech*, *D. sechellia*; *D. sim*, *D. simulans*; *D. tei*, *D. teisseri*; *D. trop*, *D. tropicalis*; *D. yak*, *D. yakuba*.

(*SI Appendix*, *Supplemental Materials and Methods* and Table S1). Using immunohistochemistry, we quantified the frequency of *Wolbachia*'s niche tropism in the germaria of all 11 *Wolbachia* strain–*Drosophila* species pairs. In every ovary analyzed, we found that *Wolbachia* preferentially infect the border region (BR) between regions 2a and 2b of the germarium (Fig. 1*A;* for controls, see *SI Appendix*, Fig. S1). This region contains the SSCN, and preferential *Wolbachia* infection at the BR characterizes SSCN tropism (*SI Appendix*, *Supplemental Materials and Methods*). By comparing *Wolbachia* levels at the BR to the neighboring somatic regions 2a and 2b, we found that *Wolbachia* was enriched in the SSCN in 100% of individuals for each species ($n = 119$ flies; Fig. 1 *B–L*). Visual assessment of confocal imaging of ∼10 randomly sampled germaria from each ovary showed a frequency of SSCN tropism of greater than 80% ($n = 1{,}194$ total germaria; Fig. 1*M*; $P = 0.0012$). To quantify levels of *Wolbachia* enrichment at the SSCN, representative confocal Z stacks were subjected to image analysis of *Wolbachia* voxel density in the soma of the different germarial regions (*SI Appendix*, *Supplemental Materials and Methods*, Fig. S2*A*). In every species analyzed, there was an increase in *Wolbachia* load in the soma of the SSCN region normalized to the somatic cells in adjacent region 2b ranging from 2- to 59-fold (*SI Appendix*, Fig. S2*B*; *t* test between BR and 2b statistically significant, $P < 0.01$ for all species). This analysis indicates a strong selective pressure for an evolutionarily conserved *Wolbachia* tropism to the SSCN.

***Wolbachia* Target the Germ-Line Stem-Cell Niche in a Subset of Species.** In addition to *Wolbachia* tropism to the SSCN, we observed *Wolbachia* infection in the GSCN (Fig. 1*A*, green TF and CC), characterized as GSCN tropism (*SI Appendix*, *Supplemental Materials and Methods*). Six of eleven *Drosophila–Wolbachia* pairs analyzed showed GSCN tropism (Fig. 1 *G–L*). Occurrence of GSCN tropism is more variable than SSCN tropism, with frequencies ranging from 37% to 99% of GSCNs targeted (Fig. 1*N* and *SI Appendix*, Table S2; $n = 647$ total germaria). ANOVA analyses defined three distinct groups: high frequency (HF) of GSCN targeting (Fig. 1 *J–L* and *N*; $P = 0.80$), moderate frequency (MF) of GSCN targeting (Fig.1 *G–I* and *N*; $P = 0.087$), and low/no frequency (LF) of GSCN targeting (Fig. 1 *B–F* and *N*; $P = 0.44$). Voxel intensity measurements showed that *Wolbachia* density is from 2.5- to 26.5-fold enriched in the GSCN normalized to region 2b soma (*SI Appendix*, *Supplemental Materials and Methods* and Fig. S2*C*). In addition, *Wolbachia* infection of the escort cells was also noted in some species (*SI Appendix*, *Supplemental Results* and Fig. S3, and Movie S1). Escort cells are a stable, nondividing, stromal population of cells that are attached to the basement membrane of the germarium and support the progression of early germ-line cysts in region 1 and 2A of the germarium (see gray cells in Fig. 1*A*). Relative to SSCN tropism, targeting of the GSCN occurred at a lower frequency and density. These observations show that, although targeting of stem-cell niches in the *Drosophila* ovary is a widespread occurrence, the patterns of distribution are not the same in all *Drosophila* host–*Wolbachia* strain pairs.

Phylogenetic Analyses Suggest That Differential Niche Tropisms Are Mediated by *Wolbachia*-Encoded Factors. In broad terms, we see two different patterns of stem-cell niche tropism in the *Drosophila* ovary: (*i*) targeting of only the SSCN (herein referred to as SSCN pattern) or (*ii*) targeting of both the SSCN and the GSCN (herein referred to as GSCN pattern). This observation of differential patterning of stem-cell niches led us to investigate the relative contributions of host factors and bacterial factors toward the distinct *Wolbachia* tropism patterns. We reconstructed the evolution of niche tropism on phylogenetic trees of both *Wolbachia* and *Drosophila* (Fig. 2*A*) (22, 23) to determine whether patterns of niche tropism were primarily determined by factors derived from the *Wolbachia* strains or derived from the *Drosophila* host species. To quantify the correlation of niche tropism pattern to the two different phylogenies, we used a computer simulation model of randomized character distributions to compare with the distribution of niche tropism pattern on each of the phylogenies (*SI Appendix*, *Supplemental Results* and Fig. S4) (24). This analysis indicated that there is an ∼10-fold lower probability that the association of niche tropism with the *Wolbachia* phylogeny is due to random chance than the association with the *Drosophila* phylogeny. Therefore, closely related *Wolbachia* strains are more likely to display similar patterns of tropism compared with the tropism

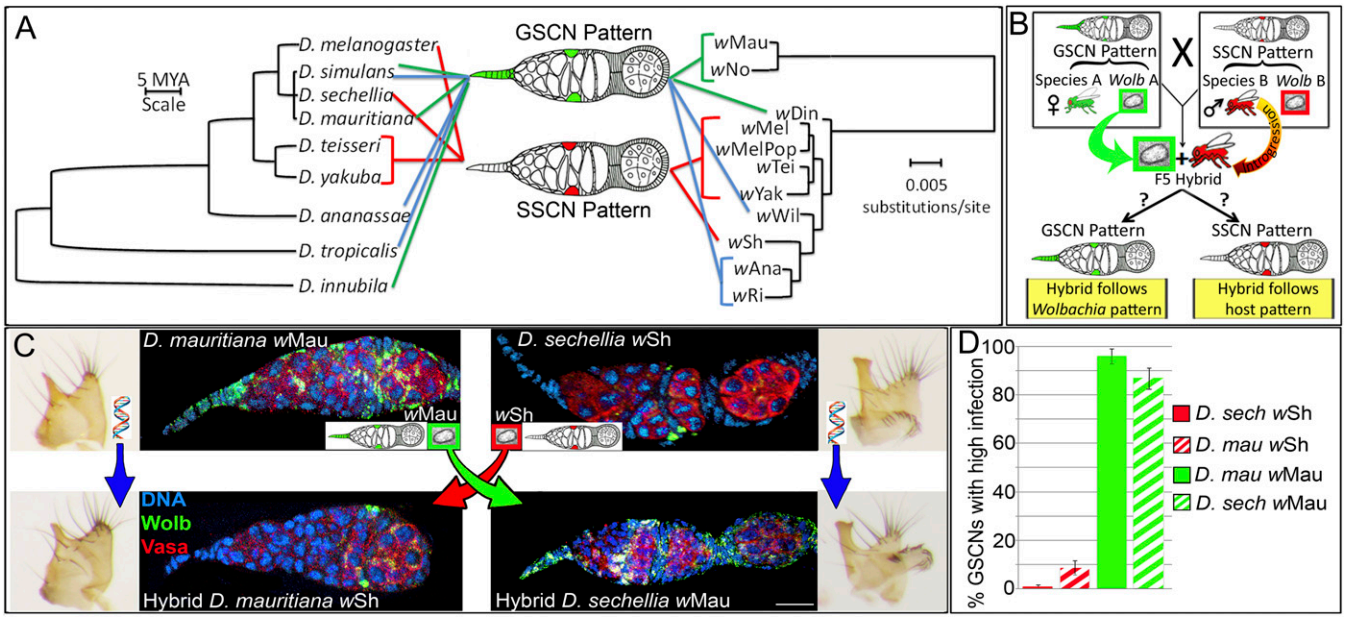

Fig. 2. *Wolbachia* strain determines differential targeting of the germ-line stem-cell niche. (*A*) Different patterns of niche targeting are correlated with *Drosophila* and *Wolbachia* phylogenies (22, 23). MYA, million years ago. Green, blue, and red lines indicate high, moderate, and low frequency of GSCN tropism, respectively. (*B*) Diagram showing experimental design of the hybrid cross to introgress *Wolbachia* A into species B genetic background. (*C*) *Wolbachia* strains *w*Mau and *w*Sh were introgressed into *D. sechellia* and *D. mauritiana,* respectively. Representative images of *Wolbachia* niche targeting in the parental (*Upper*) and F_5 hybrid (*Lower*) host germaria. The red and green arrows represent the direction of *Wolbachia* transfer. The male genital arch is shown to confirm successful introgression of the male genetic background. (Scale bar: 10 μm.) (*D*) Quantification of GSCN targeting in parental (solid bars) and hybrid (striped bars) species (Log reg, $P_{wolb} = 4.7 \times 10^{-22}$ and $P_{host} = 0.18$). $N_{D.sech\ wSh} = 120$, $N_{D.mau\ wSh} = 140$, $N_{D.mau\ wMau} = 100$, $N_{D.sech\ wMau} = 109$ (N = number of germaria). Error bars represent SEM.

patterns observed in closely related *Drosophila* species. Furthermore, the phylogenetic analysis suggests that the different patterns of niche tropism evolved in *Wolbachia* and that the pattern of shared *Wolbachia* niche tropism in *Drosophila* results from characteristics of the infecting *Wolbachia* strain rather than characteristics of the host *Drosophila* species.

