**Developmental Cell *15***

# Supplemental Data

# Plasticity and Errors of a Robust Developmental System in Different Environments

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## 1. SUPPLEMENTAL MATERIALS AND METHODS

### 1.1 Strains and General Experimental Procedures

For experimental procedures, worm breeding, genetics and materials, we followed the commonly used protocols in *C. elegans* research (Brenner, 1974; Hope, 1999; Wood, 1988). Strains were obtained from the CGC, several laboratories and from our own collections or crosses. The reference strain N2 used in all experiments (except for the analysis of mutation accumulation lines) was obtained from the CGC ("N2 ancestral"). From this stock, a single individual was used to initiate a population, which was allowed to expand and was kept at high population density for 10-15 generations. Samples from this population (kindly provided by H. Teotonio, Lisbon) were then frozen until use in experiments. The same method was used for additional *C. elegans* (AB1, CB4856, JU258, PB303, PB306, PS2025) and *C. briggsae* (AF16, HK104, JU439) isolates. Both species reproduce mostly by selfing and the strains are isogenic. For information on geographic origin and more details on these strains, see www.wormbase.org. We also established frozen stocks of *C. elegans* mutants and transgenic (reporter) strains. Only recently thawed worms were used for all experiments (< 6 weeks maintenance at 20°C until experiment). Prior to each experiment (i.e. experimental block), a single individual was isolated (from freshly thawed worm stock kept at -80°C) to initiate the experimental population, which was kept under standard conditions for 4-6 generations prior to the experiment. All individuals used for a given experiment were derived from identical maternal and grandmaternal environmental conditions. Experimental individuals were derived from the same parental populations and randomly allocated to the different environments. For a given environmental condition, either a single or several replicates were established. For a detailed description of the design of different experiments, see Supplemental Data, sections 1.3 to 1.9.

### 1.2 Experimental Environments

Our principal objective was to study the effects of ecologically relevant environmental stimuli – i.e. stimuli likely to occur in the natural habitat of *Caenorhabditis* worms – on vulval cell fate patterning. Although little is known about *Caenorhabditis* ecology, there are a number of environmental conditions that vary within the natural habitat and among locations from which different *Caenorhabditis* isolates have been sampled (Braendle et al., 2008). While our experimental environments remain highly artificial and simple in structure, they encompass a number of stimuli that occur in the natural habitat. Our experimental environments are therefore likely to mimic relevant ecological conditions. We selected six environmental conditions for detailed experimental analysis (Figure S1):

*Experimental environments 1-3: NGM plates at 15°C, 20°C, 25°C.* Temperature is an apparent variable of the *Caenorhabditis* environment, both within and among different locations where these nematodes have been found. We selected different temperatures that are within the range of temperatures found in natural habitats of all tested isolates.

Methods: Standard protocol, 55 mm Petri dishes, NGM, seeded with *E. coli* OP50 (Hope, 1999). Main characteristics: decreased developmental time with increase in temperature; NGM plates at 20°C represent the reference environment (standard *C. elegans* laboratory environment).

*Experimental environment 4: Liquid culture at 20°C*: We chose liquid medium as an experimental environment because worms were frequently found in decomposing, very moist vegetal matter (Barrière and Félix, 2005; Barrière and Félix, 2006). Worms therefore likely experience variable substrate conditions, including changes between solid and liquid substrates. In the laboratory, worms can be maintained in liquid media. When developing in liquid, *C. elegans* shows stereotyped physiological (Suo et al., 2006) and behavioural (Mullen et al., 2007) differences compared to development on solid NGM plates. Methods: M9 solution (Hope, 1999) (50 µl) containing 5 µl of 4 % (w/v) *E. coli* OP50 solution in a 1.5 ml Eppendorf tube. Main characteristics: Change in overall phenotype (thin, small worms), slight increase in developmental time relative to NGM plates at 20°C, likely causing hypoxic conditions and inducing stress responses (Hong et al., 2004; Jewitt et al., 1999; Suo et al., 2006).

*Experimental environment 5: Starvation (agar plates) at 20°C:* The natural environment of *C. elegans* and other *Caenorhabditis* species is highly ephemeral in food sources (Barrière and Félix, 2005; Barrière and Félix, 2007). Starvation is consequently a likely naturally occurring environmental factor. This scenario is further supported by the observation that most isolated *C. elegans* individuals were found as dauer larvae (Barrière and Félix, 2005), a long-lived and stress-resistant alternative developmental stage, which is induced by starvation and high population density (see also below) (Cassada and Russell, 1975; Golden and Riddle, 1984). Non-dauer individuals that were freshly isolated from natural habitats further showed signs of malnourishment probably due to starvation or infection by pathogens (Barrière and Félix, 2005; M.-A.F, unpublished data). We therefore defined a starvation environment in which individuals were deprived of food in the L1/L2 stage without causing them to enter the dauer stage. Methods: NGM plates containing 50 µl/ml ampicillin without *E. coli* OP50 (the antibiotic was added to prevent growth of bacteria attached to the animals). Animals at the late L1 to lethargus L1/L2 stage were transferred to these plates in a small drop of M9 solution (5-25 µl). After 48 hours, animals were transferred (collection in M9 and centrifugation) to regular NGM plates seeded with *E. coli* OP50. In the starvation environment, overall development was drastically slowed down: at the end of the starvation treatment, most individuals were in the mid to late L2 stages, rarely in lethargus L2. After transfer to food, individuals reached the L4 stage after approximately 15-20 hours. Main characteristics: Change in overall phenotype (small), induction of stress responses (Jewitt et al., 1999; Suo et al., 2006), drastic increase in developmental time relative to NGM plates at 20°C.

*Experimental environment 6: Dauer-inducing pheromone plates at 25°C:* In this environment we used conditions to induce the formation of the dauer larva. The frequent observation of this developmental stage in natural populations (Barrière and Félix, 2005) is an indicator that dauer-inducing conditions occur frequently in the natural habitat. Methods: Dauer-inducing plates were made using pheromone extracted from liquid worm cultures as previously described (Golden and Riddle, 1984). Petri dishes were 35 mm in diameter. The antibiotic streptomycine (50 µl/ml) was added to NGM medium lacking bacto-peptone. Extracted dauer pheromone was applied to the agar surface using an amount causing > 50% of N2 individuals to adopt the dauer stage (usually around 20-60 µl per plate). 24-48 hours later, plates were inoculated with 5-10 µl of a 4% stock solution of OP50. Approximately 50 adults were allowed to lay eggs for 4-6 hours at the same time as adults on NGM plates 20°C. Individuals were then transferred to a 25°C incubator. After three days, a proportion of the offspring had developed into dauer larvae (recognized by morphological criteria) and non-dauer individuals were removed from the plates. 10-20 presumptive dauer individuals

were collected and their survival in 1% SDS medium examined (dauer but not any of the non-dauer stages survive such an SDS treatment (Wood, 1988)). If at least 5 presumptive dauer individuals survived 15 min of SDS exposure, we considered dauer induction to have been successful (tested individuals were excluded from further analyses). After an additional three days (i.e. 5 days after egg laying), individuals in the dauer stage were transferred to standard 55 mm NGM plates seeded with OP50 (at 20°C). After transfer, individuals reached the L4 stage within approximately 10-20 hours. Main characteristics: The dauer represents an alternative L3 developmental stage resistant to stressful conditions (Golden and Riddle, 1984), change in overall phenotype (thin, dark, small), drastic increase in developmental time relative to NGM plates at 20°C.

*Experimental procedures:* We used the *E. coli* strain OP50 as food source in all experimental environments. Although *E. coli* is very unlikely to represent a natural food source for *Caenorhabditis*, we decided to use OP50 because the natural food sources of *Caenorhabditis* worms still remain unknown. All individuals examined in a given experiment/block were grown using the same materials (NGM stock solutions, bacterial solutions, etc.). Several days after pouring, NGM plates were inoculated with 25-50 µl of *Escherichia coli* OP50 culture (grown from a single bacterial colony overnight at 37°C). After 3 days at room temperature, plates were transferred to a 4°C chamber until the start of experiments (maximum storage of 14 days).

For a given experiment/block a fresh worm stock (kept at -80°C) was thawed. A single individual from this population was then used to initiate an isogenic experimental population. Adults of this population were then hypochlorite treated (Hope, 1999) to decontaminate the cultures. After 5 generations, approximately 10-30 young adults were allowed to lay eggs for 4-6 hours on NGM and dauer-inducing plates at 20°C, after which adults were removed. For each environment, several replicates (Petri dishes) were usually established. Animals were then transferred to different incubators at 15°C, 20°C and 25°C. For liquid and starvation environments (20°C), animals were transferred to their respective environments only when they had reached the late L1 stage to lethargus L1/L2 (after approximately 24 hours, e.g. for the isolate N2). Animals were handled using M9 solution and a micropipetter and were collected using brief (30 s) centrifugation at 6,000 rpm in 1.5 ml Eppendorf tubes. (For slight modifications of these procedures, see individual sections of experiments outlined in Text S1.).

### 1.3 Precision and Deviations of Vulval Cell Fate Patterns in Different Environments (Isolate N2) (Figure 2)

*Methods:* Experimental procedures were followed as described in the Materials and Methods section. For a given experimental block, we examined several replicates per environment and approximately 20-60 animals per replicate. We performed a total of nine experimental blocks and scored a total of 1,000 individuals for their vulval cell fate pattern in each of the six environments.

*Statistical Analysis:* To test whether the proportion of developmental deviations different from the canonical wild type vulval pattern varied among different environments we used a generalized linear model (GLM) assuming a binomial response variable and a logit link function (JMP 6.0). Effects included in the model were *block*, *environment* and the *block* by *environment* interaction. Tested dependent variables included the number of developmental deviations (per number of individuals of the replicate). An analogous analysis was carried out to test for environmental effects on P3.p division frequency.

### 1.4 Environmental Effects on Vulval Mutant Phenotypes (Figures 2, S3 to S8 and Tables S1 and S2)

*Methods*: We selected 41 previously isolated mutant strains (covering 31 genes) defective for components of different signalling processes involved in vulval cell fate patterning

(Egf, Ras, Notch, Wnt, SynMuv, downstream effectors). All mutant strains had been derived from the reference wild isolate N2. More detailed information on these mutations is provided in Table S1. We followed the basic experimental procedures outlined in the Materials and Methods section. However, as many vulval mutants exhibit impaired egg laying, we hypochlorite treated (Hope, 1999) experimental animals and randomly allocated the released eggs to individual NGM and dauer-inducing plates. The plates were kept at 20°C for the next 24 hours and animals were then transferred to different incubators at 15°C, 20°C and 25°C. For liquid and starvation environments (20°C), animals were transferred to their respective environments only when the majority of individuals had reached the late L1 to lethargus L1/L2 stage. Individuals of each mutant strain were assayed in the six different environmental conditions in parallel and the vulval phenotype (including the fate of cells P3.p to P8.p) was scored in the L4 stage. For each environment, several replicates (usually 2-3) were established.
*Statistical analysis:* We performed Two-Way-ANOVAs testing for the fixed effect of *environment* and the random effect of *replicate* (nested within *environment*) on the number of induced vulval cells (JMP 6.0).

**1.5 Quantification of Pathway Activities Using Transcriptional Reporters (Figures 4 and S9 to S11)**
*Methods:* We used previously generated transgenic strains (derived from N2) containing transcriptional reporter constructs for the LET-60/Ras pathway (*egl-17::cfp-lacZ*, strain JU480) (Yoo et al., 2004), LIN-12/Notch pathway (*lip-1::gfp*, strain AH142) (Berset et al., 2001) and the downstream effector LIN-39 (responsive to both Ras and Wnt signals) (*lin-39::gfp*) (Wagmaister et al., 2006) (Figures 4 and S9 to S11). The *egl-17::cfp-lacZ* strain JU480 was derived from the strain GS3582 by genetically removing the transformation marker *unc-4(e120)*. The *egl-17::cfp-lacZ* and *lip-1::gfp* constructs contain nuclear localization sequences upstream of the XFP coding sequence. The transformation construct of the *lin-39::gfp* strain also contains *ajm-1::gfp*, a cell junction marker (Wagmaister et al., 2006). The *egl-17::cfp-lacZ* and *lip-1::gfp* strains show nuclear localization and the *lin-39::gfp* strain shows cytoplasmic localization.

