

Caenorhabditis elegans and *Serratia marcescens* host-parasite coevolution model strains derived
through mutagenesis and experimental evolution

Samuel P. Slowinski¹✉ and Levi T. Morran²

✉Corresponding author sslowinski@une.edu

¹School of Biological Sciences, University of New England, Biddeford, ME 04005; ²Department
of Biology, Emory University, Atlanta, Georgia 30322

Key words: *Caenorhabditis elegans*, *Serratia marcescens*, host, parasite, coevolution,
experimental evolution

Abstract

Here we present a detailed description of the derivation of a collection of *Caenorhabditis elegans*
and *Serratia marcescens* strains and populations that have been mutagenized and passaged in a
series of experimental evolution projects over the past two decades. These experimental strains
and populations provide a powerful resource for the study of host-parasite coevolution and have
so far been used to demonstrate (among other things) that coevolutionary interactions with a
virulent parasite can select for biparental sex in host populations (Morran et al. 2011, Slowinski
et al. 2016, 2023); that local adaptation by parasites depends on host mating system (Morran et
al. 2014), and that gene flow can accelerate adaptation to parasites (Lewis et al. 2023). We hope
this paper clarifies the history and context of these strains and populations, and standardizes the
nomenclature used to describe them by researchers who use them in the future.

24

25 *Terminology*

26 In this paper we use the term “strain” to refer to a named, genetically defined and genetically
27 homogenous stable stock. We use the term “population” to refer to a genetically heterogeneous
28 group undergoing mutagenesis and/or experimental evolution.

29

30 *Derivation of the experimental nematode strains and populations*

31 Experimental nematode strains and populations were derived from the CB4856 strain, originally
32 from Hawaii, which was provided by the *Caenorhabditis* Genetics Center (University of
33 Minnesota, Minneapolis, MN). A strain, PX382, was derived by systematic inbreeding of strain
34 CB4856 (Morran et al. 2009). An obligately outcrossing strain called PX386 was derived from
35 the inbred strain by systematically backcrossing the mutant allele *fog-2(q71)* into the genetic
36 background of PX382 (Morran et al. 2009). The *fog-2(q71)* allele confers obligate outcrossing by
37 blocking the production of viable sperm in hermaphrodites, functionally transforming
38 hermaphrodites into females (Schedl and Kimble 1988). The *fog-2(q71)* mutation has no known
39 effect on males (Schedl and Kimble 1988). Over three consecutive generations, five near-
40 isogenic populations of PX386 were independently mutagenized with 40mM EMS for four hours
41 to create five genetically diverse obligately outcrossing populations, designated F1-F5, fixed for
42 the obligate-outcrossing allele *fog-2(q71)* (Morran et al. 2011). Five near-isogenic populations of
43 PX382 were also mutagenized (same protocol) to create five genetically diverse mixed-mating
44 populations, fixed for the mixed-mating allele *fog-2(wt)* (Morran et al. 2011). This procedure
45 induced approximately 1,000 point mutations per lineage in each population (Epstein and Shakes
46 1995, Morran et al. 2011). The genetically diverse mixed-mating and obligately outcrossing

nematode populations were passaged with the virulent bacterial pathogen *Serratia marcescens* (strain Sm2170) for 30 host generations on *Serratia* selection plates (SSPs) under three experimental evolution treatments (Morran et al. 2011). Briefly, nematode populations in the heat-killed parasite treatment were passaged on SSPs seeded with heat-killed (avirulent) Sm2170 bacteria. Nematode populations in the fixed parasite treatment were passaged on SSPs seeded with the virulent, but non-evolving Sm2170 stock (maintained in the freezer). Nematode populations in the copassaged treatment were copassaged with an evolving (and potentially coevolving) population of Sm2170, originally seeded from the same stock used in the fixed parasite treatment, but which evolved over the course of the study.

Slowinski et al. (2016) introgressed the mixed-mating allele from the PX382 strain (see above) into the genetic background of 12 obligately outcrossing populations, from the Morran et al. (2011) study, through a series of seven backcrosses. Four of these populations (designated “CF”) had been previously passaged in the heat-killed treatment (Morran et al. 2011). Four of these populations (designated “EF”) had been previously passaged in the fixed-parasite treatment (Morran et al. 2011). Four of these populations (designated “F”) had served as the ancestors in the (Morran et al. 2011) experimental evolution. This introgression created 12 mixed-mating populations (designated “WT” for wildtype), each representing a genetic background derived from one of the obligately outcrossing populations (Slowinski et al. 2016). Slowinski et al. (2023) combined nematodes from one of those obligately outcrossing populations (designated CF3, which had evolved under the heat-killed parasite treatment in the (Morran et al. 2011) study), with nematodes from its associated mixed-mating population WT CF3 (i.e. with nematodes bearing the mixed-mating allele and the CF3 genetic background), at a starting

frequency of 5% mixed-maters, to create a trioecious experimental population (i.e., composed of males, females, and hermaphrodites). The trioecious population was divided into four replicate populations, and each replicate was divided into three experimental treatments, which were passaged on *Serratia* selection plates (SSPs) for 24 host *C. elegans* generations (Slowinski et al. 2023).