Hybrid Crosses Confirm That Bacterial Factors Mediate Stem-Cell Niche Tropism. The phylogenetic analyses suggest that *Wolbachia* factors mediate differential stem-cell niche tropism patterns. To experimentally evaluate this hypothesis, we generated hybrid flies between *Drosophila* species harboring two different *Wolbachia* strains that display the two different *Wolbachia* tropism patterns, using genetic introgression (*SI Appendix*, Fig. S5). The rationale for this experiment is as follows: if the pattern of tropism is mediated by the *Wolbachia* strain, the *Wolbachia* patterning in the germaria in the hybrid host will be the same as the original maternal host, regardless of the introgressed male host genetic background (Fig. 2*B*).

Hybrid fly lines were created by crossing *D. mauritiana* flies infected with *Wolbachia w*Mau, which display a GSCN tropism pattern, and *Drosophila sechellia* flies infected with *Wolbachia w*Sh, which display a SSCN tropism pattern. *Wolbachia w*Mau, infecting both the parental *D. mauritiana* and hybrid *D. sechellia*, display a high frequency of GSCN tropism pattern (greater than 85%; Fig. 2 *C* and *D*; $n = 209$ total germaria). In contrast, *Wolbachia w*Sh, infecting both the parental *D. sechellia* and hybrid *D. mauritiana*, display high frequencies of the SSCN tropism pattern, with greater than 90% of germaria analyzed only infecting the SSCN (Fig. 2 *C* and *D*; $n = 260$ total germaria). Regardless of genetic background, both *Wolbachia* strains maintain the maternal niche tropism pattern in the hybrid host. Logistic regression analysis was performed to evaluate the relative contributions of the *Wolbachia* strain and the host genetic background to the differential patterns of stem-cell niche tropism. We found no evidence of host influence on niche tropism pattern ($P = 0.18$); however, the *Wolbachia* strain does have a highly statistically significant effect ($P = 4.7 \times 10^{-22}$). Image analysis of representative images confirms GSCN tropism in *w*Mau-infected flies and SSCN tropism in *w*Sh-infected flies (*SI Appendix*, Fig. S6).

During the hybrid crosses, together with the *Wolbachia* strain, other maternally inherited components, such as the mitochondria, are also transmitted. To eliminate the possibility that maternally transmitted organelles and other factors have a role in determining *Wolbachia* niche tropism pattern, we analyzed a fly line whose *Wolbachia* infection was established via microinjection (*Drosophila simulans* artificially infected with *w*Mel) (25). The results indicate that the *Wolbachia* strain is necessary and sufficient to determine the pattern of niche tropism in a nonnative host. *w*Mel-infected flies always display the SSCN tropism pattern only, regardless of genetic background and maternally inherited components (*SI Appendix*, *Supplemental Results* and Fig. S7; $n =$ 246 total germaria).

These results are in agreement with our phylogenetic analysis and support the hypothesis that stem-cell niche tropism is largely mediated by *Wolbachia* factors rather than the host genetic background.

***Wolbachia* Factors also Direct Qualitative Differences Within Niche Tropism Pattern.** We also observed variability in the pattern of *Wolbachia* distribution in the TF cells. Some TFs were fully infected, with all cells densely infected with *Wolbachia*; others had a discontinuous pattern of infection, with only some TF cells densely infected, interspersed with noninfected TF cells. Interestingly, two *Wolbachia* strains that naturally infect *D. simulans* had this noticeable difference, which was most evident in young flies. *Wolbachia w*Ri displays a discontinuous TF pattern of infection (Figs. 1*H* and 3*A*); *Wolbachia w*No fully infects the TF (Figs. 1*J* and 3*A*).

Because we have shown that *Wolbachia* factors are mediating the overall patterns of niche tropism, we investigated whether they

also influence qualitative differences within the same pattern. After backcrossing to introgress the host genetic backgrounds (Fig. 2*B* and *SI Appendix*, Fig. S5), we observed that *w*Ri-infected flies, regardless of host strain genetic background, display a high frequency of discontinuous terminal filament infection, with ~80% of highly infected niches having a discontinuous pattern (Fig. 3; n = 230 total germaria). *Wolbachia* *w*No-infected flies display a low frequency of discontinuous terminal filament infection, with ~20% of infected niches having a discontinuous pattern, regardless of host strain genetic background (Fig. 3; n = 242 total germaria). Logistic regression analysis confirms that the *Wolbachia* strain plays a more significant role in the discontinuous GSCN pattern than the fly genetic background ($P = 6.5 \times 10^{-11}$ and $P = 0.54$, respectively). These results demonstrate that *Wolbachia*-encoded factors also direct specific differences in the distribution of bacteria within the GSCN.

***Wolbachia* Levels in the Germ Line Increase with Proximity to Infected Niches.** To assess the contribution of stem-cell niche tropism toward *Wolbachia* enrichment in the germ line, we quantified the *Wolbachia* density in the germ line in the different germarial regions of each of the *Drosophila–Wolbachia* pairs (*SI Appendix*, Fig. S2*C*). For contribution from the SSCN, we compared the density of *Wolbachia* in germ-line cysts in region 2a to the density of *Wolbachia* in germ-line cysts in region 2b (*SI Appendix*, *Supplemental Materials and Methods*). These two regions contain germ-line cells before (2a) and after (2b) developing cysts pass through the niche (Fig. 1*A*). In all species, except *Drosophila tropicalis*, we observed a similar trend: after passage through the border region containing the highly infected SSCNs, the levels of *Wolbachia* in germ-line cysts in region 2b are higher than the levels of *Wolbachia* in region 2a, with fold-changes (2b/2a) ranging from 1.3 to 25 (*SI Appendix*, Fig. S8*M*). Although there is high variability in *Wolbachia* load from germ-line cyst to germ-line cyst, 7 of 11 species, have a statistically significant increase of *Wolbachia* load from 2a to 2b [see white arrows in *SI Appendix*, Fig. S8 *B–F*, *J*, *K*, and *M* (quantification); *t* test, $P < 0.05$].

For contribution from the GSCN, we compared the relative fraction of *Wolbachia* in region 1 of the germ line across species with GSCN tropism and without GSCN tropism (*SI Appendix*, Fig. S8*N*). Species with GSCN tropism had a higher relative density of *Wolbachia* in region 1 (compared with the whole germarium) than species with only SSCN [green asterisks in *SI Appendix*, Fig. S8 *G–L* and *N* (quantification)].

In the majority of *Drosophila* species analyzed, *Wolbachia* tropism to the stem-cell niches correlates with higher densities of *Wolbachia* in the adjacent germ line. These results agree with previous work (17, 19–21) supporting a passage of *Wolbachia* from the niche into the germ line.

Increase of *Wolbachia* Density from Regions 2a to 2b Is Contributed to by *Wolbachia* Proliferation in the Niche and Germ Line. For the niche to be a source for *Wolbachia* into the germ line, we expect *Wolbachia* to be dividing in the niche. Using an antibody against the conserved bacterial cell division protein FtsZ (named after filamenting temperature sensitive mutant Z), we observed substantial *Wolbachia* division within the SSCN (*SI Appendix*, *Supplemental Materials and Methods* and Fig. S9*A*) (20, 26). In addition to passage from the SSCN, *Wolbachia* are actively dividing in the germ line, which also contributes to the increase in *Wolbachia*'s density in region 2b. Region-specific differences in the rate of *Wolbachia* division could play a major role in the increase of *Wolbachia* in region 2b. However, our analysis indicates that the fraction of *Wolbachia* dividing in both regions 2a and 2b of the germarium is the same (*SI Appendix*, Fig. S9 *B* and *C*). Even with the same division rate of *Wolbachia* in these regions, differences in cyst development timing could also play a role in the increase of *Wolbachia* density in region 2b. However, studies in *D. melanogaster* demonstrate that the developmental time that germ-line cysts remain in region 2b is not significantly different from the time the germ-line cysts are present in the surrounding regions 2a and 3, ruling out this possibility in at least *D. melanogaster* (27). These data suggest that *Wolbachia* division within the germ line, in combination with *Wolbachia* passage from the niche, contributes to the increase of *Wolbachia* density in region 2b.

Discussion

To understand the spread of *Wolbachia* in nature, it is important to elucidate the mechanisms of horizontal and vertical transmission. Because the majority of transmission events are maternal, to effectively infect a population, *Wolbachia* must infect the female's germ line during both long-term stable vertical transmission and recent horizontal introduction into a new host. Here, we provide evolutionary, cytological, genetic, and developmental evidence for a mechanism in which stem-cell niche tropism promotes germ-line colonization across the *Drosophila* genus. We also demonstrate that factors encoded by the *Wolbachia* strain, rather than the host species, are the major determinants of the type of stem-cell niche that is infected.