We followed the basic experimental procedures outlined in the Materials and Methods section; however, this experiment was performed in only two environments: standard (food) NGM plates at 20°C and starvation environment at 20°C. To minimize age variation among experimental animals, we synchronized experimental populations at the beginning of the experiment. Young adults were hypochlorite treated and the released eggs were placed on NGM plates without food, so that individuals would hatch but not develop further due to lack of food (Hope, 1999). After approximately 15 hours, age-synchronized animals (early L1 stage) were transferred to NGM plates with OP50. When individuals had reached the late L1 to lethargus L1/L2 stage, they were randomly allocated to either starvation or standard (food) environment. Individuals from the food environment were examined during the proceeding 10-20 hours from mid L2 to early L3. Individuals from the starvation environment were transferred to NGM plates containing food after the 48-hour starvation period. After 6-12 hours development on these plates, individuals (mostly in the mid to late L2 stage) were examined until the early L3 stage.

Developmental stages (mid L2, lethargus L2/L3, early L3, i.e. L3 prior to first division of precursor cells) were identified using several morphological criteria, in particular gonad development (Kimble and Hirsh, 1979; Sulston and Horvitz, 1977). For each experiment, single-plane digital images were taken under identical exposure using a Zeiss AxioImager microscope equipped with a camera (Coolsnap ES, Princeton Instruments). The focal plane was chosen in such way that the nuclei of relevant Pn.p cells were in focus under Nomarski optics. Image analysis was performed using the software Metaview 6.3r7 (Visitron Systems GmbH, Puchheim, Germany).

For each individual/image, we quantified signal (pixel) intensity of P5.p, P6.p and P7.p. Because heterogeneity in signal background caused difficulties in determining the outline of nuclei/cells with low signal intensity (i.e. difficulties in using the thresholding tool based on differences in pixel intensity), we manually selected a subregion of fixed size within nuclei (*egl-17::cfp-lacZ* and *lip-1::gfp*) or cytoplasm (*lin-39::gfp*) of P5.p, P6.p, and P7.p using the ellipse tool. For each image, we also measured the signal intensity of the image background. After background subtraction, we used the mean signal intensity of this region as a measure of expression level in the corresponding cell. For the above measures, we ignored nucleus/cell size because there was no effect of the environment on nucleus size: Using a partial data set (*egl-17::cfp-lacZ* strain, cell P6.p only) where we could determine the nucleus outline using the thresholding tool, we tested for the effects of *environment* and *developmental stage* (and their interaction) on nucleus size (measured as nucleus area). Nucleus size was not affected by the environment at any developmental stage (2-Way ANOVA, main effect *environment*: $F_{1,110}$=0.24, P=0.6254, interaction effect *environment* by *developmental stage*: $F_{2,110}$=0.40, P=0.6735). Nucleus size was increased in the early L3 stage relative to the earlier stages (2-Way ANOVA, main effect *developmental stage*: $F_{2,110}$=45.15, P<0.0001; post-hoc comparisons: eL3 versus mid L2 and lethargus L2/L3, Tukey's HSD, P<0.05); however, this difference was accounted for in our statistical analyses (see below). In addition, the same partial data set (*egl-17::cfp-lacZ* strain, cell P6.p only) showed that our measures using the mean signal intensity of a subregion are very strong correlates of measures using the integrated signal intensity of the total nucleus size (Pearson Correlation on log-transformed data, R=0.94, P<0.0001, N=112). Statistical analyses (see below) using either of these measures resulted in identical outcomes (*egl-17::cfp-lacZ* strain, cell P6.p only). We therefore concluded that mean signal intensity as measured by a subregion is a reliable estimate of the total signal intensity of a given nucleus/cell.

*Statistical analysis*: For each examined developmental stage, we carried out an ANOVA (JMP 6.0) testing for the fixed effects of *environment*, *individual* (nested in *environment*), *cell*, and the interaction between *environment* and *cell type* using mean signal intensity as a response variable. The inclusion of the effect *individual(environment)* allowed us to control for the non-independence between measures of P5.p, P6.p, and P7.p taken from a single individual. Post-hoc tests (Tukey's HSD) were then performed for the *environment-by-cell type* interaction effect to determine differences in signal expression between environments (standard "food", starvation) and cells (P5.p. P6.p, P7.p).

### 1.6 Precision and Deviations of Vulval Cell Fate Patterns in Different Environments (Isolate JU258) (Figure S12)

*Methods:* We followed the basic experimental procedures outlined in the Materials and Methods section. For a given experimental block, we examined 1-5 replicates and approximately 20-60 animals per replicate. We carried out six experimental blocks and scored a total of 500 individuals for their vulval cell fate pattern in each of the six environments.

*Statistical analysis*: We performed GLM analyses as for the N2 isolate described above in section 1.3.

### 1.7 Precision and Deviations of Vulval Cell Fate Patterns of *C. elegans* And *C. briggsae* Isolates in Standard versus Starvation Environment (Figures 5A, 5B and S13)

*Methods*: We followed the basic experimental procedures outlined in the Supplemental data section 1.3. However, this experiment was performed in only two environments: standard NGM plates at 20°C and starvation environment at 20°C. For each of three experimental blocks, samples of the six examined wild isolates (*C. elegans* CB4856, JU258, N2 and *C. briggsae* AF16, HK104, JU439) were thawed at the same time point and then kept under

identical conditions for 4-6 generations until the start of the experiment. To initiate experimental populations, 50-100 young adults were picked to lay eggs for approximately 4-6 hours (for all isolates in parallel). The number of adults was adjusted according to the isolate to obtain approximately 1,000 eggs per plate. When animals had reached the late L1 stage to lethargus L1/L2 (approximately 24 hours after egg laying; however, the developmental time differed slightly between wild isolates), individuals of the starvation environment were transferred to ampicillin plates for 48 hours (see Materials and Methods). For each experimental block, 100 individuals of a single replicate (plate) from each standard and starvation environment were examined. Three experimental blocks were conducted and we examined a total of 3,600 individuals.

**1.8 Vulval Centering Shifts (Figures 5C and 5D)**

*Methods*: To examine the alignment process between anchor cell (AC) and vulval precursor cells, we analyzed individuals (wild isolates N2 and AF16) in the mid to late L2 stage collected from standard NGM plates at 20°C (the AC develops in the early L2 stage). For each individual (anaesthetized with sodium azide), we estimated the distances between AC and the three vulval precursor cells P5.p, P6.p and P7.p using digital Nomarski images (Figure 5C). Using the line tool of the software program Image J 1.38x (http://rsb.info.nih.gov/ij/), we measured the distances between the centre of the AC nucleus and the centre of the nuclei of P5.p, P6.p P7.p. An individual was only considered for measurement analysis when all cell nuclei of interest were in the same focal plane. To determine the measurement error we repeatedly (10 times) measured the distances between anchor cell and vulval precursor cells of a single individual. Two distance measures were considered to differ when their absolute difference was greater than the measurement error.

**1.9 Introgression of *lin-3(n378)* into Different *C. elegans* Wild Isolates (Figure S14)**

*Methods:* The mutation *lin-3(n378)* (in background N2) was introgressed into different wild strains by repeated crossing of the mutant with genetically divergent *C. elegans* wild isolates (AB1, CB4856, JU258, PB303, PB306, PS2025) (Denver et al., 2003; Stewart et al., 2004). The mutation was also backcrossed to the examined N2 strain ("N2 ancestral"). After eight backcrosses, each introgressed line was made isogenic by selfing for several generations. The presence of the mutation in the homozygous state in all introgressed lines was verified by PCR and sequencing (Liu et al., 1999). The strains were then frozen.

We followed the basic experimental procedures outlined in the Materials and Methods section; however, this experiment was performed in only four environments: standard NGM plates at 20° and 25°C, starvation environment at 20°C and dauer-inducing environment at 25°C. We omitted 15°C and liquid environment because these environments did not cause apparent effects on *lin-3(rf)* mutants in the N2 background. All introgressed strains were then thawed at the same time and kept under identical conditions for 4-6 generations until the start of the experiment. To initiate experimental populations, we hypochlorite treated 50-100 young adults per strain. The number of adults was adjusted according to the isolate to obtain approximately 500-1000 eggs per plate. When the majority of animals had reached the late L1 stage to lethargus L1/L2, individuals assigned to the starvation environment were transferred to ampicillin plates for 48 hours (see Materials and Methods). For each experimental environment, we examined 40-60 individuals from a single replicate (plate). Strains derived from CB4856, PB303 and PB306 showed a reduced propensity to enter the dauer stage and we therefore omitted analyses of the dauer-inducing environment for these strains. This experiment was performed once and we examined a total of 1440 individuals.

*Statistical analysis:* Individuals of the six introgressed strains were assayed for the number of induced vulval cells in the four environments. We performed a Two-Way-ANOVA

(JMP 6.0) testing for the fixed effects of *genotype* and *environment* and their interaction effect on the number of induced vulval cells.

## 2. SUPPLEMENTAL RESULTS

### 2.1 Effects of Vulval Hypo- And Hyperinduction on Offspring Production

To examine the effects of hypo- and hyperinduction on offspring production, we made use of the inter-individual variation in the penetrance of mutant vulval phenotypes due to reduction (*lin-3(e1417)*) or gain-of-function (*let-60(n1046)*) mutations affecting the activity of the Egf/Ras/Mapk pathway. Individuals showed a variable number of induced vulval cells, ranging from 0.0 to 3.0 induced Pn.p cells for *lin-3(e1417)* and from 3.0 to 6.0 induced Pn.p cells for *let-60(n1046).* These mutations allowed us to measure offspring number in animals of identical genotype but with different vulval phenotypes raised in the same environment. Individuals of each mutant line were grown under the same condition for several generations, and the number of induced vulval cells was scored in the L4 stage using Nomarski optics. The individuals were then placed back to NGM plates and total lifetime offspring production was scored.

*Hypoinduction*: The *lin-3(e1417)* mutation causes reduction of LIN-3 signal in the anchor cell due to a mutation in an anchor cell-specific enhancer element. This mutant allele causes very few pleiotropic effects, so that viability and offspring production of this mutant are similar to the wild type condition (Hwang and Sternberg, 2004; Liu et al., 1999). There was a highly significant positive correlation between the number of induced Pn.p cells and the number of offspring produced (Spearman's Rank Correlation, $R=0.62$, $P<0.0001$, $N=51$). In general, *C. elegans* hermaphrodites without a functional vulva cannot lay eggs. However, they nevertheless generate a reduced number of offspring that hatch within the mother thereby killing her. In the *lin-3(e1417)* mutant background, individuals lacking a functional vulva produced a highly reduced total offspring number (50.83±0.31, N=35) compared to individuals capable of egg laying (185.13±3.47, N=16) (Mann-Whitney-U-Test, $U=3.5$, $P<0.0001$, $N=51$). In addition, we found 6/41 of individuals with mild hypoinduction (2.0-2.5 induced vulval cells) that were capable of egg laying and generated relatively high offspring numbers. All these individuals had a fully induced 1° fate (P6.p) but showed partial induction of the 2° fates (P5.p or P7.p).

*Hyperinduction:* The *let-60(n1046)* causes overactivation of Ras signalling (Beitel et al., 1990), leading to induction of vulval fates in P3.p, P4.p or P8.p. There was only a weakly significant negative correlation between the number of cells induced and the number of offspring produced (Spearman's Rank Correlation, $R=-0.32$, $P=0.033$, $N=44$*).* Virtually all individuals with an excess of vulval cells could maintain egg-laying function. It is however possible that ectopically induced (non-functional) vulvae cause other adverse effects, for example, by impeding efficient locomotion. In addition, an excess of Ras signalling may also induce adjacent primary fates (e.g. on P6.p and P5.p or on P6.p and P7.p) causing a non-functional vulval pattern, which prevents egg laying, for example, because of eversion of the vulva at the final moult (seen in a variety of Ras gain-of-function mutants).