Figure 1) A schematic showing the derivation of the nematode strains and populations

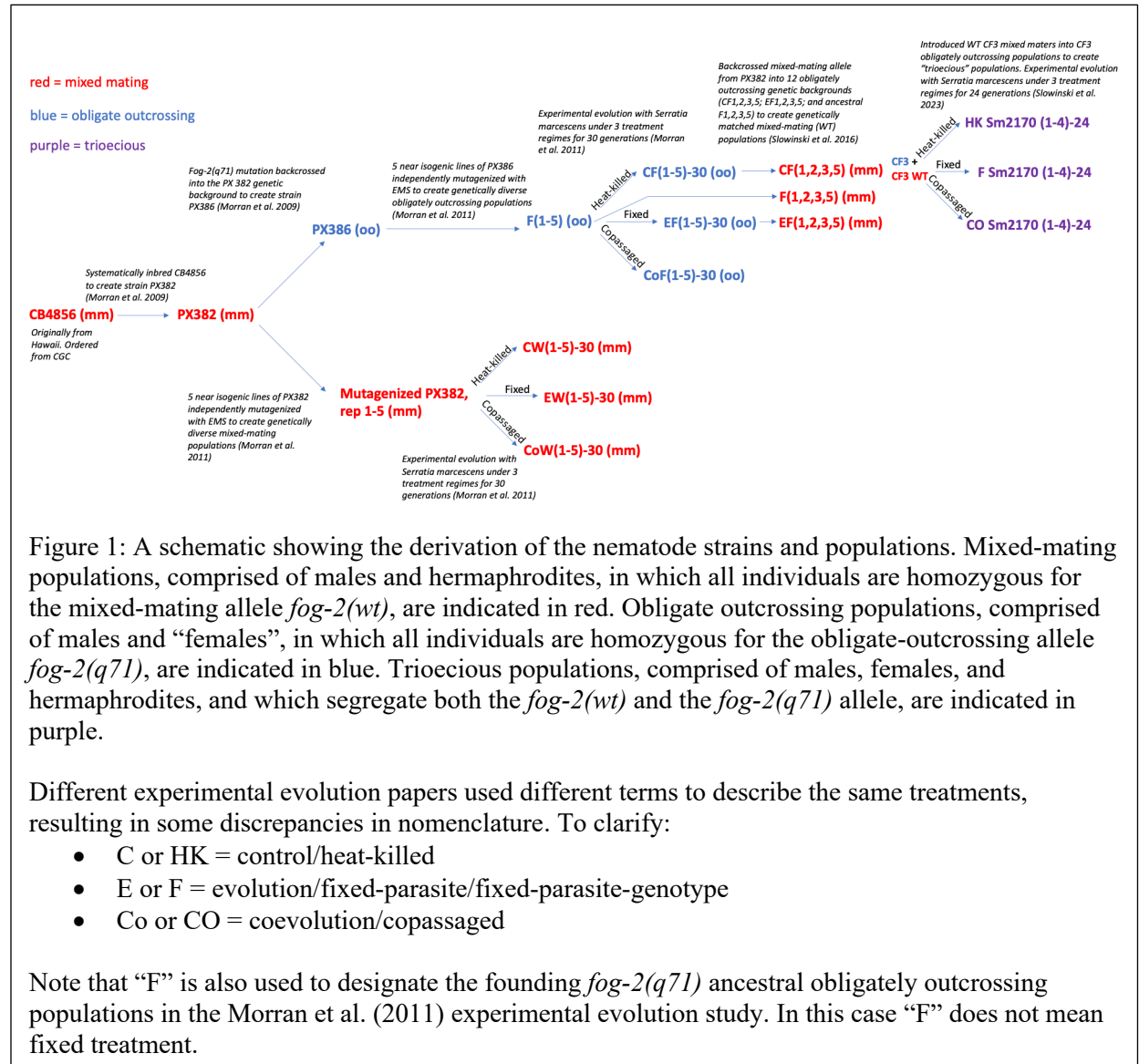


Figure 1: A schematic showing the derivation of the nematode strains and populations. Mixed-mating populations, comprised of males and hermaphrodites, in which all individuals are homozygous for the mixed-mating allele *fog-2(wt)*, are indicated in red. Obligate outcrossing populations, comprised of males and “females”, in which all individuals are homozygous for the obligate-outcrossing allele *fog-2(q71)*, are indicated in blue. Trioecious populations, comprised of males, females, and hermaphrodites, and which segregate both the *fog-2(wt)* and the *fog-2(q71)* allele, are indicated in purple.

Different experimental evolution papers used different terms to describe the same treatments, resulting in some discrepancies in nomenclature. To clarify:

- C or HK = control/heat-killed
- E or F = evolution/fixed-parasite/fixed-parasite-genotype
- Co or CO = coevolution/copassaged

Note that “F” is also used to designate the founding *fog-2(q71)* ancestral obligately outcrossing populations in the Morran et al. (2011) experimental evolution study. In this case “F” does not mean fixed treatment.

78 *Derivation of the Serratia marcescens experimental strains and populations*

79 For all experiments in which *C. elegans* hosts were copassaged with *Serratia marcescens*, the
80 evolved *S. marcescens* populations from all of the copassaged replicate lines were also saved
81 and frozen at the end of the experiment. Copassaged *S. marcescens* populations were generally
82 designated with names that matched the host population that they were copassaged with.

83
84 *Author note*

85 Portions of this manuscript were previously posted on bioRxiv as part of a preprint titled
86 'Outcrossing increases resistance against coevolving parasites'
87 (<https://doi.org/10.1101/2024.01.30.578011>). That preprint has since been divided into two
88 companion manuscripts: 1) the present paper, describing the derivation of the experimental *C.*
89 *elegans* and *S. marcescens* lines, 2) a separate manuscript (titled "Cross-fertilization confers
90 resistance against coevolving parasites", in preparation) presenting the associated empirical
91 results.

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