In a survey of niche tropism, we show that *Wolbachia* display tropism for two different stem cell niches in the *Drosophila* ovary: the SSCN and the GSCN. Several studies have described *Wolbachia* preferential infection of different tissues, host cells, and subcellular locations in the *Drosophila* genus, including adult brain, embryonic neuroblasts, specific regions of the oocyte during oogenesis, and posterior or anterior areas of the early embryo (9, 28–30). Considering *Wolbachia*'s transmission across generations, a site in the host of particular interest is the germplasm, which is a highly specialized, maternally synthesized cytoplasm that is deposited in the posterior pole of the egg and induces the formation of the germ line in the embryo (ref. 31 and reviewed by ref. 32). During late oogenesis and early embryonic development, *Wolbachia* efficiently colonize the germplasm in *D. melanogaster*, giving rise to a highly infected germ line, ensuring *Wolbachia* transmission to the subsequent generation (28, 33). However, germplasm

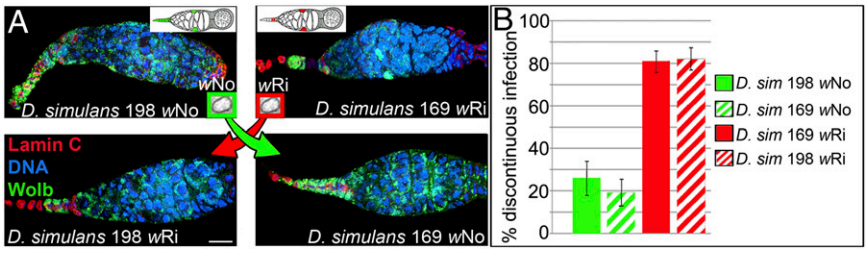

Fig. 3. *Wolbachia* strain directs patterning within the GSCN. (*A*) *Wolbachia* distribution in GSCN of *w*Ri and *w*No infected *D. simulans* 198,169 (*Upper*) and F_5 backcrossed strains (*Lower*). (Scale bar: 10 μm.) (*B*) Quantification of parental F_0 (solid bars) and F_5 (striped bars) strains. (Log reg, $P_{wolb} = 6.5 \times 10^{-11}$ and $P_{host} = 0.54$). $N_{D.sim198\ wNo} = 120$, $N_{D.sim169\ wNo} = 122$, $N_{D.sim169\ wRi} = 100$, $N_{D.sim198\ wRi} = 130$ (*N* = number of germaria). Error bars represent SEM. Lamin C labels TF and CCs.

infection is not observed in several other *Drosophila* species (28, 29). Surprisingly, targeting of the SSCN is more prevalent in the *Drosophila* genus than targeting of the germplasm. To our knowledge, with the exception of infection of the adult oocyte, the preferential infection of the SSCN reported here is the most conserved *Wolbachia* tropism reported in the *Drosophila* genus.

Given that *Wolbachia* does not colonize the germplasm of the embryo in every *Drosophila* species, there must be an alternative mechanism to ensure its vertical transmission. The strong phylogenetic conservation of patterns and the pervasive presence of tropism for stem-cell niches in the *Drosophila* germarium are suggestive of a significant role for niche tropism in transmission. Previous work has implicated stem-cell niche tropism as a mechanism facilitating horizontal transmission of *Wolbachia* in *D. melanogaster* (17). Our confocal imaging analysis suggests that stem-cell niches in the *Drosophila* germarium also play a role in vertical transmission of *Wolbachia.* Similar to our findings, there is a surprising observation from the *Wolbachia* strains infecting filarial nematodes. In the filarial worm, *Wolbachia* are excluded from the precursor of the germ-cell lineage; infection of the gonad happens later in development, through the invasion via the distal tip cell, the nematode equivalent to the stem-cell niche (20). Furthermore, studies on a bedbug and a leafhopper suggest that *Wolbachia* are transmitted to the germ line via a putative stem-cell niche (19, 21). These observations support a hypothesis of stem-cell niche tropism as a mechanism for *Wolbachia* dissemination shared during both horizontal and vertical transmission.

Our data clearly show that the SSCN prevails over the GSCN in terms of occurrence and evolutionary conservation. To provide an explanation for these observations, we propose a model that considers *Wolbachia* transmission to the germ line during development from the stem-cell niches. The differences in the anatomic features between niches and associated cells, as well as the developmental time periods in which *Wolbachia* can be transmitted from each niche, suggest that the SSCN is better suited for *Wolbachia* transmission to the germ line.

The model presented in Fig. 4 displays potential routes of *Wolbachia* entry into the germ line from the surrounding niches and other somatic cells during *Drosophila* oogenesis. The GSCN contacts the germ-line stem cell, providing a potential route for the *Wolbachia* present in this niche to enter the germ line (Fig. 4*C*, dark blue arrows). In addition, when escort cells are highly infected, it is possible to have transmission from these somatic cells into the germ line until the developing cyst reaches the BR (Fig. 4*C*, light blue arrow; see also *SI Appendix*, Fig. S3 and Movie S1). Therefore, transmission into the germ line could occur for a total of ~2.5 d, the estimated time for germ-line transit from the germ-line stem-cell niche to the BR (Fig. 4*B*, see blue line in timeline) (27, 34).

In comparison, the SSCN provides several routes for *Wolbachia* transmission into the germ line (Fig. 4 *D–G*), both direct and indirect. Because the SSCN contacts all developing germ-line cysts, it can transmit *Wolbachia* directly into the germ-line cells that must pass through the border region (Fig. 4 *B* and *D*, red arrows). The possibility of *Wolbachia* passage into the germ line was initially suggested for *D. melanogaster* by confocal analysis (see supplementary table 1 in ref. 17), further corroborated by EM studies (21). The data presented here suggest that the SSCN can deliver *Wolbachia* directly into the germ line in all species of *Drosophila* analyzed in this study.

The SSCN can also transmit *Wolbachia* indirectly. The infected niche is a constant source of *Wolbachia* into the SSC, which, in turn, divides and transmits *Wolbachia* into the developing follicle cells (Fig. 4*D*, orange arrows) (see also supplementary figure 2 b–d and supplementary movie in ref. 17). The follicle cells can transmit *Wolbachia* into the germ line of developing egg chambers through the remaining stages of germ-line development, providing an extended period of developmental time for transmission (Fig. 4*B*, developmental stages indicated by orange line; Fig. 4 *E–G*, orange arrows). Furthermore, several yolk proteins produced by the follicle cells are actively transported into the oocyte during the final stages of oogenesis (35). This process may provide a facilitated mechanism for *Wolbachia* present in the follicle cells to transfer into the oocyte (Fig. 4*G* and *SI Appendix*, Fig. S10). From the border region, it takes approximately 5 d for the completion of oogenesis (36). Compared with the previous 2.5 d of cyst development in regions 1 and 2A, where there is the potential for *Wolbachia* transmission from the GSCN and escort cells, the developmental time available for transmission of *Wolbachia* derived from the SSCN is about twice as long (Fig. 4 *A* and *B*, blue line vs. red/orange line in timeline). Ultimately, it is easier for *Wolbachia* to reach the germ line through the SSCN (rather than the GSCN) during vertical transmission and probably during horizontal transmission as well. These developmental and anatomical features of

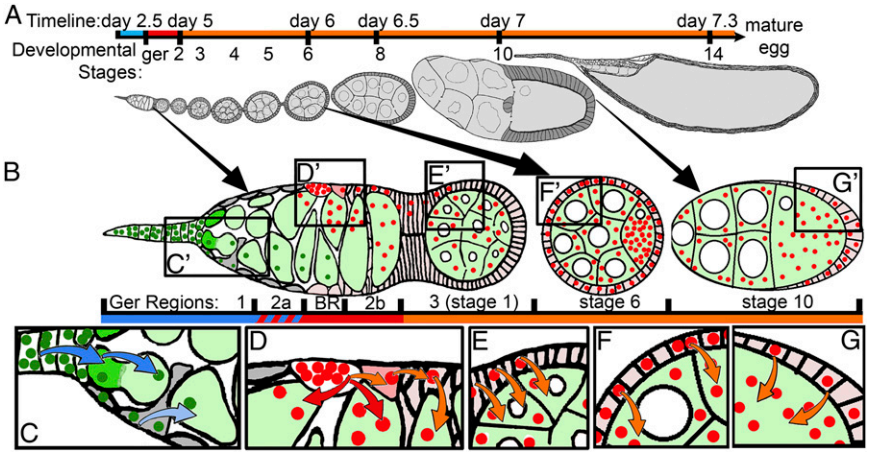

Fig. 4. Model for *Wolbachia* transmission from the stem-cell niches into the germ line. *Wolbachia* originating from the SSCN, rather than from the GSCN, are more likely to invade the germ line. (*A*) Diagram of egg formation with developmental stages and timeline in days (27, 36; diagram adapted from ref. 18). Developmental timeline is colored according to potential for *Wolbachia* transmission from the GSCN and escort cells (blue, days 0–2.5) or from the SSCN, either directly (red, day 2.5) or indirectly (orange, days 2.5–7.3). (*B*) Diagram of potential sources of *Wolbachia* transmission into the germ cells from somatic cells present in the germarium and representative egg chambers. (*C*) Magnification of *Wolbachia* transfer from the GSCN (dark blue arrows) or the escort cells (light blue arrows). (*D*) Magnification of *Wolbachia* transmission directly from the SSCN (red arrows). (*E–G*) The somatic tissue infected with *Wolbachia* originating from the SSC can indirectly transmit *Wolbachia* into the germ line for the rest of egg development (orange arrows).

the niches provide an explanation to the phylogenetic, genetic, and cytological data presented here.

This work highlights bacterial localization as a fundamental aspect of *Wolbachia*–host interactions being maintained during *Wolbachia* evolution. Our current understanding of the mechanisms involved in *Wolbachia* localization is limited (36). Toward dissecting the mechanistic basis of stem-cell niche tropism, we investigated the relative role of bacterial versus host factors in the different patterns of niche tropism. Through hybrid crosses and transinfection experiments, we showed that bacterial intrinsic factors are the major determinant of the pattern of niche tropism and also determine differences within the same pattern.