### 2.2 Environmental Effects on Vulval Mutant Phenotypes

Each tested environmental condition affected the vulval phenotype in some of the mutants (compared to the standard environment at 20°C). Frequent environmental effects on vulval mutant phenotypes were observed in starvation and dauer-inducing environments (see main text, Figures S3 to S8). In addition, temperature effects were frequently found: overall, it seemed that multiple pathways are cold sensitive, as levels of vulval induction were frequently lower in 15°C environment. While some of the mutations are known to be heat-sensitive (e.g. due to the nature of the molecular lesion), we also found cold-sensitive

effects on the vulval phenotype in presumptive null mutants (e.g. *bar-1*) (Figure 3C). Development in liquid culture, although likely inducing stress-responses (Hong et al., 2004; Suo et al., 2006), only rarely affected vulval induction in mutants: 2/41 mutants had a significantly different mean number of induced vulval cells (relative to the standard environment at 20°C).

The expression of mutant vulval phenotypes showed considerable variability, even within a given environment (indicated by significant differences in the mean number of induced vulval cells between individuals of different replicates within a tested environment, see Table S2). Such variability may be due to random stochastic variation and unknown variables within a given environment. Moreover, the vulval phenotype of many mutants showed differences from one experiment to another despite carefully controlled environmental conditions (data not shown). Although we do not know the underlying causes, this observation suggests that vulval mutants are highly sensitive to minor changes in their developmental environment (as compared to wild type animals).

Mutations showing significant differences in mean number of induced vulval cells in different environments are represented in Figures S3 to S8 and the statistical results are presented in Table S2. Note that other properties of vulval cell fate patterning (not necessarily reflected by the number of induced vulval cells) were also affected in some of the mutants. Details on the nature of such environmental effects are given for individual mutants in the legends of Figures S3 to S8. In addition, rare vulval developmental deviants (as observed for the wild type isolate N2) were also observed in a range of mutants (data not shown). The following mutants exhibited no significant variation in the mean number of vulval cells across environments: Egf/Ras/Mapk pathway: *dep-1(ok1844)*: 3.0±0.0 induced cells, N=240, *gap-1(ga133)*: 3.0±0.0 induced cells, N=286, *ksr-1(n2682)*: 3.0±0.0 induced cells, N=248, *lip-1(zh15)*: 3.0±0.0 induced cells, N=174, *sli-1(sy143)*: 3.0±0.0 induced cells, N=347; Mutations Delta/Notch pathway: *dsl-1(ok810*): 3.0±0.0 induced cells, N=240, *lst-1(ok814)*: 3.0±0.0 induced cells, N=204; Mutations Wnt pathway: *bar-1(mu63)*: 3.0±0.0 induced cells, N=320; *egl-20(n585)*: 3.0±0.0 induced cells, N=305; *lin-17(n671)*: 3.0±0.0 induced cells, N=240; Mutations downstream effectors: *lin-39(n760)*: 0.0±0.0 induced cells, N=240, *lin-31(n1053)*: 3.58±0.03 induced cells, N=189; Mutations SynMuv: *lin-8(n111)*: 3.0±0.0 induced cells, N=240, *lin-15(n309)*: 6.0±0.0 induced cells, N=218, *lin-15(n767)*: 3.0±0.0 induced cells, N=355, *lin-35(n745)*: 3.0±0.0 induced cells, N=236. Note that most of the insensitive mutants were those presenting an induction index of 3.0 (and 0.0 or 6.0) indicating a higher buffering of the system around these values.

**2.3 Interplay between Vulval Signalling Pathways in Different Environments**

The mutant analyses suggested that Ras and Wnt pathways affect vulval cell fate patterning differentially depending on the environment. A previous study (Gleason et al., 2002) showed that hyperactivation of the Wnt pathway is sufficient for induction of vulval fates when the Egf/Ras/Mapk pathway is compromised. Increasing Wnt signalling by a mutation in *pry-1*/Axin could compensate in standard laboratory conditions for severe reduction-of-function mutations in *lin-3/egf* (C.B., unpublished data) and in *let-23/egfr* or *let-60/Ras* (Gleason et al., 2002). Based on this evidence, we tested whether the observed starvation suppression of the hypoinduced phenotype in *lin-3(rf)* mutations required action of the Wnt pathway. Consistent with this hypothesis, we found that suppression of the *lin-3(n378)* mutation by the starvation environment was abolished by a null mutation in *bar-1*/β-catenin: the *lin-3(n378); bar-1(ga80)* double mutant showed a mean of 0.02±0.03 vulval cells per individual (N=50) in the starvation environment and similarly low values in any of the other environments (N=209). For the starvation environment, the induction levels of the *lin-3(n378); bar-1(ga80)* double mutant were thus significantly lower than for the *lin-3(n378)* single mutant (Mann-Whitney-U-Test, U=283.00, P<0.0001, N=130). Starvation suppression of the *lin-3(rf)* mutant phenotype was therefore Wnt pathway-dependent.

However, we also found that this suppression effect required basal LET-23/EGFR activity because the *let-23(sy1); lin-3(n378)* double mutant showed no suppression (mean: 0.00±0.00, N=360) in the starvation or in any of the other five environments. In the starvation environment, the induction levels of the *let-23(sy1); lin-3(n378)* double mutant were thus much lower than for the *lin-3(n378)* single mutant (Mann-Whitney-U-Test, U=324.50, P<0.0001, N=139).

### 2.4 Vulval Centering Shifts

To test whether vulval centering shifts affect functionality of the vulva and offspring production, we isolated L4 individuals of the N2 isolate that had developed in the starvation environment and showed either centering on P5.p (with P4.p and P6.p adopting lateral vulval fates) or P6.p (with P5.p and P7.p adopting lateral vulval fates) (using Nomarski microscopy). We then let these animals resume development and observed their capacity for egg laying and counted offspring number during adulthood. Individuals with vulval centering on P5.p were capable of egg laying and produced 205.50±16.75 offspring (range: 98-282, N=4) while individuals with vulval centering on P6.p produced 244.25±6.84 offspring (range: 243-266, N=4). Therefore, vulval centering shifts on P5.p do not seem to significantly impede functionality of the vulva.

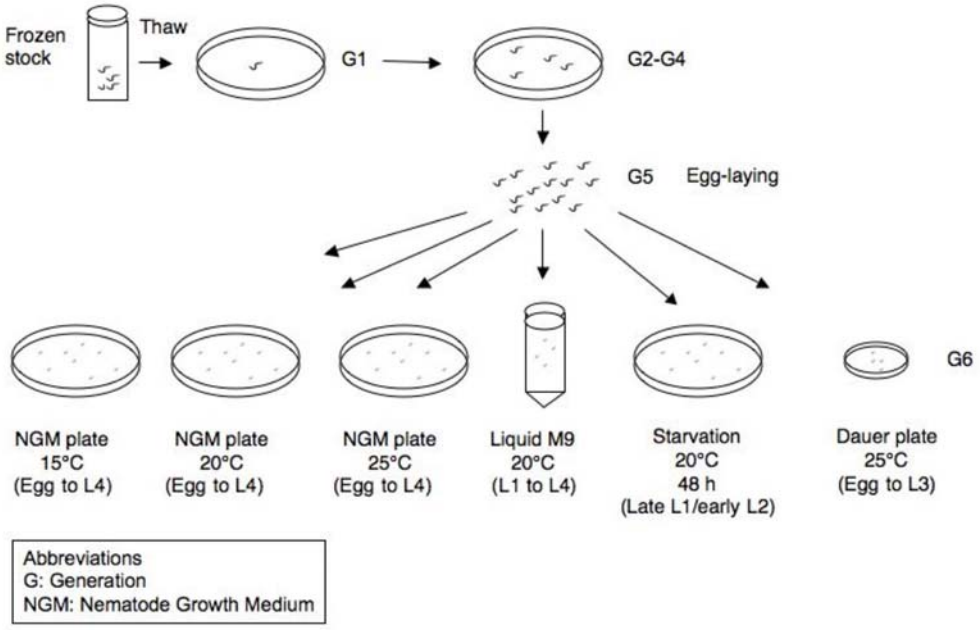


**Figure S1. Experimental Environments**

Outline of experimental procedures and of the different laboratory environments (see Materials and Methods for a detailed description).

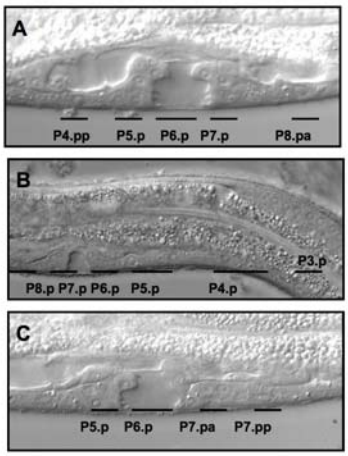


**Figure S2. Nomarski Images of Wild-Type Vulva and Developmental Deviations**
(A) Wild-type vulva (mid L4 stage).
(B) Vulval centering on P7.p (early L4 stage).
(C) Hypoinduction and extra vulval invagination of P7.p (mid L4 stage).

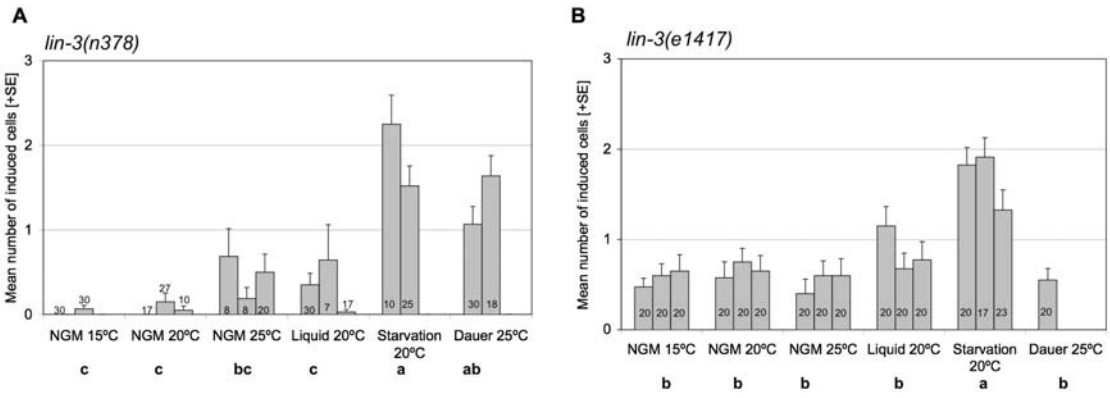


**Figure S3. *lin-3/egf* Mutants in Different Environments**
The starvation environment caused strong suppression of the hypoinduced phenotype in *lin-3(rf)* mutants. This effect was consistently observed in multiple experiments (data not shown). Numbers displayed on bars represent the number of individuals examined for a given replicate. The mean number of induced vulval cells was not significantly different in environments labelled with the same letter (Tukey's HSD, $P < 0.05$). For ANOVA results, see Table S2.
(A) *lin-3(n378)* (these data are also represented as Figure 3B in the main text).
(B) *lin-3(e1417).*

