**Fig. S1.** The effect of media choice and temperature on *S. hermaphroditum* growth.(A) Approximate nematode life cycle progression on NGM, NGM supplemented with pyruvate, and lipid agar supplemented with cholesterol at 25°C. A small population of young juveniles (J1-J3) was transferred to lawns of their bacterial symbiont HGB2511 on Petri plates and estimated for life stage progression daily. The representative observations from three replicates are shown. (B) Estimated IJ recovery on NGM, NGM supplemented with pyruvate, and lipid agar. Approximately 10-20 individual IJs were transferred onto a Petri plate containing the indicated media with a lawn of HGB2511 and incubated at 25°C. IJ recovery was monitored daily and percent IJ recovery was calculated by scoring the number of recovered IJs out of the total number of recovered and non-recovered IJs. The averages and standard errors of three replicates are shown. (C) Estimated generation time at various temperatures. Animals were grown on lawns of their bacterial symbiont HGB2511 on NGM agar. Sterile indicates animals remained alive but failed to produce progeny; lethal indicates animals died early and without producing progeny. (D) Estimated fertility at various temperatures. Four young adults were transferred onto lawns of their bacterial symbiont HGB2511 grown on NGM agar and incubated at 20-22°C (RT); 25°C, 27.5°C; or 33°C. Gravid adults were identified by the presence of embryos in the gonads and percentage of gravid adults in the total population was monitored and calculated daily as an indication of fertility. The averages and standard errors of three replicates are shown.

**Figure S2:** Inbreeding of *S. hermaphroditum* wild isolate CS34 to generate a wild-type reference strain with a stable phenotype and a homozygous genetic background.Conventional IJs recoveredfrom infected insects were cultured *in vitro* (on lipid agar) to establish ten ancestral mating groups (Ancestral lines I-X). The ancestral line number is determined by the symbiont isolate number with which the line was co-cultured (Isolate 1-10, corresponding to the ancestral line with the same number, I to X). Five ancestral lines were chosen to further produce inbred lines (Ancestral group III, IV, VI, VII, and IX). In each generation, ten individual unmated hermaphrodites each were placed on a Petri plate containing NGM agar seeded with the corresponding bacterial isolate of *X. griffiniae*. The diagram shows the ten lines of ancestral mating groups, six lines inbred for five generations, and one line originated from Ancestral IX inbred for ten generations. The resulting line PS9179 was used for further study. It was grown on bacterial isolate 9, later renamed HGB2511.