There are extensive comparative genomic analyses of different *Wolbachia* strains used in this study (37–39). At this point, we cannot attribute differences in the targeting of stem-cell niches to specific genes or proteins due to a large number of genomic differences across the *Wolbachia* strains analyzed (38, 39). Indeed, it has been suggested that *Wolbachia* is one of the most highly recombining intracellular bacterial genomes known to date (37). Nevertheless, the data presented here provide the foundation for future approaches toward the identification of genetic pathways mediating *Wolbachia*'s stem-cell niche tropism in hosts.

Wolbachia-based technologies are emerging as a promising tool for the control of vectors of deadly human diseases, including Dengue fever, West Nile virus, and malaria (3–6, 41, 42). Understanding the basis of *Wolbachia* targeting of specific tissues in the host and its consequences toward bacterial transmission will provide further mechanistic insight into their extremely successful propagation and is also relevant for developing new *Wolbachia*-based vector control approaches.

Materials and Methods

SSCN tropism was defined as *Wolbachia* accumulation in the somatic cells residing at the border between regions 2a and 2b, as previously described (17). GSCN tropism was defined as *Wolbachia* accumulation in the TF and CCs, as previously described (18). Fly stocks utilized in this study, husbandry, immunohistochemistry, FISH, introgression crosses, phylogenetic analyses, image analysis, FtsZ analysis, and statistical analysis are provided in *SI Appendix, Supplemental Materials and Methods*.

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Contents

Supplemental Results:

***Wolbachia* also target the escort cells**

In region 1 of the germarium, in addition to tropism to the GSCN, we also observed high levels of *Wolbachia* in the escort cells (see Fig. 1*A*, S3 and Movie S1). The escort cells are a stable, non-dividing, stromal population of cells that are attached to the basement membrane of the germarium and support the progression of early germline cysts in region 1 and 2A of the germarium (Fig. 1*A* and 4*B*)(1). Because the Vasa antibody staining did not consistently allow clear visualization of escort cells in all species, this analysis was not possible across the genus, and was restricted to *D. mauritiana*. We found that approximately 50% of the escort cells analyzed in *D. mauritiana* were highly infected with *Wolbachia* relative to the surrounding germline (Fig. S3*D*), indicating that there may be an additional tropism to the escort cell population promoting somatic routes for germline infection.

Phylogenetic analysis confirms niche tropism is more closely related to *Wolbachia* phylogeny.

To quantify the correlation of niche tropism pattern to the two different phylogenies, we utilized a computer simulation model of randomized character distributions to compare with the distribution of niche tropism pattern on each of the phylogenies (Fig. S4 *A* and *C*) (2). We used tree length as a measurement for goodness of fit for the distribution of a character, such as the tropism pattern, as aligned with the phylogeny. Tree length is defined as the total number of steps required to map a data set onto a phylogenetic tree. Observed niche tropism correlated with the *Wolbachia* phylogeny requires 3 steps (Tree length = 3) and out of 1000 computer simulated random characters, only 8.7% require 3 or fewer steps (Fig. S4*B*). Conversely, observed niche tropism correlated with the *Drosophila* phylogenetic tree has a tree length of 4 and out of 1000 random character distributions, 80.8% require 4 or fewer steps (Fig. S4*D*) (2). Therefore, there is an approximately 10-fold lower probability that the association of niche tropism with the *Wolbachia* phylogeny is due to random chance versus the association with the *Drosophila* phylogeny. These analyses strongly support our hypothesis that niche tropism pattern is directed by the *Wolbachia* strain, rather than the *Drosophila* host.

Maternally inherited components have no influence on stem cell niche tropism.

During the hybrid crosses, together with the *Wolbachia* strain, other maternally inherited components, such as the mitochondria, are also transmitted. To eliminate the possibility that maternally transmitted organelles and other factors have a role in determining the previously tested differences in *Wolbachia* niche tropism, we utilized a fly line whose *Wolbachia* infection was established via microinjection. This line was previously generated by *Wolbachia* isolation from one host species followed by injection into another species (3). Niche tropism of *D. simulans* flies trans-infected with *w*Mel via embryonic microinjection was assessed. The results indicate that the *Wolbachia* strain is necessary and sufficient to determine the pattern of niche tropism in a non-native host. *w*Mel infected flies always display *Wolbachia* infection in the SSCN only, regardless of genetic background and maternally inherited components (Fig. S7 *A* and *B*, N=246 total germaria). Logistic regression analysis confirms that the *Wolbachia* strain has a significantly greater effect on niche tropism pattern than the host genetic background ($P= 6.7x10^{-7}$ and P=0.76, respectively). Analysis of *Wolbachia* pixel density of representative images supports niche tropism quantification, showing high *Wolbachia* densities only in the SSCN of *w*Mel-infected flies (Fig. S7*C*).

<u>Supplemental Materials and Methods:</u>

Identification of stem cell niches for tropism analysis:

The SSCN and associated somatic stem cells (SSCs) reside at the boundary between regions 2a and 2b of the germarium. For the purpose of this analysis, this boundary was defined as the border region (BR), encompassing the SSCN and SSC, as previously done (4). Association with the adjacent somatic stem cell identified by lineage labeling is the most reliable method to identify the stem cell niche (5). Due to the general lack of genetic and cytological SSC and SSCN markers across the *Drosophila* genus, somatic stem cell niche tropism was considered as a more general tropism for the somatic tissue at the border region.

Germline stem cell niche tropism consists of tropism to two main cell types comprising the GSCN: the cap cells (CC) and the terminal filament (TF) cells. Infection of the CC vs. the TF cells was fairly similar, and when correlated, have an R^2=0.97 (Fig. S10, $P=6.6x10^{-9}$). Since the frequency of infection are similar between the two

cell types, the analysis shown of GSCN tropism refers to an average between infection of the TF cells and the CCs.

Fly stocks used for analysis:

Stocks analyzed in this study and their sources are shown in Table S1. Of the nine species comprising the *D. melanogaster* subgroup, seven are naturally infected with *Wolbachia*. We analyzed all of them except for *D. santomea*. The publicly available *D. santomea* stock that we obtained was not infected (6). However, we characterized niche tropism in natively infected *D. yakuba* and *D. teisseri* flies that are closely related to *D. santomea*, together comprising the *yakuba* complex. The *Wolbachia* strains that infect the *yakuba* host complex are closely related and have been described as identical (7). Therefore, all the major *Wolbachia* strains infecting the *D. melanogaster* subgroup are present in this study.

In addition 3 other species representative of major groups across the *Drosophila* genus (naturally infected with *Wolbachia*) were analyzed (*D. innubila, D. tropicalis,* and *D. ananassae*).

Fly husbandry:

Flies were raised at room temperature and fed a typical molasses, yeast, cornmeal, agar food, with the exception of the following: *D. sechellia* flies were supplemented with reconstituted Noni Fruit (Hawaiian Health Ohana, LLC)(8); *D. innubila* flies we raised on Instant *Drosophila* medium (Carolina Biological Supply, Burlington, NC) supplemented with a mushroom (9).

Immunohistochemistry:

Flies were aged to seven days (with the exception of the *D. simulans* hybrids for Fig. 4 which were dissected at eclosion), dissected, and fixed in a 4% paraformaldehyde solution. Ovaries were stained as previously described (4, 10). The following antibodies were used at the indicated dilutions: mouse anti-hsp60 (Sigma; 1:100), rat anti-Vasa (a gift from P. Lasko; 1:500, for non-*D.melanogaster* species), rat anti-Vasa IgM (DSHB; 1:5, for *D. melanogaster*), rabbit anti-Vasa (a gift from R. Lehmann, 1:5000), mouse anti-lamin C (1:20; DSHB), rabbit anti-FtsZ (a gift from Bill Sullivan; 1:1000). Nuclei were counterstained with Hoechst (1 µg/ml, Molecular Probes).

Fluorescent *in situ* hybridization:

In situ hybridization control staining (Fig. S1*C*) protocol: adapted from (11)-(12). Tissue was dissected in Graces and fixed in 4%PFA solution. Specific oligonucleotide probes were designed against the 16SrRNA of *Wolbachia* (Integrated DNA Technologies). Two *Wolbachia* probes labeled with Cy3 at the 5' end were used: Wpan16S887: 5'-ATCTTGCGACCGTAGTCC-3' and Wpan16S450 5'-CTTCTGTGAGTACCGTCATTATC -3'. Hybridization was performed at 37°C in 50% Formamide, 5x SSC, 250 mg/l Salmon sperm DNA, 0.5x Denhardt's solution, 20mM Tris-HCl, and 0.1% SDS. After a 30 min preincubation period, tissue was incubated in 100ng of each probe for 3 hours. Tissue was then washed twice for 15 minutes at 55°C in a 1x SSC wash with 0.1% SDS and 20mM Tris-HCl and then twice for 15 minutes in a 0.5x SSC wash with 0.1% SDS and 20 mM Tris-HCl. Hoechst was added to the second 0.5x SSC wash at a concentration of 10 µg/mL. Tissue was then washed in PBS and mounted in Prolong Gold antifade solution and imaged as described below.