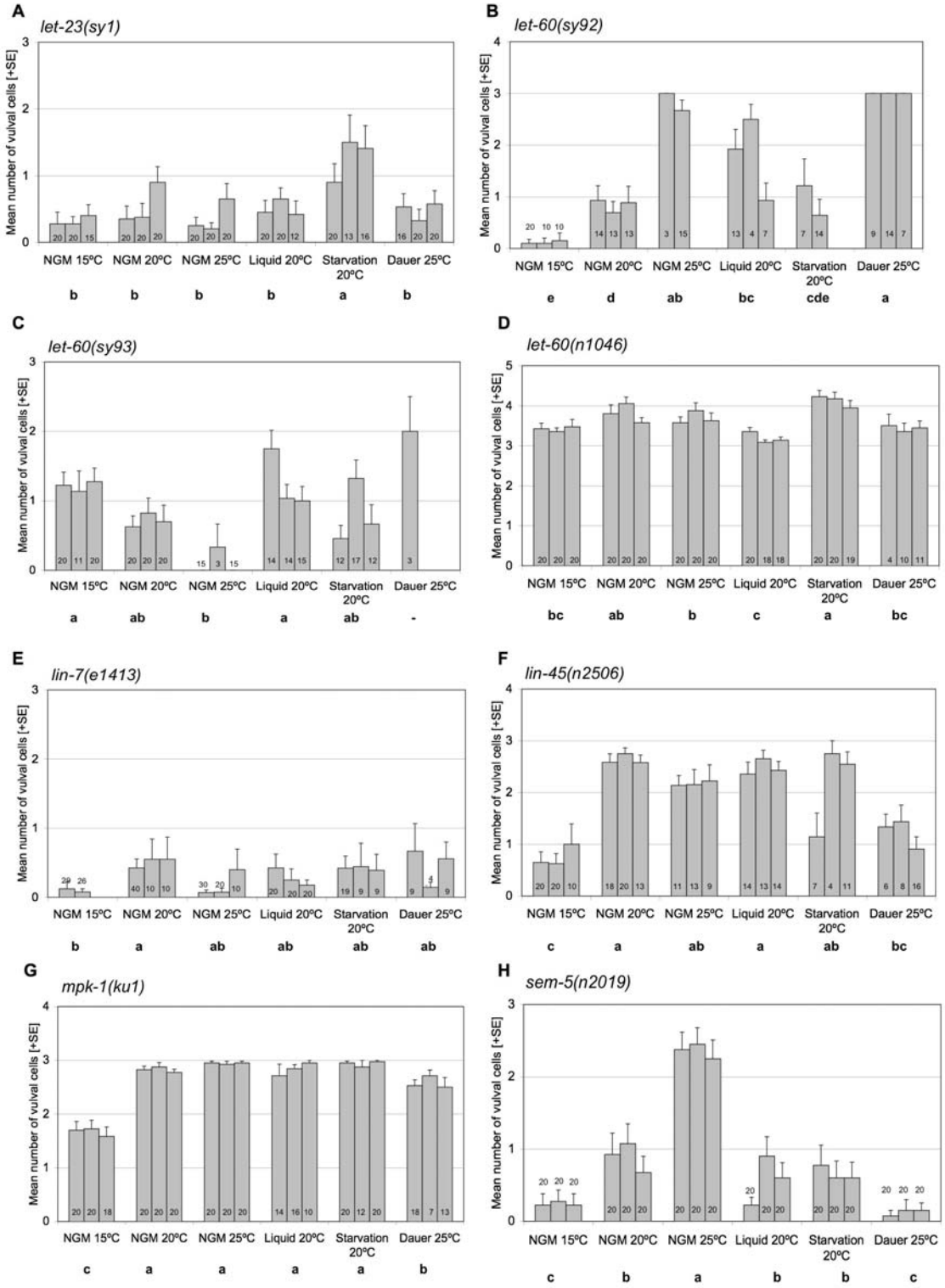


**Figure S4. Ras/Mapk Pathway Mutants in Different Environments**
For details on representation of data in figure, see legend of Figure S3. For ANOVA results, see Table S2.
(A) *let-23(sy1)*.
(B) *let-60(sy92)*.
(C) *let-60(sy93)*.
(D) *let-60(n1046)*.
(E) *lin-7(e1413)*.
(F) *lin-45(n2506)*.
(G) *mpk-1(ku1)*.
(H) *sem-5(n2019)*.

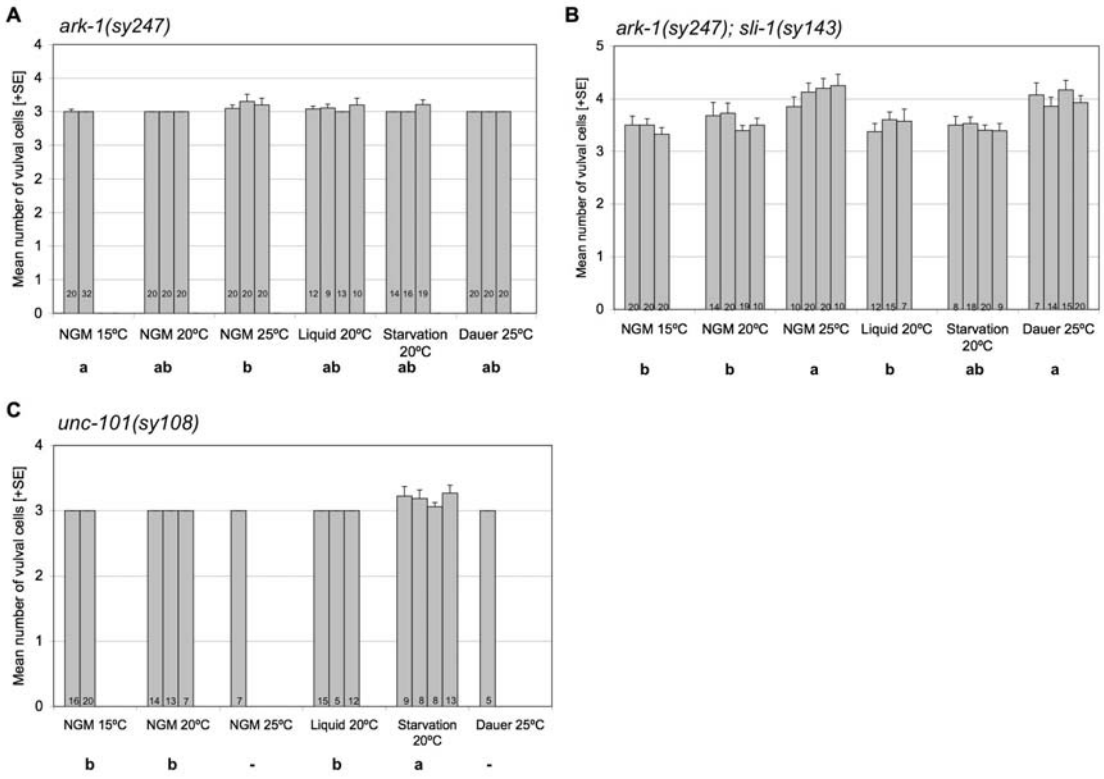


**Figure S5. Ras/Mapk Regulator Mutants in Different Environments**
For details on representation of data in figure, see legend of Figure S3. For ANOVA results, see Table S2.
(A) *ark-1(sy247).*
(B) *ark-1(sy247); sli-1(sy143).*
(C) *unc-101(sy108)*: Hyperinduction was consistently observed in the starvation but not in other environments, with mainly P8.p (and rarely P4.p) adopting a partial or full vulval fate.

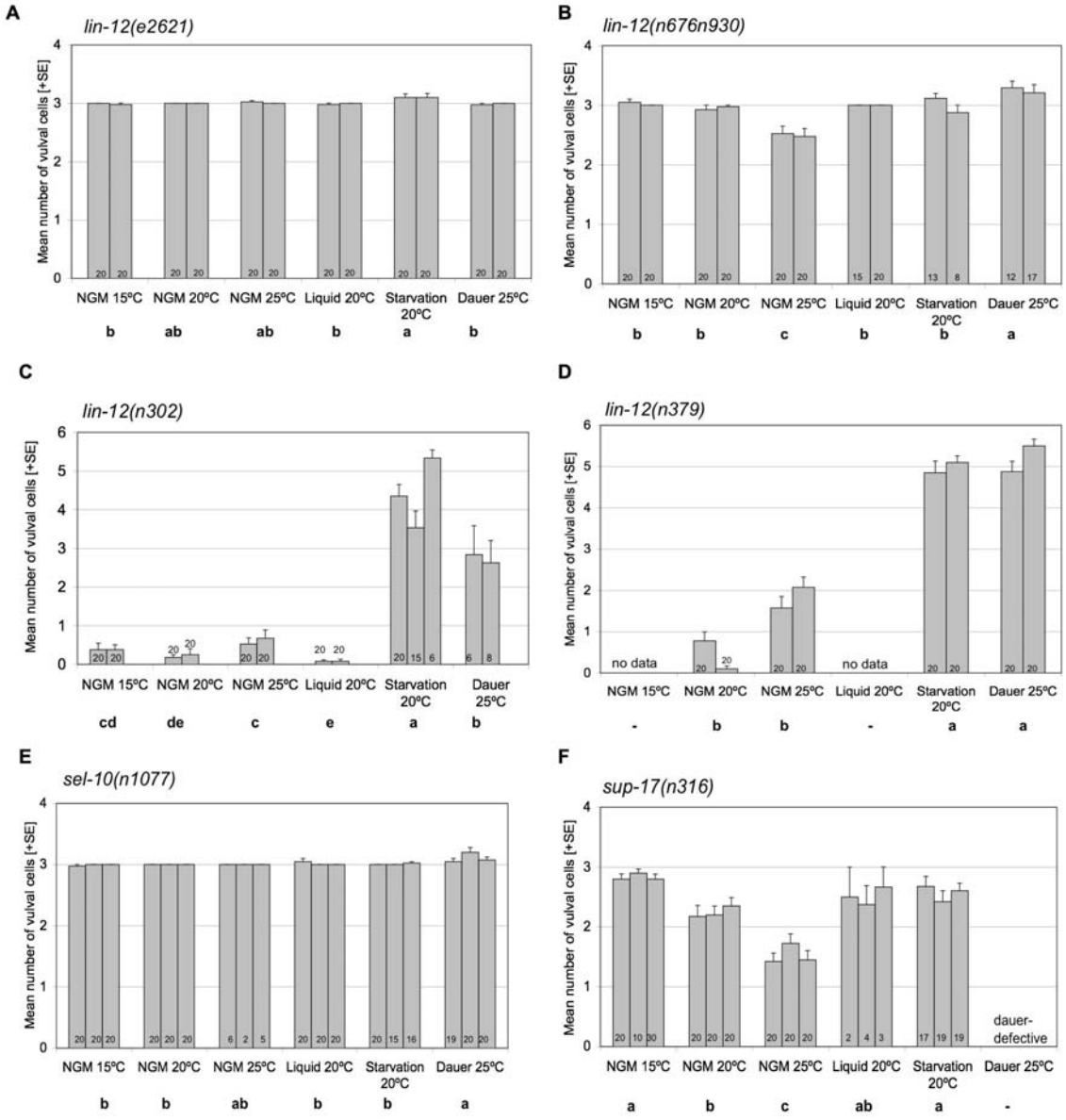


**Figure S6. Delta-Notch Pathway Mutants in Different Environments**
For details on representation of data in figure, see legend of Figure S3. For ANOVA results, see Table S2.
(A) *lin-12(e2621).*
(B) *lin-12(n676n930).*
(C) *lin-12(n302)* (these data are also represented as Figure 3D in the main text).
(D) *lin-12(n379).*
(E) *sel-10(n1077).* This mutation caused an increase in vulval centering on P5.p in both starvation and dauer environments. Hyperinduction in the dauer environment was predominantly due to individuals with a wild type vulval pattern (centered on P6.p) and partial or full induction of P4.p.
(F) *sup-17(n316).*

