

# C. elegans srf-6 and nsy-1 mutations result in a similar 2AWC<sup>ON</sup> phenotype and do not complement (srf-6 is nsy-1 II)

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Genotype	% 2AWC ON (n)	% 1AWC ON (n)	% 0AWC ON (n)	Total number scored
Wild-type	1 (1)	99 (97)	0 (0)	98
srf-6(yj13)	92 (108)	5 (6)	3 (3)	117
nsy-1(ok593)	89 (110)	9 (10)	2 (3)	123
srf-6 (yj13) / nsy-1 (ok593)	82 (50)	11 (7)	7 (4)	61
+ / nsy-1 (ok593)	0 (0)	100 (33)	0 (0)	33

**Figure 1**. Comparison of AWC phenotypes of srf-6(yj13) and nsy-1(ok593) mutants, and complementation testing of srf-6(yj13) and nsy-1(ok593) mutants using the 2AWC<sup>ON</sup> phenotype. The nsy-1(ok593) mutation is a large complex rearrangement that completely deletes the catalytic domain of nsy-1 (C. elegans Deletion Mutant Consortium 2012). Homozygous srf-6 and nsy-1 mutants also contained homozygous unc-4 (e120) II and kyls140 I. Table entries represent percentages of worms with each phenotype, followed by the actual number of worms scored in parentheses. The right-hand column indicates the total number of worms scored per genotype.

# **Description**

C. elegans srf-6 mutants were isolated using altered surface immunofluorescence as phenotype (Hemmer et al., 1991; Grenache et al., 1996). In Van Sciver et al., 2019, we showed by whole genome sequencing that three different srf-6 mutants carry mutations in gene nsy-1. Well-characterized mutant alleles of nsy-1 result in hermaphrodites that express the olfactory receptor gene str-2 in both AWC neurons (2AWC<sup>ON</sup>, Troemel et al., 1999), unlike wild type hermaphrodites, which express str-2 asymmetrically in only one of the two AWC neurons (1AWC<sup>ON</sup>, Troemel et al., 1999). Our sequencing results suggested that srf-6 mutants might have a similar 2AWC<sup>ON</sup> phenotype. We therefore examined srf-6(yi13) for its AWC phenotype.

In order to introduce an str-2::GFP marker into the srf-6 genotype, first srf-6(yj13) unc-4(e120) II and nsy-1(ok593) unc-4(e120) II double mutants were constructed as described (Hemmer et al., 1991). Homozygous males carrying the construct kyIs140, which contains an str-2::GFP fusion integrated on chromosome I (Troemel et al., 1999), were mated with srf-6(yj13) unc-4(e120) II hermaphrodites, and an Unc F2 hermaphrodite expressing GFP in chemosensory neuron AWC was cloned. An individual hermaphrodite descendant, all of whose offspring expressed GFP, was isolated to establish a strain of genotype kyIs140 I; srf-6(yj13) unc-4(e120) II. A strain of genotype kyIs140 I; nsy-1(ok593) unc-4(e120) was constructed similarly. Adult hermaphrodites from these strains were examined in a fluorescent microscope for their AWC phenotype. Figure 1 shows that srf-6(yj13) adults exhibited a  $2AWC^{ON}$  phenotype similar to that of nsy-1(ok593).

To test whether srf-6(yj13) and nsy-1(ok593) affect the same or different genetic functions, a complementation test was performed (Figure 1, last two lines). Males of genotype srf-6(yj13) were mated with  $kyls140\ I$ ; nsy-1(ok593) unc- $4(e120)\ II$  hermaphrodites, and non-Unc offspring were scored in a fluorescent microscope for the AWC phenotype. The complementation heterozygotes showed a  $2AWC^{ON}$  phenotype similar to that of nsy-1(ok593). This result included data from two separate crosses. In contrast, when wild type males were mated with  $kyls140\ I$ ; nsy- $1(ok593)\ unc$ - $4(e120)\ II$  hermaphrodites, the  $1AWC^{ON}$  phenotype was observed. These results included worms from one cross.



These results indicate that srf-6(yj13) and nsy-1(ok593) mutations do not complement each other for the 2AWC<sup>ON</sup> phenotype, and together with the srf-6 mutant sequencing results (Van Sciver et al, 2019), we conclude that srf-6 and nsy-1 are the same gene.

### Reagents

C. elegans Strains

N2 C. elegans wild type

CX3695 kyIs140 [str-2::GFP + lin-15(+)] I

AT18 srf-6(yj13) II

AT19 srf-6(yj13) unc-4(e120) II

VC390 nsy-1(ok593) II

AT28: srf-6(yj13) unc-4(e120) II; kyIs140 [str-2::GFP + lin-15(+)] I

AT29: nsy-1(ok593) unc-4(e120) II

AT30: nsy-1(ok593) unc-4(e120) II; kyIs140 [str-2::GFP + lin-15(+)] I

Strains N2, CX3695, and VC390 are available from the CGC. Strains AT28 and AT30 will be submitted to the CGC.

### References

C. elegans Deletion Mutant Consortium (69 authors) (2012). Large-scale screening for targeted knockouts in the Caenorhabditis elegans genome. 2: 1415-25. PMID: 23173093.

Grenache DG, Caldicott I, Albert PS, Riddle DL, and Politz SM. (1996). Environmental induction and genetic control of surface antigen switching in the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci USA*. 93: 12388-93. PMID: 8901591.

Hemmer RM, Donkin SG, Chin KJ, Grenache DG, Bhatt H, and Politz SM. (1991). Altered expression of an L1-specific, O-linked cuticle surface glycoprotein in mutants of the nematode *Caenorhabditis elegans*. *J Cell Biol*. 115: 1237-1247. PMID: 1955471.

Olsen DP, Phu D, Libby LJ, Cormier JA, Montez KM, Ryder EF, Politz SM. (2007). Chemosensory control of surface antigen switching in the nematode *Caenorhabditis elegans*. *Genes Brain Behav*. 6: 240-52. PMID: 11287957.

Troemel, ER, Sagasti, A, and Bargmann, CI. (1999). Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in *C. elegans*. *Cell* 99: 387-398. PMID: 10571181.

Van Sciver, ND, Pulkowski, JO, and Politz, SM. 2019. Three C. elegans srf-6 mutants carry nsy-1 mutations (srf-6 is nsy-1 I). microPublication Biology. 10.17912/micropub.biology.000127.

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