Image analysis of *Wolbachia* niche tropism

Visual identification of niche tropism: Presence of fluorescent labeling for *Wolbachia* was visually identified and counted using epifluorescence at 600x magnification using Olympus Fluoview 1000 Confocal microscope. Representative images of niche tropism for each species were acquired using a FV1000 confocal microscope (Olympus). Visual identification of niche tropism was confirmed in a subset of representative confocal images (N=10 for each *Drosophila*/*Wolbachia* pair) using MatLab software for image processing.

Wolbachia density analysis: Z stacks of representative images (N=10 for each *Drosophila*/*Wolbachia* pair) were analyzed for *Wolbachia* density in the soma and germline in several regions of the germarium using MatLab software, as defined by Frydman, et al. 2006. *Wolbachia* in the soma and germline were distinguished via overlap with Vasa marking the germline. Manual masks were drawn to separate the following regions of the germarium: GSCN, 1, 2a, border region, 2b, and 3. The GSCN was considered separately from region 1. Manual corrections were applied for unclear or ambiguous Vasa staining.

Quantification of *Wolbachia* density: GSCN and SSCN tropism was assessed relative to *Wolbachia* density in the somatic cells of region 2b as a base level of *Wolbachia* in the soma. Region 2b was chosen based on overall consistent levels of *Wolbachia* across species and because differentiating between germline and soma based on Vasa staining is the most consistent in this region. Infection of the stem cell niche was considered tropism if the relative levels were increased by at least 1.5 fold.

Introgression crosses:

Introgression crosses were performed according to Fig. 2*B* and Fig. S5. Female flies with the *Wolbachia* strain of interest were backcrossed for 5 generations to males with the genetic background of interest. To confirm the introgression, the morphology of the male genital arch was observed, which is genetically controlled by approximately 40 loci scattered throughout the genome (13). The corresponding hybrid flies' genital arches matched the appropriate genetic background, as indicated by the blue arrows in Fig. 2*C*, demonstrating a successful introgression of most of the paternal genome into the F_5 hybrid.

FtsZ analysis

In dividing bacteria, FtsZ creates a ring structure during septation and is required through the final step of division. In non-dividing bacteria, FtsZ is not localized and is distributed throughout the bacterial cell (14). Thus, by quantifying the localization of FtsZ in each *Wolbachia* cell, the fraction of dividing *Wolbachia* can be determined (15, 16). For a precise measurement, it is important to determine the distribution of FtsZ within each individual *Wolbachia*. Therefore, it is very difficult in situations where the density of *Wolbachia* is high, so we conducted this experiment in the *Drosophila* species that has the lowest density of *Wolbachia* (*D. sechellia w*Sh).

Statistical analysis of data

To determine if the frequencies of niche targeting for each *Drosophila* species – *Wolbachia* strain pair were statistically significantly different (or not) for both SSCN tropism and GSCN tropism, values were transformed using arcsine transformation and Anova analyses were performed according to Hoffman *et al.*, 1998 (Figs. 1*M* and 1*N*) (17).

To measure the relative contribution of the host genetic background and the *Wolbachia* strain on the frequency of niche tropism pattern in Figs. 2*D*, 3*B*, and S7 logistic regression analysis was performed.

To analyze if changes in levels of *Wolbachia* in the germline related to SSCN tropism (Fig. S8*M*) is statistically significant, a T-test between *Wolbachia* density in regions 2a and 2b was performed for each species.

To assess significance of GSCN tropism towards *Wolbachia* levels in region 1 of the germarium a t-test was performed between the two *Drosophila*-*Wolbachia* pairs that had the closest fractions of *Wolbachia* in region 1 of the germline, but different niche tropism patterns (*D. simulans w*Ri and *D. yakuba w*Yak, Fig. S8*N*).

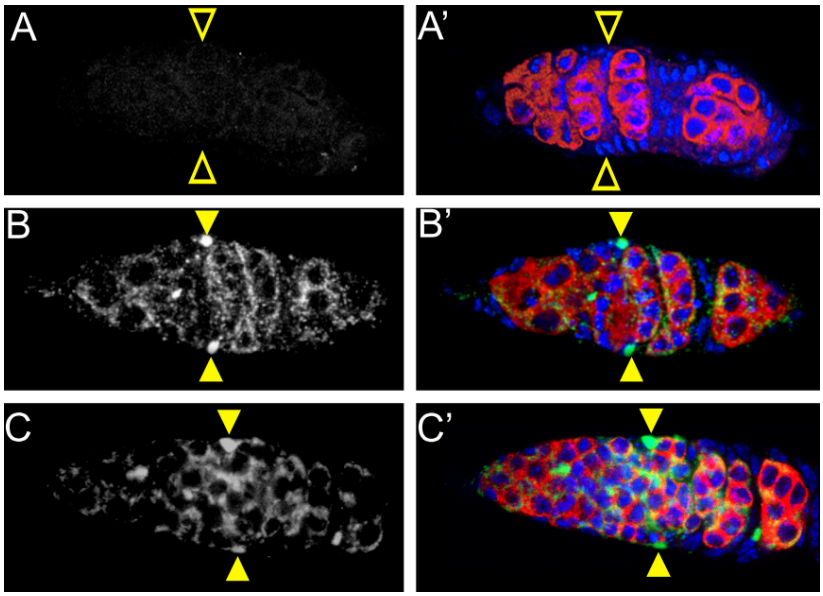

Fig. S1: *Wolbachia* antibody staining controls. A-C. Gray scale image of *Wolbachia* channel only. **A'-C'.** Overlay of all channels. Germline marker (Vasa) in red, DNA in blue and *Wolbachia* in green. **A.** Uninfected *D. sechellia* germaria showing *Wolbachia* antibody staining which gives very low background in the absence of bacteria. Empty yellow arrowheads point to SSCN with no *Wolbachia* staining. **B.** Antibody staining of *D. melanogaster* germaria infected with *w*Mel showing high levels of *Wolbachia* in the SSCNs (yellow arrowheads). **C.** *In situ* hybridization of infected *D. melanogaster* germaria with probe against *Wolbachia* 16S rRNA in green showing the same staining pattern as seen with the antibody.

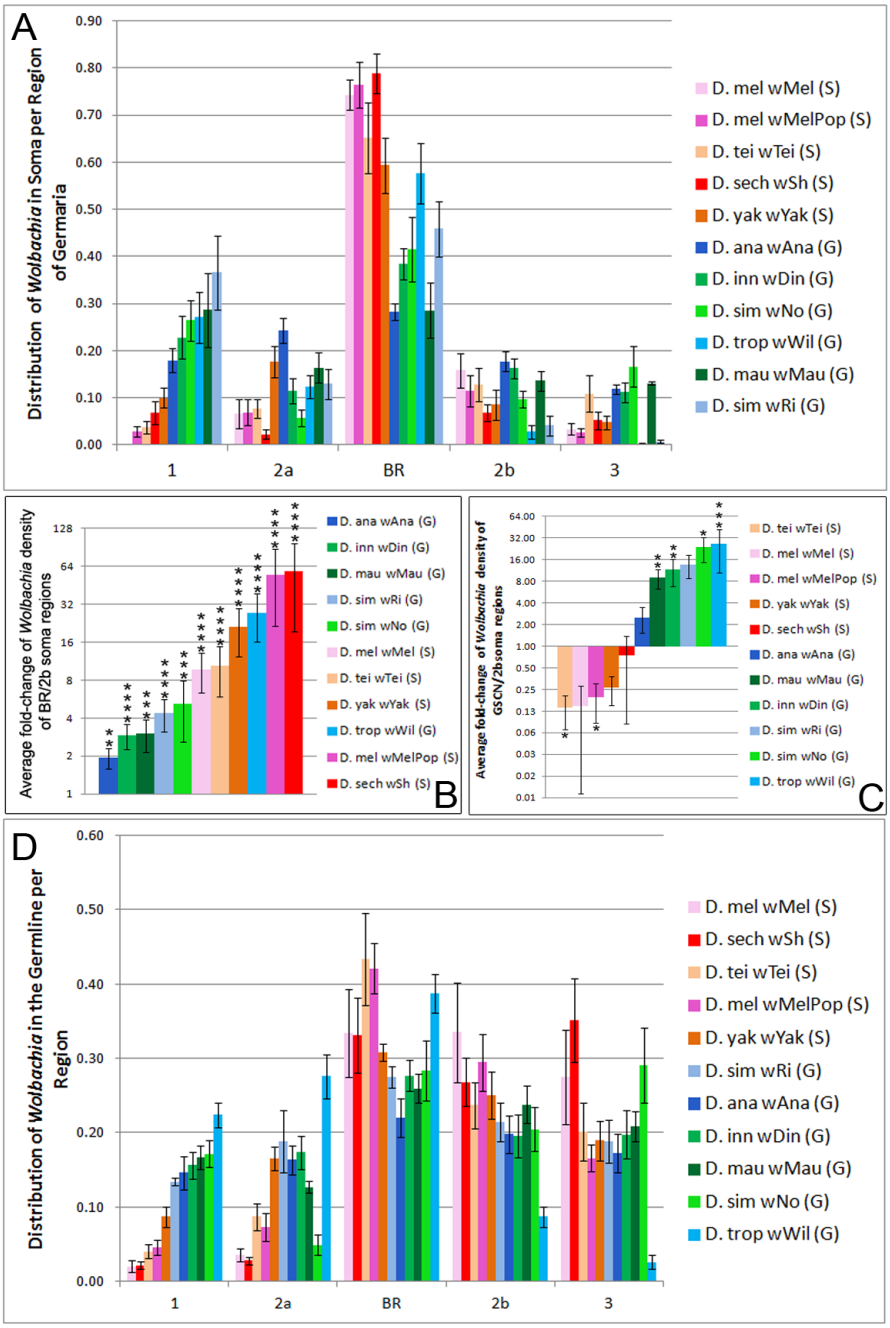

Fig. S2: *Wolbachia* distribution in somatic and germline regions of the germarium. Representative images for each *Drosophila-Wolbachia* were analyzed using MatLab to measure the *Wolbachia* pixel density in each of the regions of the germaria as defined in the materials and methods (N=10 for each species). Error bars represent SEM and *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. **A.** In every species analyzed, the fraction of *Wolbachia* in the soma is the highest in the border region, varying from 30% to 80% of total *Wolbachia* infecting somatic cells. **B.** *Wolbachia* density of the soma in the BR containing the SSCN is significantly higher than the adjacent somatic cells in region 2b. P-values represent that the differences in *Wolbachia* density between BR and 2b are statistically significantly different (T-test). **C.** *Wolbachia* density in the GSCN is statistically significantly higher in most species with GSCN tropism (T-test between GSCN and somatic region 2b). **D.** Density of *Wolbachia* infection in the germline per germarial region.