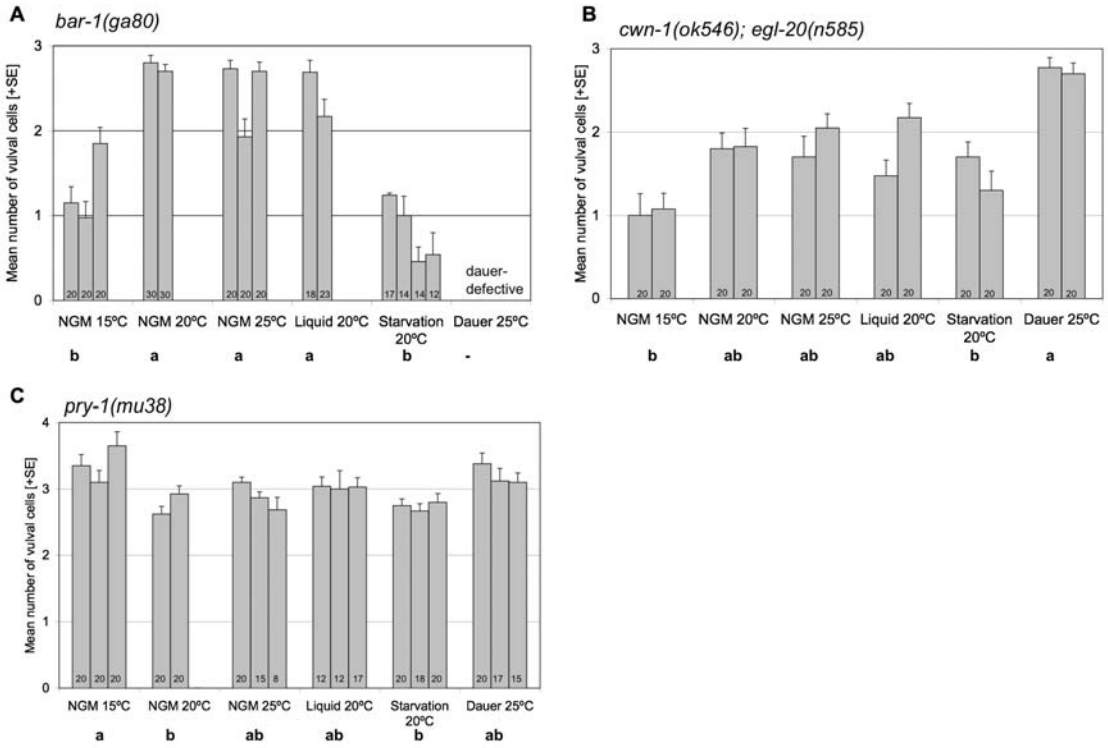


**Figure S7. Wnt Pathway Mutants in Different Environments**
For details on representation of data in figure, see legend of Figure S3. For ANOVA results, see Table S2.
(A) *bar-1(ga80)* (these data are also represented as Figure 3C in the main text). Note that aggravation of the hypoinduced phenotype in the starvation environment was primarily due to P(5-7).p adopting F fates, while at 15ºC the aggravation was primarily due to P(5-7).p adopting 3º fates.
(B) *cwn-1(ok546); egl-20(n585).*
(C) *pry-1(mu38).*

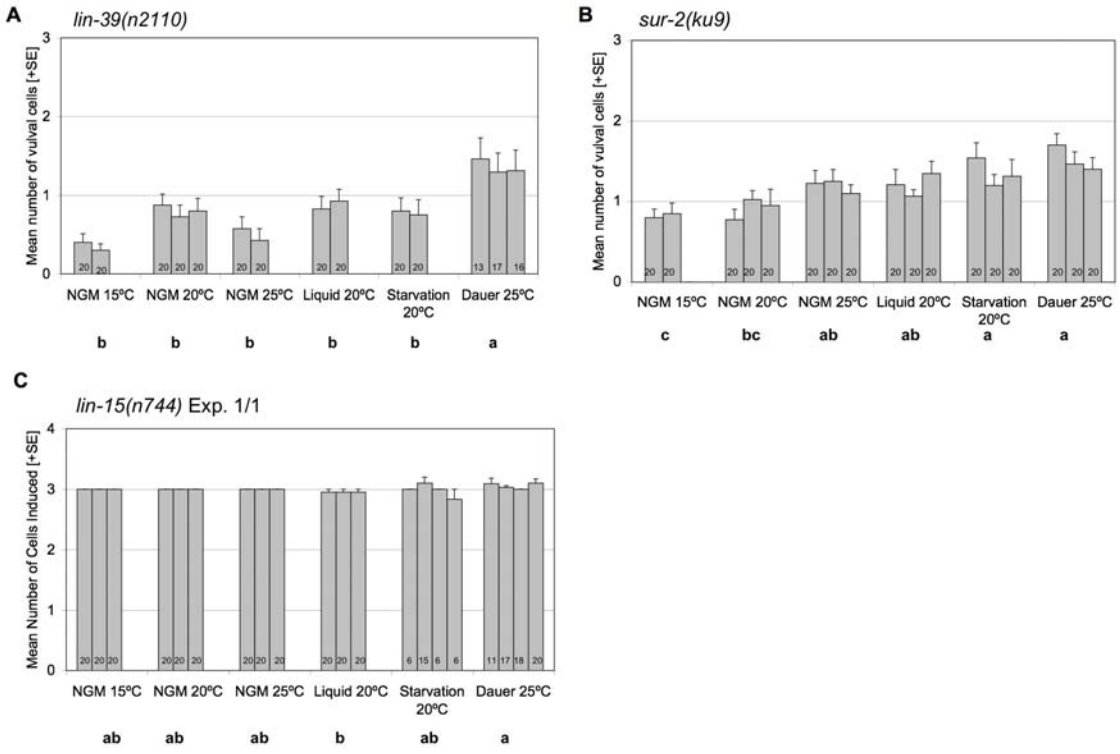


**Figure S8. Downstream Effector and SynMuv Mutants in Different Environments**
For details on representation of data in figure, see legend of Figure S3. For ANOVA results, see Table S2.
(A) *lin-39(n2110).*
(B) *sur-2(ku9).*
(C) *lin-15(n744).*

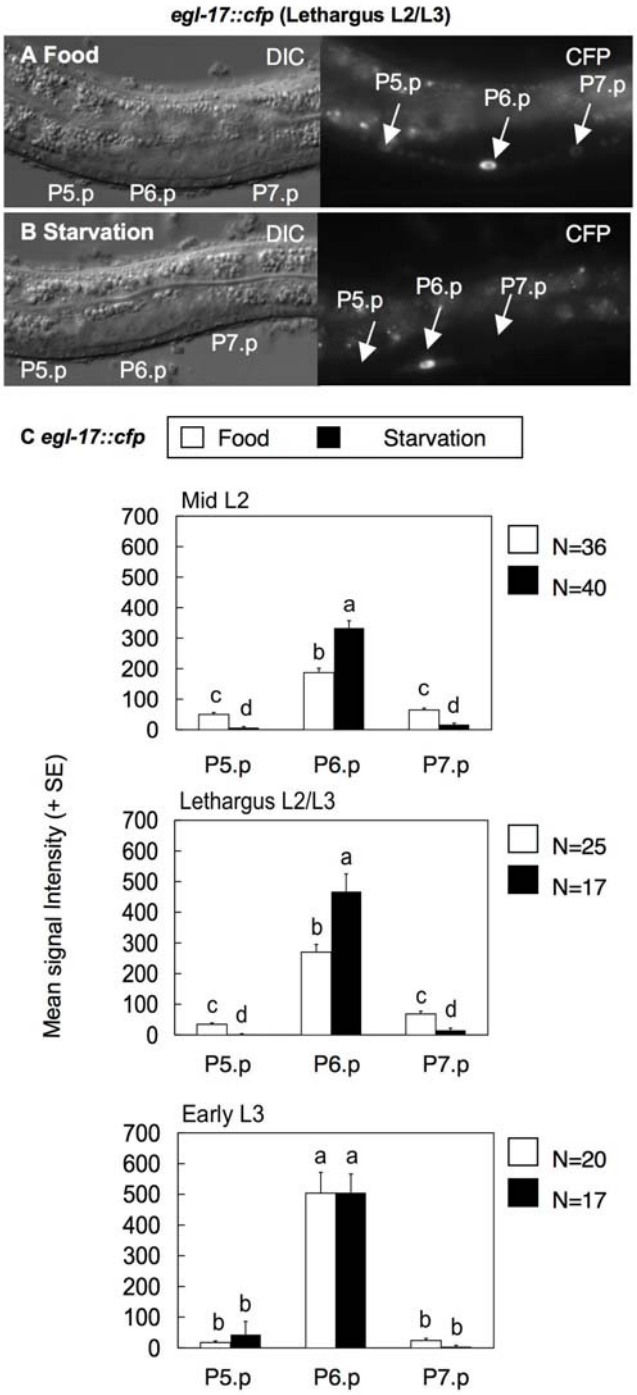


**Figure S9. Ras Pathway Transcriptional Reporter (*egl-17::cfp-lacZ*)**
(A and B) Nomarski and fluorescence images of animals bearing an integrated Ras pathway reporter transgene (*egl-17::cfp-lacZ)*, in the L2/L3 lethargus period. An arrow indicates the position of the nucleus for each of the Pn.p cells.
(A) NGM 20°C environment ("food").
(B) Starvation environment. The graded induction of *egl-17::cfp* in P5.p and P7.p (Yoo et al., 2004) was still visible at this stage in the food environment, but not in the starvation environment. In the L3 stage, expression levels were similar in both environments. However, expression level differences observed in the L2 stage may have nevertheless contributed to differences in overall levels of vulval induction. Specifically, we observed that a main downstream target of the Ras pathway, the Hox gene *lin-39*, is significantly increased at all developmental stages, including the L3 stage (Fig. S11). Increased *lin-39* expression thus may have resulted through the starvation-induced upregulation of the Ras pathway during the L2 stage.

(C) Quantification of environmental effects on Ras reporter activity at different developmental stages (results for the lethargus L2/L3 stage are also displayed as Figure 4B in the main text). Note: At the L3 stage, one individual was observed with exclusive and strong signal activity in P5.p (starvation environment), indicating that this cell had adopted a 1° cell fate (instead of P6.p). Bars labelled with the same letter did not show significant differences in expression levels (Tukey's HSD, $P < 0.05$). For ANOVA results, see Table S3.

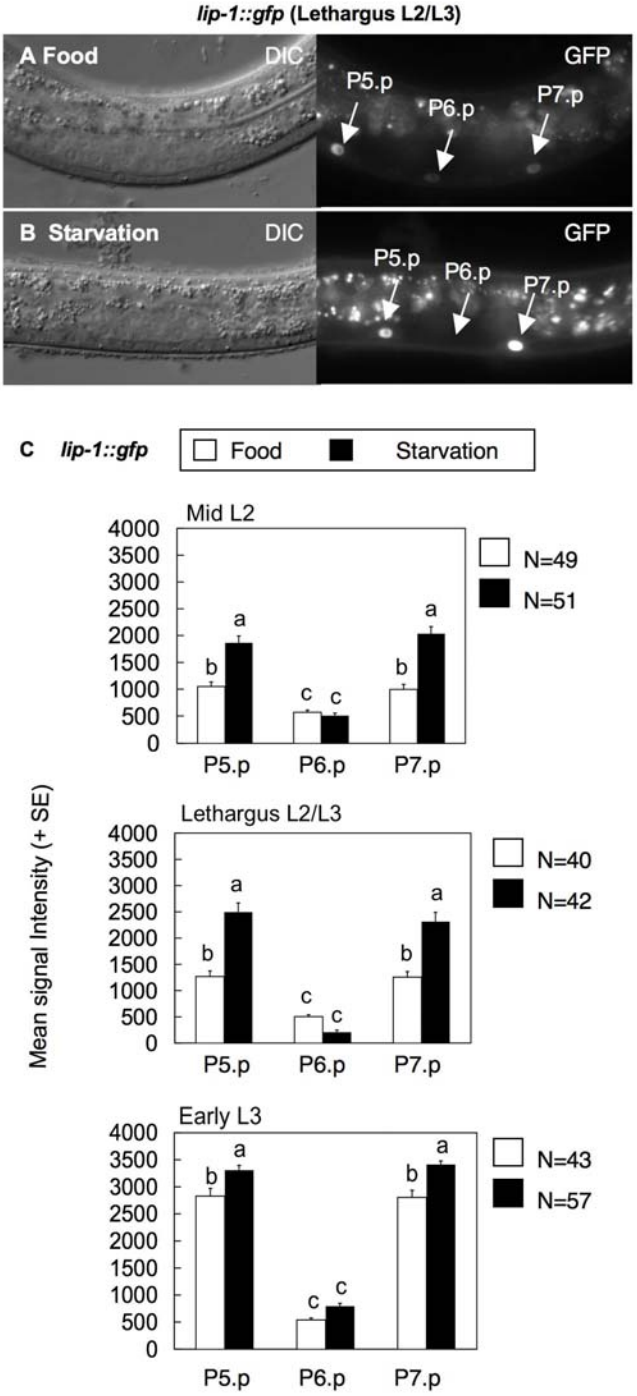


**Figure S10. Notch Pathway Transcriptional Reporter (*lip-1::gfp*)**
(A and B) Nomarski and fluorescence images of animals bearing an integrated Notch pathway reporter transgene (*lip-1::gfp*), in the L2 lethargus period. An arrow indicates the position of the nucleus of each Pn.p cell.
(A) NGM 20°C environment (“food”).
(B) Starvation environment.

(C) Quantification of environmental effects on Notch reporter activity at different developmental stages (results for the lethargus L2/L3 stage are also displayed as Figure 4C in the main text). Bars labelled with the same letter did not show significant differences in expression levels (Tukey's HSD, $P < 0.05$). For ANOVA results, see Table S3.

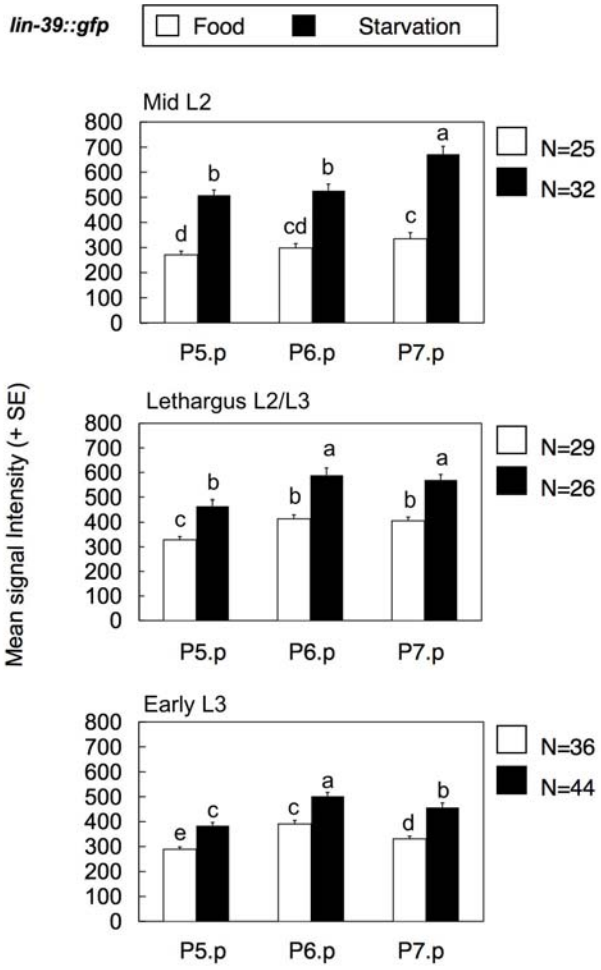


**Figure S11. Transcriptional Reporter Activated by the Ras and Wnt Pathways (*lin-39::gfp*)**

Quantification of environmental effects on *lin-39* transcriptional reporter activity at different developmental stages. Bars labelled with the same letter did not show significant differences in expression levels (Tukey's HSD, $P < 0.05$). For ANOVA results, see Table S3.

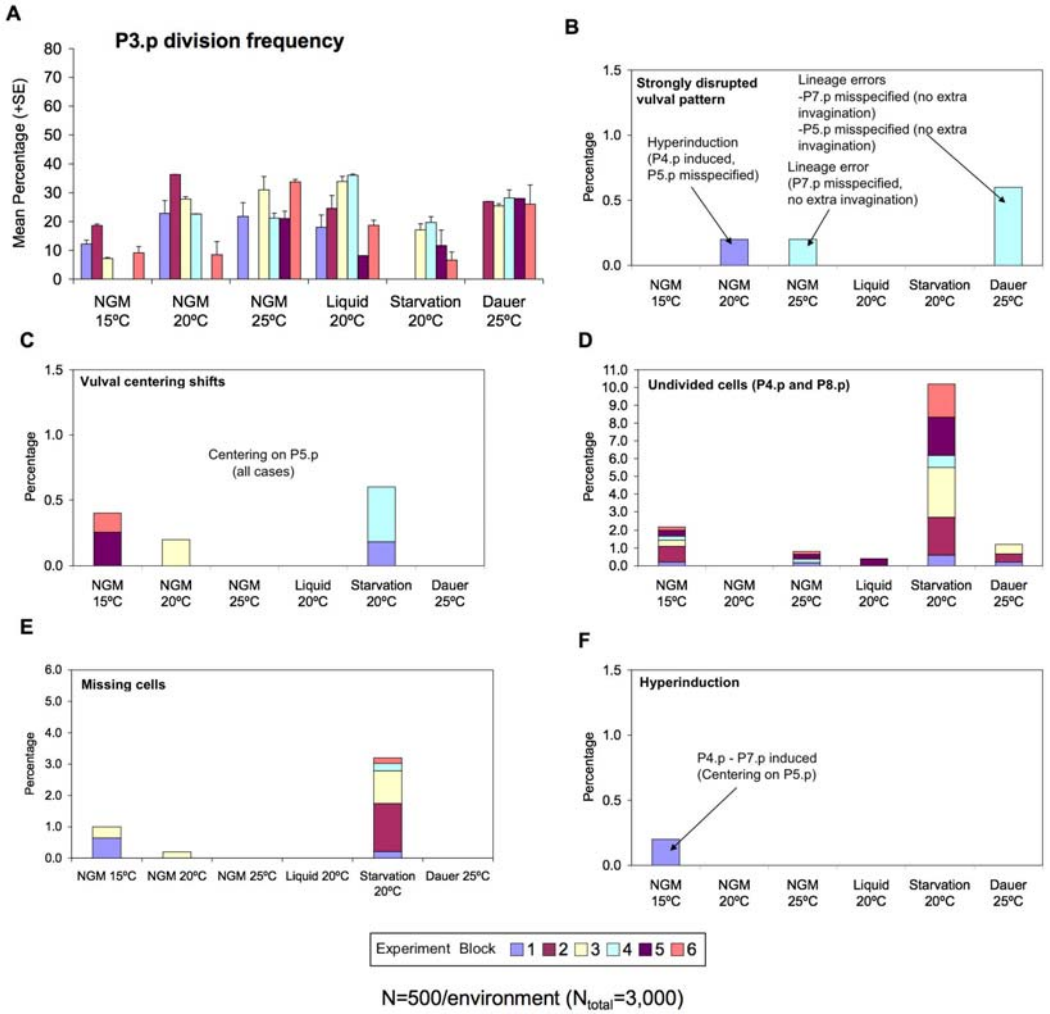


**Figure S12. Precision and Deviations of Vulval Cell Fate Patterns in Different Environments (Isolate JU258)**

Vulval developmental deviations in different environments (isolate JU258). The data are represented as described in the legend of Figure 2. Note that the scale of the Y-axis differs between panels. The combined proportion of all developmental deviations different from the canonical cell fate pattern for P3.p to P8.p varied significantly among environments (GLM, main effect *environment*: df=5, $\chi^2$=101.51, P<0.0001) and also among experimental blocks as indicated by the significant interaction term (GLM, interaction effect *environment* x *block*: df=25, $\chi^2$=39.47, P=0.0330; main effect *block*, df=5, $\chi^2$=4.90, P=0.43).

(A) P3.p division frequency. P3.p division frequency varied significantly among environments (GLM, main effect *environment*: df=5, $\chi^2$=54.50, P<0.0001) and also among experimental blocks as indicated by the significant interaction term (GLM, interaction effect *environment* x *block*: df=25, $\chi^2$=56.55, P=0.0003; main effect *block*, df=5, $\chi^2$=7.70, P=0.17).

(B) Strongly disrupted vulval patterns likely leading to a non-functional vulva.

(C) Vulval centering shifts.

(D) Undivided cells (P4.p and P8.p.). Undivided Pn.p cells likely correspond to cells with reduced competence (Eisenmann et al., 1998). In JU258, P3.p and P4.p fuse to hyp7 in the L2 stage when they do not divide (observed using a cell junction marker, *ajm-1::gfp* (Mohler et al., 1998)). 75 individuals (2.5%, N=3,000) showed such developmental deviations (undivided P4.p: 50/75; undivided P8.p: 19/75; undivided P4.p and P8.p: 6/75).

(E) Missing cells. Missing cells were more frequently observed for anterior Pn.p cells (P3.p to P5.p) than for posterior cells (P7.p and P8.p).

(F) Hyperinduction.

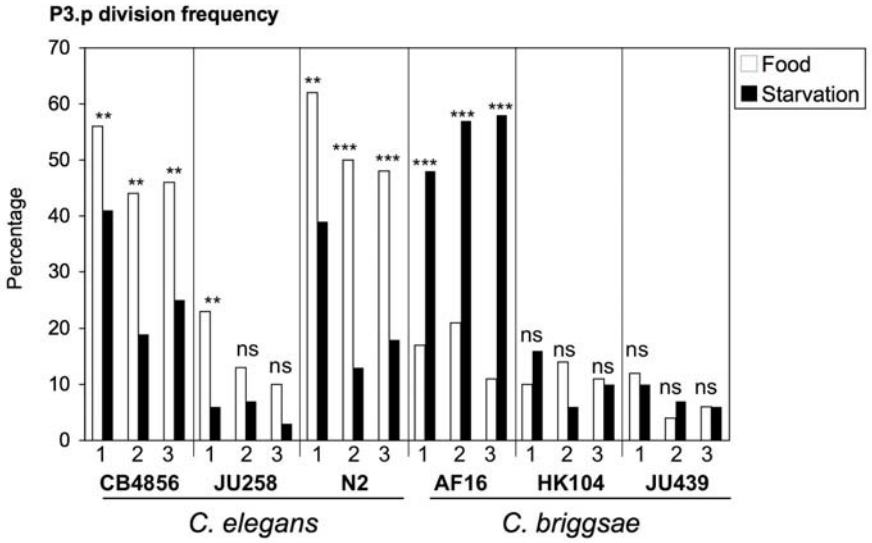


**Figure S13. Precision and Deviations of Vulval Cell Fate Patterns in *C. elegans* and *C. briggsae* Isolates in Standard versus Starvation Environment: P3.p Division Frequency**

For each of three experimental blocks, six isolates of *C. elegans* (CB4856, JU258, N2) and *C. briggsae* (AF16, JU439, HK104) were assayed in food and starvation environments in parallel (N=100/isolate/environment/block, $N_{total}$=3600). For each isolate, the results of experimental blocks 1-3 are listed sequentially.

P3.p division frequency (for other phenotypes, see Figures 5A and 5B). *C. elegans* isolates showed a decreased P3.p division frequency in the starvation environment compared to the standard (food) environment. In contrast, the *C. briggsae* AF16 isolate showed an increased P3.p division frequency in the starvation environment, while *C. briggsae* HK104 and JU439 showed a similarly low P3.p division frequency in both environments. Asterisks indicate significantly different proportions of individuals with divided P3.p in the two environments for each block (*: P < 0.05, **: P < 0.001, ***: P < 0.0001, ns: non-significant, Fisher's Exact Test).