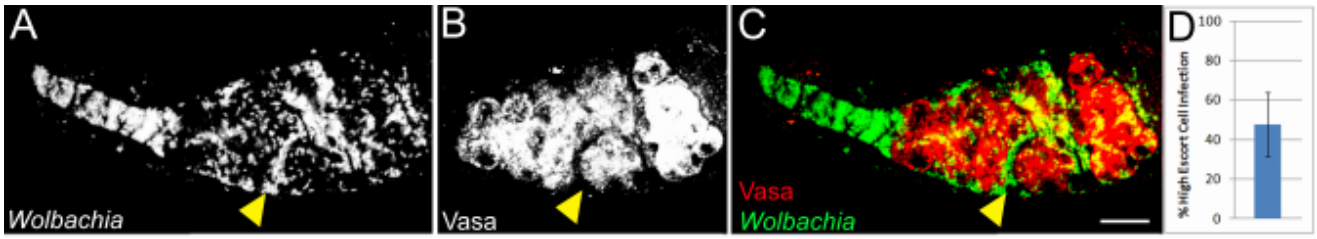

Fig. S3: *Wolbachia* target the escort cells in *Drosophila mauritiana*. Yellow arrowhead indicates *Wolbachia* highly targeting an escort cell. **A.** Grey scale image of *Wolbachia* channel only. **B.** Grey scale image of Vasa channel only. **C.** Merge, showing *Wolbachia* highly infecting an escort cell. **D.** Quantification of *Wolbachia* tropism to escort cells (N=22). Error bar represents SEM, scale bar 10μm.

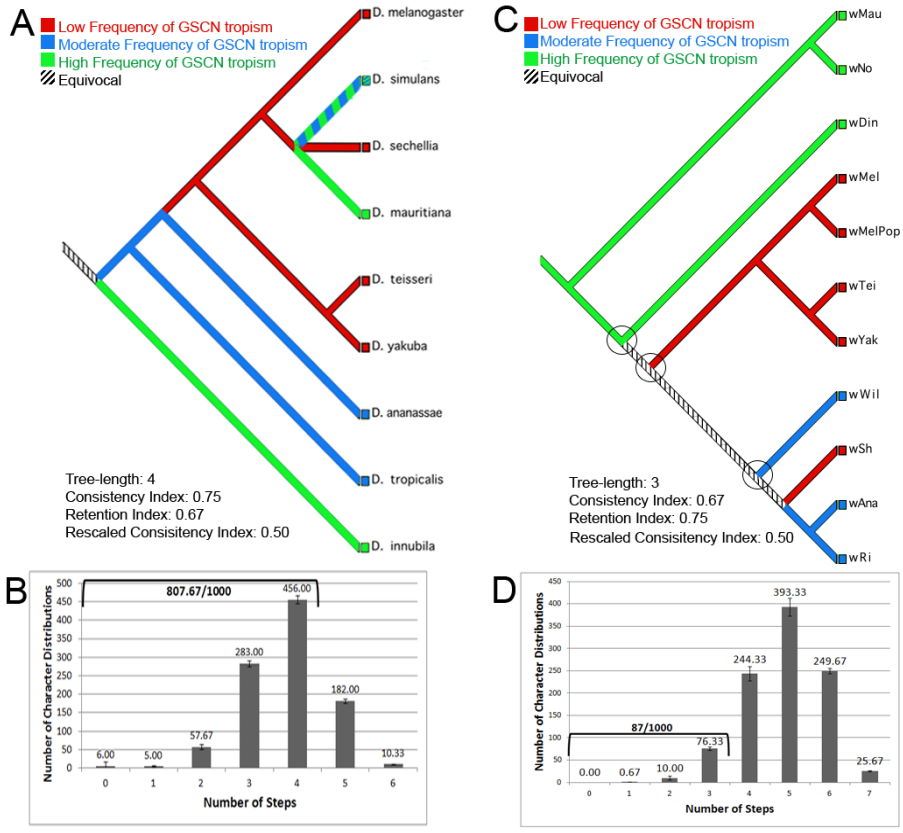

Fig. S4: Random fit distribution of niche tropism on *Wolbachia* and *Drosophila* phylogenies. GSCN tropism character is traced and character fit to the *Drosophila* and *Wolbachia* phylogenies using MacClade software (2). **A.** Stem cell niche tropism character fit to the *Drosophila* phylogeny. Phylogeny based on *alcohol dehydrogenase* gene (18). **B.** and **D.** A set of 1000 random characters was evolved to assess the probability of the GSCN tropism character fit to the phylogeny due to chance. The probability of a fit as good, or better than the true character was calculated for each phylogeny. **B.** There is an 80.7% probability that the GSCN tropism character distribution on the *Drosophila* phylogeny is due to random chance. **C.** Stem cell niche tropism character fit to *Wolbachia* phylogeny. Circles represent nodes with a maximum likelihood boot strap value of less than 50. *Wolbachia* phylogeny based on multilocus sequence typing (19). **D.** There is an 8.7% probability that the GSCN tropism character distribution on the *Wolbachia* phylogeny is due to random chance.

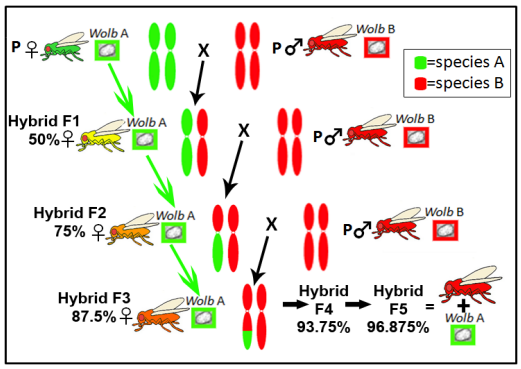

Fig. S5: Diagram of genetic introgression. Female flies of species A carrying the *Wolbachia* A are backcrossed to male flies of species B for 5 generations to introgress the species B genetic background into a fly carrying *Wolbachia* A.

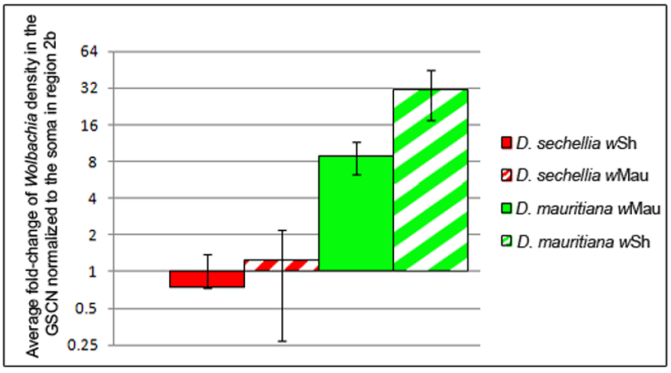

Fig. S6: *Wolbachia* density at the GSCN correlates with *Wolbachia* strain. Voxel density analysis shows that regardless of host genetic background, *Wolbachia* *w*Mau consistently densely infects the GSCN, as compared to *Wolbachia* *w*Sh. Measurements were acquired using MatLab software (N=10 for each). For each species the values were normalized to region 2b. Error bars represent SEM.