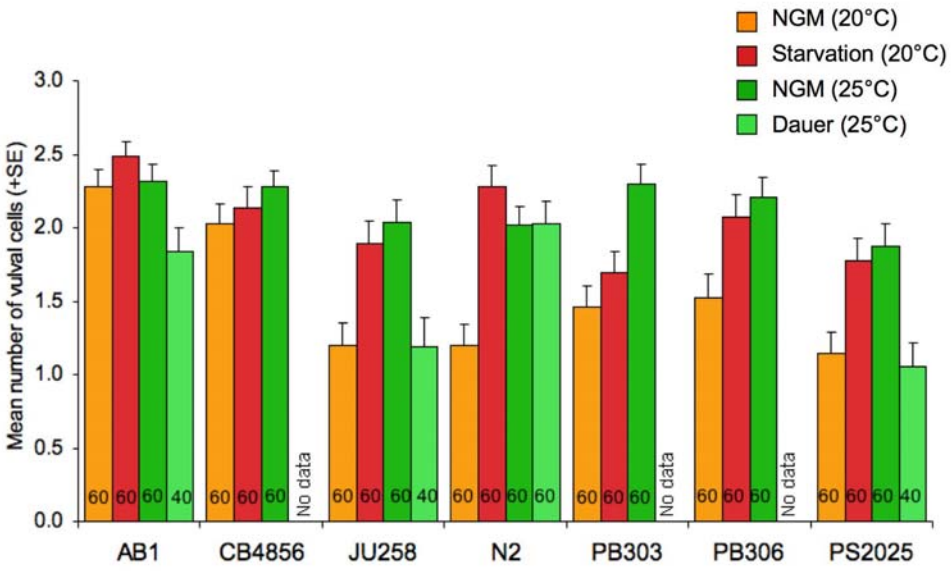


**Figure S14. Introgression of *lin-3(n378)* into Different *C. elegans* Wild Isolates**

Mean number of induced vulval cells of the *lin-3(n378)* mutation introgressed into seven

wild isolates *C. elegans*, examined in four different environments. Different genetic backgrounds strongly modified the effect of the mutation under standard conditions (NGM plates at 20°C). The observed mutational effects were further dependent on the environment. All introgressed genotypes tend to show higher levels of vulval induction in the starvation environment. In the dauer-inducing environments, higher levels of vulval induction are observed for N2 but not for other genotypes (AB1, JU258, PS2025). A Two-Way-ANOVA testing for the fixed effects of *genotype* and *environment* on the number of vulval cells reveals significant main effects (*environment*: $F_{2,1415}$=36.35, P<0.0001); *genotype*: $F_{3,1415}$ = 21.10, P < 0.0001) and a significant genotype-by-environment interaction (*environment* X *genotype*: $F_{15,1415}$ = 2.90, P = 0.0002).

# Table S1. Overview of Examined Vulval Mutants

| Genotype/Chromosome | Function/Homologues | Molecular lesion | Effect | Vulval phenotype (NGM, 20°C) | References | Strain |
|---|---|---|---|---|---|---|
| **LIN-3/EGF** | | | | | | |
| *lin-3(n378) IV* | EGF ligand | Missense | rf | Hypoinduction | (Ferguson and Horvitz, 1985; Liu et al., 1999) | MT378 |
| *lin-3(e1417) IV* | EGF ligand | AC enhancer | rf | Hypoinduction | (Ferguson and Horvitz, 1985; Hwang and Sternberg, 2004) | CB1417 |
| **Ras/MAPK pathway** | | | | | | |
| *let-23(sy1) II* | EGF receptor tyrosine kinase | Nonsense | rf | Hypoinduction | (Aroian et al., 1990; Aroian et al., 1994; Aroian and Sternberg, 1991) | JU605 |
| *let-60(sy92) IV** | Ras | Missense | rf, sdn | Hypoinduction | (Han and Sternberg, 1990; Han and Sternberg, 1991) | PS538 |
| *let-60(sy93) IV* | Ras | Missense | rf, sdn | Hypoinduction | (Han and Sternberg, 1990; Han and Sternberg, 1991) | PS436 |
| *let-60(n1046) IV* | Ras | Missense | gf | Hyperinduction | (Beitel et al., 1990; Ferguson and Horvitz, 1985) | MT2124 |
| *lin-7(e1413) II* | Cell junction protein/EGFR localization | Nonsense | null | Hypoinduction | (Ferguson and Horvitz, 1985; Simske et al., 1996) | CB1413 |
| *lin-45(n2506) IV** | Raf | Missense | rf | Hypoinduction | (Hsu et al., 2002) | WU49 |
| *mpk-1(ku1) III* | MAP kinase | Missense | rf | Hypoinduction | (Wu and Han, 1994) | MH15 |
| *sem-5(n2019) X* | SEM-5/Grb2 adaptor protein | Splice | rf | Hypoinduction | (Clark et al., 1992) | MT4755 |
| **Regulators Ras/MAPK pathway** | | | | | | |
| *ark-1(sy247) IV* | Negative regulator *sem-5* | Nonsense | rf | WT | (Hopper et al., 2000) | PS1461 |
| *ark-1(sy247) IV, sli-1(sy143) X* | Negative regulators | | rf | Hyperinduction | (Hopper et al., 2000) | PS2995 |
| *dep-1(ok1844) II* | Tyrosine phosphatase/Negative regulator *let-23* | Deletion | rf/null | WT | (Berset et al., 2005), new KO allele | RB1536 |
| *gap-1(ga133) X* | GTPase-activating protein/Negative regulator *let-60* | Deletion | null | WT | (Hajnal et al., 1997) | AH12 |
| *ksr-1(n2682) X* | Protein kinase/Positive regulator | Nonsense | rf | WT | (Kornfeld et al., 1995; Ohmachi et al., 2002) | MT8673 |
| *lip-1(zh15) IV* | Phosphatase/Negative regulator *mpk-1* | Deletion | null | WT | (Berset et al., 2001) | AH102 |
| *sli-1(sy143) X* | *cbl* oncogene homologue/Negative regulator | Nonsense | null | WT | (Jongeward et al., 1995; Yoon et al., 1995) | PS2728 |
| *unc-101(sy108) I* | Clathrin adaptor protein/ Negative regulator *let-23* | Insertion/deletion | rf/null | WT | (Lee et al., 1994) | PS529 |
| **Delta/Notch pathway** | | | | | | |
| *dsl-1(ok810) IV* | Delta | Deletion | null | WT | (Chen and Greenwald, 2004) | GS3662 |
| *lin-12(e2621) III* | Notch receptor | Unknown | weak rf | WT | (Hodgkin and Doniach, 1997; Wu et al., 1998) | CB5216 |
| *lin-12(n676n930) III** | Notch receptor | Missenses | *n676*gf, *n930*rf | Hypoinduction | (Sundaram and Greenwald, 1993; Wen and Greenwald, 1999) | GS60 |
| *lin-12(n302) III* | Notch receptor | Missense | gf, sd | Hypoinduction (no AC) | (Greenwald and Seydoux, 1990) | MT302 |
| *lin-12(n379) III* | Notch receptor | Missense | gf, sd | Hypoinduction (no AC) | (Greenwald and Seydoux, 1990) | GS3196 |
| *lst-1(ok814) I* | Lateral signalling target | Deletion | rf | WT | (Yoo et al., 2004) | RB977 |
| *sel-10(n1077) V* | E2-E3 ubiquitin ligase /Negative regulator | Missense | dn | WT | (Desai and Horvitz, 1989; Jager et al., 2004) | MT2244 |
| *sup-17(n316) I* | ADAM/KUZBANIAN protein/Positive regulator | Splice | rf, weak sd | Hypoinduction | (Ferguson and Horvitz, 1985; Wen et al., 1997) | MT1789 |

| Genotype/Chromosome | Function/Homologues | Molecular lesion | Effect | Vulval phenotype (NGM, 20°C) | References | Strain |
|---|---|---|---|---|---|---|
| **Wnt pathway** | | | | | | |
| *bar-1(ga80) X* | Transcriptional co-activator/β-Catenin | Nonsense | null | Hypoinduction | (Eisenmann and Kim, 2000; Eisenmann et al., 1998) | EW15 |
| *bar-1(mu63) X* | Transcriptional co-activator/β-Catenin | Missense | rf | WT | (Maloof et al., 1999; Natarajan et al., 2004) | CF376 |
| *egl-20(n585) IV* | Wnt ligand | Missense | rf | WT | (Maloof et al., 1999; Trent et al., 1983) | MT1215 |
| *cwn-1 (ok546) II, egl-20(n585) IV* | Wnt ligands | Deletion/Missense | rf | Hypoinduction | (Gleason et al., 2006; Zinovyeva and Forrester, 2005) | KN594 |
| *lin-17(n671) I* | Wnt receptor/Frizzled | Nonsense | rf/null | P7.p misspecification | (Ferguson and Horvitz, 1985; Sawa et al., 1996) | MT1306 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *pry-1(mu38) I* | Axin/negative Wnt regulator | Nonsense | rf | Hyperinduction | (Maloof et al., 1999) | CF491 |
| **Downstream effectors** | | | | | | |
| *lin-31(n1053) II* | Transcription Factor HNF3/Forkhead family | Nonsense | rf/null | Hyperinduction | (Ferguson and Horvitz, 1985; Miller et al., 2000) | MT2131 |
| *lin-39(n1760) III* | Hox Gene/ Def Scr family | Nonsense | null | Hypoinduction | (Clark et al., 1993) | MT4007 |
| *lin-39(n2110) III* | Hox Gene/ Def Scr family | Missense | rf | Hypoinduction | (Clark et al., 1993) | MT5101 |
| *sur-2(ku9) I* | Novel protein | Nonsense | rf | Hypoinduction | (Singh and Han, 1995) | MH17 |
| **Syn Muv** | | | | | | |
| *lin-8(n111) II* | Unknown?/SynMuv A | Missense | rf | WT | (Davison et al., 2005; Ferguson and Horvitz, 1985) | MT111 |
| *lin-15(n309) X* | Unknown?/SynMuv AB | Deletion | null | Hyperinduction | (Clark et al., 1994; Ferguson and Horvitz, 1985; Huang et al., 1994) | MT309 |
| *lin-15(n744) X* | SynMuv B | Unknown | rf | WT | (Ferguson and Horvitz, 1989; Kim and Horvitz, 1990) | MT2495 |
| *lin-15(n767) X* | SynMuv A | Deletion | *lin-15a(null)* | WT | (Clark et al., 1994; Ferguson and Horvitz, 1989; Huang et al., 1994) | MT1806 |
| *lin-35(n745) I* | Rb orthologue (SynMuv B) | Unknown? | rf | WT | (Ferguson and Horvitz, 1989; Lu and Horvitz, 1998) | MT10430 |
| **Other** | | | | | | |
| *let-23(sy1) II, lin-3(n378) IV** | - | - | - | Hypoinduction | (Moghal et al., 2003) | PS3815 |
| *lin-3(n378) IV; bar-1(ga80) X* | - | - | - | Hypoinduction | this study | JU1193 |

***Full genotypes:**
*unc-32(e189) lin-12(n676n930) III*
*lin-45(n2506) unc-24(e138) IV*
*let-23(sy1) unc-4(e120) II; lin-3(n378) IV*
*unc-24(e138) let-60(sy92) IV/nT1 [let-?(m435)](IV;V).*

## Table S2. Results of Statistical Tests for Mutant Assays in Different Environments

### Mutations *lin-3/egf*

*lin-3(n378)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 19.76 | 15.98 | 0.0003 |
| Replicate (Environment) | 9 | 2.21 | 2.59 | 0.0070 |
| Error | 272 | 25.73 | | |

*lin-3(e1417)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 27.04 | 10.75 | 0.0009 |
| Replicate (Environment) | 10 | 5.03 | 1.14 | 0.3324 |
| Error | 304 | 134.26 | | |

### Mutations Ras/Mapk pathway

*let-23(sy1)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 21.90 | 3.59 | 0.0273 |
| Replicate (Environment) | 14 | 17.04 | 1.50 | 0.1096 |
| Error | 337 | 273.85 | | |

*let-60(sy92)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 132.84 | 39.31 | <.0001 |
| Replicate (Environment) | 10 | 6.80 | 1.07 | 0.3873 |
| Error | 157 | 99.65 | | |

*let-60(sy93)* [dauer not tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 4 | 19.53 | 9.02 | 0.0016 |
| Replicate (Environment) | 10 | 5.50 | 1.25 | 0.2589 |
| Error | 213 | 93.51 | | |

*let-60(n1046)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 23.37 | 13.15 | 0.0001 |
| Replicate (Environment) | 12 | 4.27 | 1.05 | 0.4054 |
| Error | 300 | 101.94 | | |

*lin-7(e1413)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 0.53 | 3.33 | 0.0278 |
| Replicate (Environment) | 16 | 0.50 | 0.90 | 0.5694 |
| Error | 330 | 11.53 | | |

*lin-45(n2506)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 131.20 | 19.52 | <.0001 |
| Replicate (Environment) | 12 | 16.64 | 2.32 | 0.0083 |
| Error | 209 | 124.83 | | |

*mpk-1(ku1)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 49.60 | 116.90 | <.0001 |
| Replicate (Environment) | 13 | 1.09 | 0.63 | 0.8241 |
| Error | 297 | 39.09 | | |

*sem-5(n2019)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 47.32 | 59.40 | <.0001 |
| Replicate (Environment) | 12 | 1.91 | 0.60 | 0.8433 |
| Error | 342 | 91.05 | | |

### Mutations regulators Ras/Mapk pathway

*ark-1(sy247)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 0.23 | 4.13 | 0.0300 |
| Replicate (Environment) | 12 | 0.14 | 0.47 | 0.9291 |
| Error | 307 | 7.74 | | |

*ark-1(sy247); sli-1(sy143)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 17.30 | 14.86 | <.0001 |
| Replicate (Environment) | 17 | 3.71 | 0.64 | 0.8568 |
| Error | 306 | 103.97 | | |

*unc-101(sy108)* [25 C and dauer not tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 3 | 0.65 | 127.40 | 0.0008 |
| Replicate (Environment) | 8 | 0.02 | 0.12 | 0.9982 |
| Error | 137 | 2.86 | | |

### Mutations Delta/Notch pathway

*lin-12(e2621)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 0.23 | 7.17 | 0.0163 |
| Replicate (Environment) | 6 | 0.04 | 0.50 | 0.8065 |
| Error | 228 | 2.86 | | |

*lin-12(n676n930)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 14.78 | 25.00 | 0.0005 |
| Replicate (Environment) | 6 | 0.71 | 0.92 | 0.4827 |
| Error | 193 | 24.83 | | |

*lin-12(n302)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 93.12 | 206.07 | <.0001 |
| Replicate (Environment) | 7 | 0.63 | 0.29 | 0.9582 |
| Error | 202 | 63.36 | | |

*lin-12(n379)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 3 | 630.08 | 65.77 | 0.0007 |
| Replicate (Environment) | 4 | 12.77 | 3.15 | 0.0161 |
| Error | 152 | 154.26 | | |

*sel-10(n1077)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 0.32 | 5.95 | 0.0047 |
| Replicate (Environment) | 12 | 0.13 | 1.06 | 0.3970 |
| Error | 285 | 2.88 | | |

*sup-17(n316)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 4 | 49.90 | 60.87 | <.0001 |
| Replicate (Environment) | 10 | 1.95 | 0.57 | 0.8372 |
| Error | 229 | 78.19 | | |

***Table S2 continued***

## Mutations Wnt pathway

*bar-1(ga80)* [dauer not tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 4 | 191.03 | 29.39 | <.0001 |
| Replicate (Environment) | 9 | 13.79 | 3.60 | 0.0003 |
| Error | 264 | 112.45 | | |

*cwn-1(ok546); egl-20(n585)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 63.44 | 10.07 | 0.0070 |
| Replicate (Environment) | 6 | 7.56 | 1.68 | 0.1269 |
| Error | 228 | 170.95 | | |

*pry-1(mu38)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 14.90 | 5.34 | 0.0098 |
| Replicate (Environment) | 11 | 6.15 | 1.51 | 0.1255 |
| Error | 277 | 102.25 | | |

## Mutations downstream effectors

*lin-31(n1053)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 19.46 | 5.34 | 0.0864 |
| Replicate (Environment) | 6 | 4.40 | 0.98 | 0.4407 |
| Error | 177 | 132.52 | | |

*lin-39(n2110)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 18.24 | 8.17 | 0.0030 |
| Replicate (Environment) | 10 | 4.45 | 1.14 | 0.3340 |
| Error | 290 | 113.50 | | |

*sur-2(ku9)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 14.06 | 11.46 | 0.0004 |
| Replicate (Environment) | 11 | 2.70 | 0.78 | 0.6623 |
| Error | 253 | 79.74 | | |

## Mutations SynMuv genes

*lin-15(n744)* [all 6 environments tested]

| Source | DF | SS | F | P |
|---|---|---|---|---|
| Environment | 5 | 0.45 | 3.50 | 0.0311 |
| Replicate (Environment) | 14 | 0.37 | 0.87 | 0.5881 |
| Error | 319 | 9.53 | | |

We performed Two-Way-ANOVAs testing for the fixed effect of *environment* and the random effect of *replicate* (nested within *environment*) on the number of induced vulval cells. For a graphical presentation of the data, see Figures S3 to S8.

**Table S3. Results of Statistical Tests for Transcriptional Reporter Assays in Different Environments**

## *egl-17::cfp-LacZ*

| Mid L2 | | | | | Lethargus L2/L3 | | | | | Early L3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Source** | **DF** | **SS** | **F** | **P** | **Source** | **DF** | **SS** | **F** | **P** | **Source** | **DF** | **SS** | **F** | **P** |
| Environment | 1 | 38494 | 53.08 | <.0001 | Environment | 1 | 30614 | 27.88 | <.0001 | Environment | 1 | 10652 | 5.06 | 0.0277 |
| Individual(Environment) | 74 | 93231 | 1.74 | 0.0023 | Individual(Environment) | 40 | 39222 | 0.89 | 0.6475 | Individual(Environment) | 35 | 31726 | 0.43 | 0.9963 |
| Cell | 2 | 361499 | 249.26 | <.0001 | Cell | 2 | 366906 | 167.06 | <.0001 | Cell | 2 | 538203 | 127.73 | <.0001 |
| Cell x Environment | 2 | 64716 | 44.62 | <.0001 | Cell x Environment | 2 | 50928 | 23.19 | <.0001 | Cell x Environment | 2 | 4241 | 1.01 | 0.3707 |
| Error | 148 | 107323 | | | Error | 80 | 87851 | | | Error | 70 | 147479 | | |

## *lip-1::gfp*

| Mid L2 | | | | | Lethargus L2/L3 | | | | | Early L3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Source** | **DF** | **SS** | **F** | **P** | **Source** | **DF** | **SS** | **F** | **P** | **Source** | **DF** | **SS** | **F** | **P** |
| Environment | 1 | 6754042 | 45.38 | <.0001 | Environment | 1 | 6754042 | 45.38 | <.0001 | Environment | 1 | 2821499 | 28.98 | <.0001 |
| Individual(Environment) | 98 | 26925644 | 1.85 | 0.0002 | Individual(Environment) | 98 | 26925644 | 1.85 | 0.0002 | Individual(Environment) | 98 | 12616754 | 1.32 | 0.0509 |
| Cell | 2 | 29286415 | 98.38 | <.0001 | Cell | 2 | 29286415 | 98.38 | <.0001 | Cell | 2 | 98249405 | 504.54 | <.0001 |
| Cell x Environment | 2 | 6612478 | 22.21 | <.0001 | Cell x Environment | 2 | 6612478 | 22.21 | <.0001 | Cell x Environment | 2 | 50298 | 0.26 | 0.7726 |
| Error | 196 | 29171976 | | | Error | 196 | 29171976 | | | Error | 196 | 19083755 | | |

## *lin-39::gfp*

| Mid L2 | | | | | Lethargus L2/L3 | | | | | Early L3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Source** | **DF** | **SS** | **F** | **P** | **Source** | **DF** | **SS** | **F** | **P** | **Source** | **DF** | **SS** | **F** | **P** |
| Environment | 1 | 2951300 | 437.11 | <.0001 | Environment | 1 | 807390 | 127.85 | <.0001 | Environment | 1 | 754135 | 166.54 | <.0001 |
| Individual(Environment) | 55 | 1631419 | 4.39 | <.0001 | Individual(Environment) | 53 | 993730 | 2.97 | <.0001 | Individual(Environment) | 78 | 1305079 | 3.70 | <.0001 |
| Cell | 2 | 292949 | 21.69 | <.0001 | Cell | 2 | 324047 | 25.66 | <.0001 | Cell | 2 | 516837 | 57.07 | <.0001 |
| Cell x Environment | 2 | 39692 | 2.94 | 0.0571 | Cell x Environment | 2 | 907 | 0.07 | 0.9308 | Cell x Environment | 2 | 4392 | 0.48 | 0.6167 |
| Error | 110 | 742712 | | | Error | 106 | 669382 | | | Error | 156 | 706399 | | |

For each developmental stage, we carried out an ANOVA (JMP 6.0) testing for the fixed effects of *environment*, *individual(environment)*, *cell*, and the interaction between *environment* and *cell* using mean signal intensity as a response variable. The inclusion of the effect *individual(environment)* allowed us to control for the non-independence between measures of P5.p, P6.p, and P7.p taken from a single individual. For a graphical presentation of the data, see Figures S9 to S11.

**Table S4. Deviant Vulval Cell Fate Patterns in Mutation Accumulation Lines (Derived from N2) (Vassilieva et al., 2000)**

| MA line | N | Hypo-induction | Lineage Error | Hyper induction | Centering P5.p | Centering P7.p | P4.p undivided | P8.p undivided | P4.p missing | P8.p missing | Extra divisions | Gonad/ Other |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2.406 | 51 | 12 | 1 | 1 | 2 | | 2 | none | 1 | | | |
| 3.424 | 41 | | | | | 1 | 3 | | | | | 1 |
| 4.407 | 46 | | | | | | | | | | | |
| 9.423 | 58 | 1 | | | | | | | | | | |
| 13.427 | 56 | | | | | | | | | | | |
| 14.42 | 55 | | | | | | | | | | | |
| 16.419 | 56 | | | | | | | | | | | |
| 18.423 | 45 | | | | | | 1 | | | | | |
| 21.414 | 50 | | | | | | 2 | | | | | |
| 23.423 | 44 | | | 1 | 1 | | | | | | | |
| 28.413 | 49 | | | | | | | | | | | |
| 29.428 | 49 | | | | | | | | | | | |
| 30.413 | 46 | | | | | | | | | | | |
| 31.425 | 58 | | | | | | | | | | | |
| 32.389 | 49 | | | | **1** | | | | | | | |
| 35.411 | 51 | | | | | | | | | | **1** | |
| 38.418 | 46 | | | | | | **1** | | | | | |
| 39.413 | 56 | | | | | **1** | **1** | | | | | |
| 40.425 | 46 | 1 | | 1 | | | 2 | | | | | |
| 41.399 | 52 | 1 | | | 1 | | 1 | | 1 | | | |
| 42.429 | 45 | | | | | | | | | | | |
| 46.425 | 50 | 1 | | | | | | | | | | |
| 47.427 | 44 | | | 1 | 1 | | | | | | | |
| 49.402 | 55 | | | | | | | | | | | |
| 50.415 | 42 | | | | | | 2 | | | | | |
| 51.425 | 45 | | | | | | | | | | | |
| 53.415 | 60 | | | | | 1 | 9 | | | | | |
| 59.28 | 42 | | | | | | 2 | | | | | 14 |
| 60.428 | 43 | | | | | | | | | | | |
| 61.42 | 43 | | | | | | | | | | | |
| 62.422 | 49 | | | | | | | | | | | |
| 63.384 | 45 | | | | | | | | | | | |
| **Control Lines** | **N** | **Hypo-induction** | **Lineage Error** | **Hyper induction** | **Centering P5.p** | **Centering P7.p** | **P4.p undivided** | **P8.p undivided** | **P4.p missing** | **P8.p missing** | **Extra divisions** | **Gonad/ Other** |
| 1 | 50 | | | | | | | | | | | |
| 2 | 50 | | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 50 | | | | 1 | | 1 | | | | | |
| 4 | 50 | | | | | | | | | | | |
| 5 | 50 | | | | | | | | | | | |
| 6 | 50 | | | | | | 1 | | | | | |
| 7 | 50 | | | | | | | | | | | |
| 8 | 50 | | | | | | 1 | | | | | |
| 9 | 50 | | | | | | | | | | | |
| 10 | 50 | | | | | | | | | | | |
| 11 | 50 | | | | | | | | | | | |
| 12 | 50 | | | | | | | | | | | |
| 13 | 50 | | | | | | | | | | | |
| 14 | 50 | 1 | | | | | | | | | | |
| 15 | 50 | | | | | | | | | | | |
| 16 | 50 | | | | | | | | | | | |
| 17 | 50 | | | | | | | | | | | |
| 18 | 50 | | | | | | | | | | | |
| 19 | 50 | | | | | | | | | | | |
| 20 | 50 | | | | | | | | | | | |

See also Figure 6.
For each MA and control line, the number of individuals with a given developmental variant are shown.

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