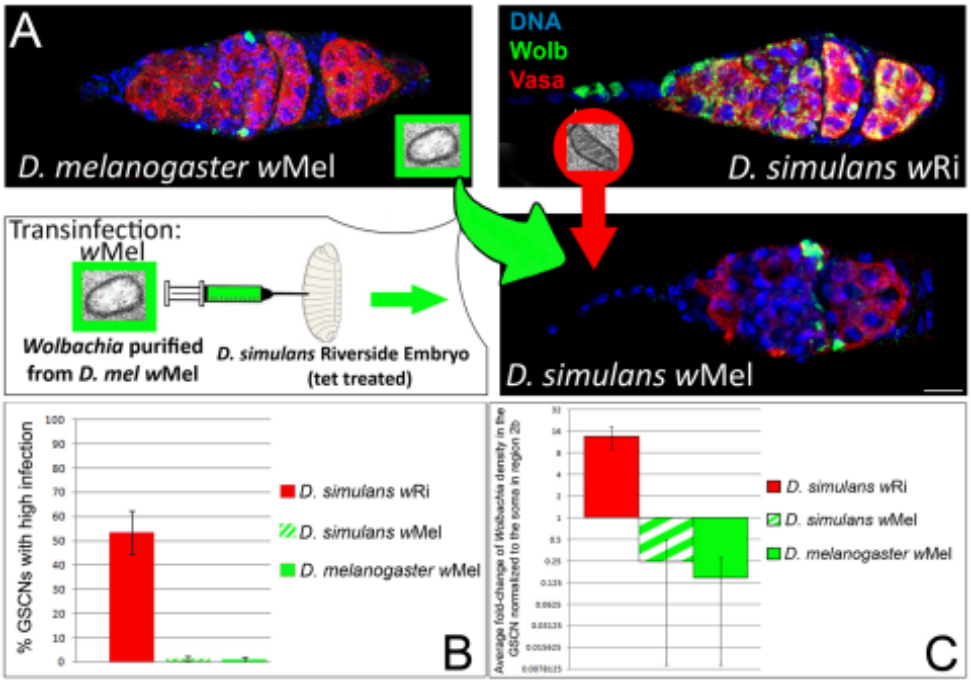

Fig. S7: Maternally inherited components do not influence GSCN tropism. A. Niche tropism of *w*Mel transinfected into *D. simulans* via embryonic microinjection confirms results from hybrid introgression crosses. Scale Bar 10 μm. **B.** *D. simulans* naturally infected with *w*Ri targets the GSCN at a higher frequency (N=99) than either *D. simulans* transinfected with *w*Mel (N=142) or *D. melanogaster* naturally infected with *w*Mel (N=104). *Wolbachia* strain significantly effects GSCN targeting (or lack of) as compared to host genetic background (Logistic regression, $p=6.7x10^{-7}$ and p=0.76, respectively). **C.** Voxel density analysis shows that regardless of host genetic background, *Wolbachia w*Mel does not densely infect the GSCN, as compared to *Wolbachia w*Ri. Measurements were acquired using MatLab software (N=10 for each). For each species the values were normalized to region 2b. Error bars represent SEM.

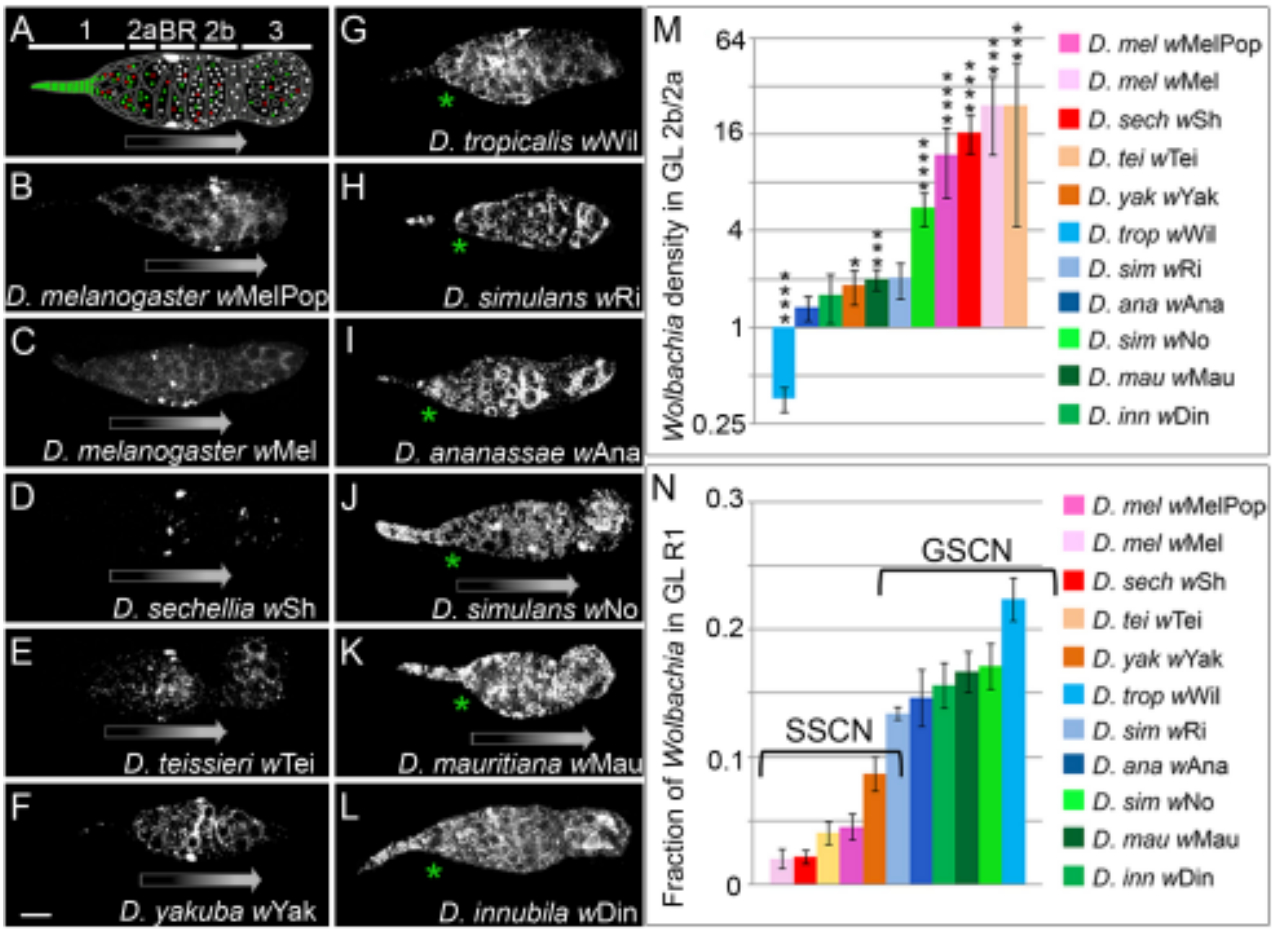

Fig. S8: *Wolbachia* distribution in the germarium of the various *Drosophila* species. A. Schematic of *Wolbachia* in the germarium. Green and white dots represent *Wolbachia* derived from the GSCN and SSCN, marked in green and white, respectively. Red dots represent *Wolbachia* naturally in the germline. **B-L.** In species with only SSCN tropism (no *Wolbachia* in the GSCN) there is a statistically significant increase of *Wolbachia* density from Region 2a to 2b (as well as in a few species with GSCN tropism; indicated by gradient arrow); quantified in **M** (*P<0.05, ***P<0.001, ****P<0.0001; T-test between region 2a and 2b for each sample). **G-L.** As compared to species with only SSCN tropism, there is a statistically higher fraction of *Wolbachia* in Region 1 in species with GSCN tropism (indicated by green asterisk); quantified in **N** (P=0.0043, T-test between *D. simulans w*Ri and *D. yakuba w*Yak). N=10 germaria each.

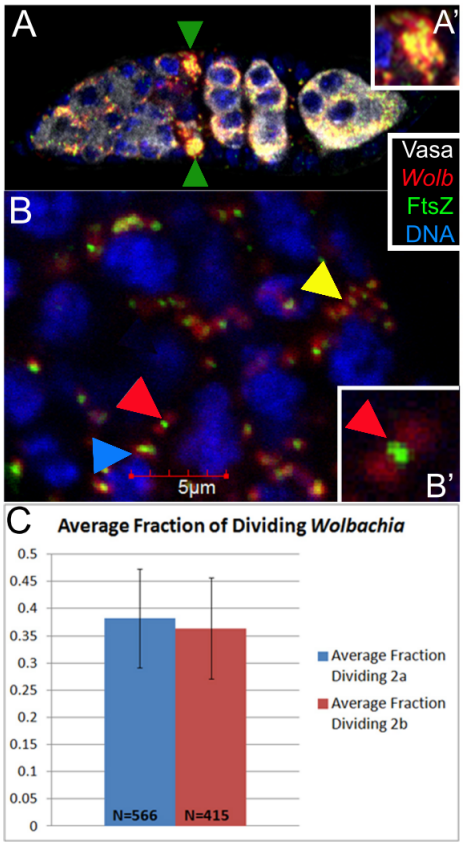

Fig. S9: *Wolbachia* division in the germaria. A. Representative image showing *Wolbachia w*Mel with an abundance FtsZ puncta in the SSCN of a *D. melanogaster* germarium similar to what is seen at the septum, suggesting that *Wolbachia* in the niche are dividing. Scale bar 10µm **B.** Representative confocal image with *Wolbachia* in red, FtsZ in green, and DNA in blue. *Wolbachia* is dividing if FtsZ is clearly localized to the center of the *Wolbachia* cell (red arrowhead, magnification **B'**). Non-dividing *Wolbachia* do not have FtsZ localized to the center (blue arrowhead). *Wolbachia* in clumps (yellow arrowhead) were not counted because it was not possible to determine the FtsZ localization. **C.** Quantification of the fraction of *Wolbachia w*Sh dividing in regions 2a and 2b of *D. sechellia* germaria (N=35 germaria from 7 ovaries). A total of 981 individual *Wolbachia* cells were counted, and the fraction of those *Wolbachia* that were dividing was calculated. There is no statistically significant difference in the fraction of *Wolbachia* dividing between regions 2a and 2b (P= 0.41, two-tailed t-test).

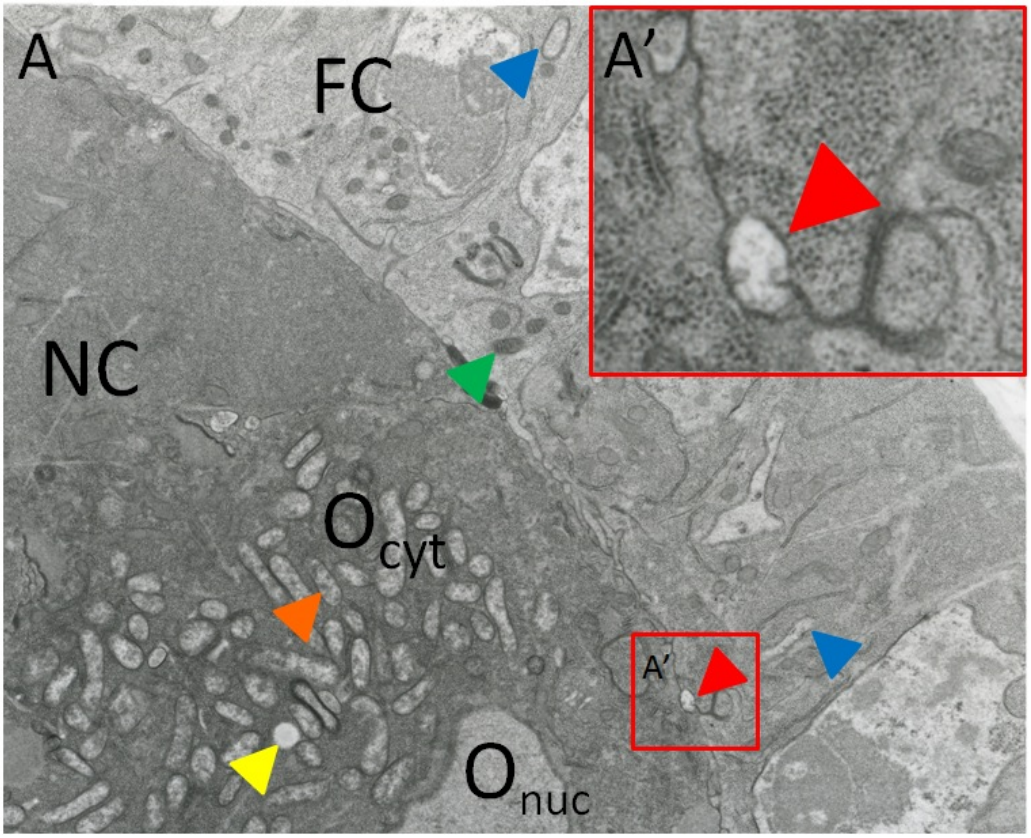

Fig. S10: Potential passage of *Wolbachia* from the follicle cells into the germline. **A.** Electron micrograph showing an early stage 8 egg chamber. *Wolbachia* (orange arrowhead) are present at high concentrations in the oocyte cytoplasm (O_{cyt}). *Wolbachia* also infect follicle cells (blue arrowheads). During vitellogenesis, there is endocytosis of yolk proteins and lipid droplets (yellow arrowhead) by the oocyte. A significant fraction of yolk proteins and lipid droplets enter the oocyte from the surrounding follicle cells (FC), suggesting that *Wolbachia* present in the FC may also be actively uptaken by the oocyte (red arrowhead). **A'.** Magnification of region outlined in red showing the *Wolbachia* found entering the oocyte from the apical side of the FC. Mitochondria are pointed for comparison (green arrows). NC, nurse cells; O_{cyt}, oocyte cytoplasm; O_{nuc}, oocyte nucleus; FC, follicle cells.

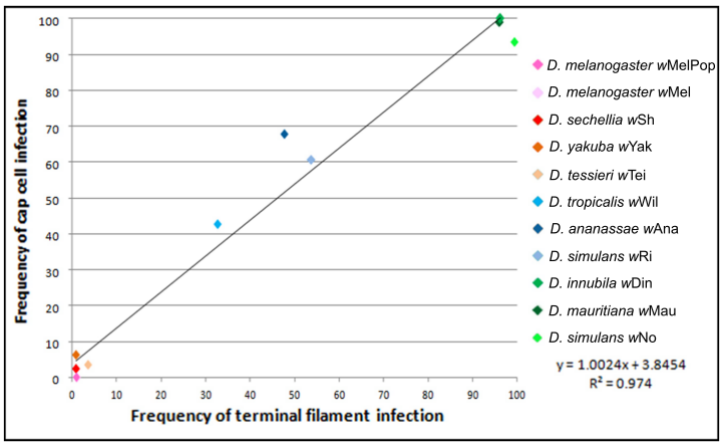

Fig. S11: Correlation of tropism to the cap cells and terminal filament. Germline stem cell niche tropism consists of tropism to two main cell types comprising the GSCN: the cap cells (CC) and the terminal filament (TF) cells. Infection of the CC vs. the TF cells is fairly similar, and has an R^2=0.97 ($P=6.6x10^{-9}$) (N≈100 germaria each, for details see Table S2).

Drosophila Species	*Wolbachia* Strain	Source	Stock Center #
D. melanogaster yw	*w*Mel	Frydman Lab	–
D. melanogaster yw	*w*Melpop	Sullivan Lab	–
D. simulans	*w*No	San Diego Stock Center	14021-0251.198
D. simulans	*w*Ri	San Diego Stock Center	14021-0251.169
D. sechellia	*w*Sh	San Diego Stock Center	14021-0248.08
D. mauritiana	*w*Mau	San Diego Stock Center	14021-0241.01
D. teissieri	*w*Tei	San Diego Stock Center	14021-0257.00
D. yakuba	*w*Yak	Virginie Orgogozo	–
D. tropicalis	*w*Wil	San Diego Stock Center	14030-0801.01
D. innubila	*w*Din	John Jaenike	–
D. ananassae	*w*Ana	Jack Werren/Michael Clark	–
D. mauritiana	***w*Sh**	Frydman Lab	–
D. sechellia	***w*Mau**	Frydman Lab	–
D. simulans	***w*Mel**	Kostas Bourtzis (via Bill Sullivan)	–
D. simulans	***w*Ri**	Frydman Lab	–
D. simulans	***w*No**	Frydman Lab	–

Table S1: Fly stocks and sources used for analysis. *Drosophila* species and their corresponding *Wolbachia* strains used for analysis are listed, along with their source and San Diego stock center number if applicable. **BOLD** indicates fly species with non-native *Wolbachia* strains introduced via hybrid crossing or embryonic microinjection.

***Drosophila* Species**	***Wolbachia* Strain**	**# Ovaries**	**Total # Germaria**	**% High GSCN ± SEM**	**% High SSCN ± SEM**
D. sechellia	*w*Sh	12	120	0.83 ± 0.83	89.17 ± 2.88
D. melanogaster	*w*Mel	10	104	0.96 ± 1.00	93.27 ± 2.60
D. melanogaster	*w*MelPop	11	110	0.91 ± 0.95	94.55 ± 2.07
D. yakuba	*w*Yak	10	103	0.97 ± 0.91	82.52 ± 4.91
D. teissieri	*w*Tei	11	110	3.64 ± 2.03	84.55 ± 4.12
D. tropicalis	*w*Wil	11	110	32.73 ± 7.02	97.27 ± 1.41
D. ananassae	*w*Ana	9	92	51.09 ± 5.27	94.57 ± 1.64
D. simulans	*w*Ri	10	99	53.54 ± 8.78	83.84 ± 4.99
D. mauritiana	*w*Mau	10	100	96.00 ± 3.22	99.00 ± 1.05
D. innubila	*w*Din	11	108	96.30 ± 1.56	99.07 ± 0.77
D. simulans	*w*No	14	138	99.28 ± 0.71	94.93 ± 2.55

Table S2: Frequency of *Wolbachia* stem cell niche tropism in diverse *Drosophila-Wolbachia* pairs. Tropism for the GSCN and BR of seven day old flies was assessed via visual quantification of confocal images. Approximately 10 germaria from each ovary (and one ovary from each fly) were analyzed and an average frequency of niches highly infected was calculated.

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Supporting Information

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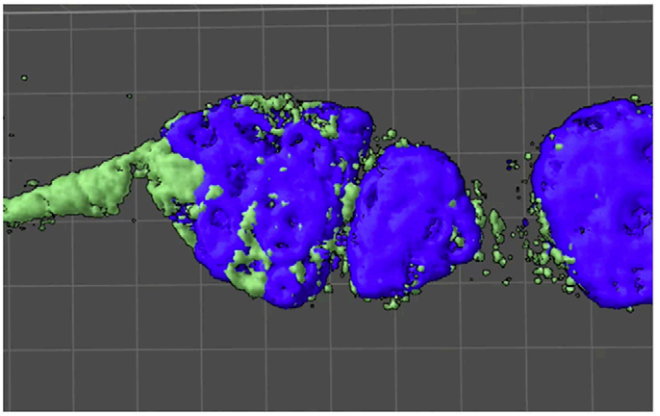

Movie S1. Rotating view of a 3D reconstruction of a *Drosophila mauritiana* germaria. Confocal Z sections are shown in "surface view" mode. In this mode, voxels that are more cortical obliterate signal originating from more internal voxels. Therefore, only the *Wolbachia* (shown in green) outside of the germ line is visible. The *Wolbachia* present in the germ line are masked by the signal of the germ-line marker Vasa (shown in blue). *Wolbachia* are visible at high levels in regions corresponding to the escort cells, with extensions protruding in between the early germ-line cysts, as well as the germ-line stem-cell niche.

Movie S1

Other Supporting Information Files

SI Appendix (PDF)