The other side of phenotypic plasticity: a developmental system that generates an invariant phenotype despite environmental variation

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Understanding how the environment impacts development is of central interest in developmental and evolutionary biology. On the one hand, we would like to understand how the environment induces phenotypic changes (the study of phenotypic plasticity). On the other hand, we may ask how a development system maintains a stable and precise phenotypic output despite the presence of environmental variation. We study such developmental robustness to environmental variation using vulval cell fate patterning in the nematode *Caenorhabditis elegans* as a study system. Here we review both mechanistic and evolutionary aspects of these studies, focusing on recently obtained experimental results. First, we present evidence indicating that vulval formation is under stabilizing selection. Second, we discusss quantitative data on the precision and variability in the output of the vulval developmental system in different environments and different genetic backgrounds. Third, we illustrate how environmental and genetic variation modulate the cellular and molecular processes underlying the formation of the vulva. Fourth, we discuss the evolutionary significance of environmental sensitivity of this developmental system.

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1. Introduction

1.1 *Definitions*

Phenotypic plasticity describes the property of a genotype to generate phenotypic variation in response to environmental variation (Stearns 1989). When the environment induces such phenotypic variation at the developmental level, we speak of developmental plasticity. However, the term plasticity usually only refers to variation that leads to distinct and irreversible phenotypic outcomes. It is obvious that many environmental factors, e.g. temperature, cause variation in some features of any developmental process (e.g. in its timing) without necessarily causing variation in the final product of this process. Thus, development is always environmentally sensitive but its phenotypic outcome may not be. To describe such insensitivity of the phenotypic outcome to environmental (as well as mutational and stochastic) variation, many different terms have been used, including robustness, developmental stability, canalization or homeostasis (Waddington 1942; Debat and David 2001; Nijhout 2002; de Visser *et al*. 2003; Kitano 2004; Flatt 2005; Wagner 2005). Phenotypic plasticity and environmental robustness thus refer to environmental sensitivity and insensitivity of the phenotypic outcome, respectively. Note that environmental sensitivity or insensitivity depends on a relevant set of environments, which should therefore be defined when the term "robustness" is used.

1.2. *Evolutionary significance of interactions between development and the environment*

Despite important exceptions (e.g. Goldschmidt 1938; Waddington 1942; Schmalhausen 1949; Bradshaw 1965), it was only relatively recently that interactions between

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development and environment received increased attention, particularly in evolutionary biology. It has become evident that adaptive plasticity of developmental mechanisms and resulting phenotypes is common in virtually all organisms (Stearns 1989; Sultan 1992; Scheiner 1993; Nijhout 1999; Pigliucci 2001; West-Eberhard 2003). Moreover, such plasticity has been hypothesized to play major roles in the process of evolutionary diversification (West-Eberhard 1989, 2003), for example, through processes of genetic accommodation (Waddington 1942; Braendle and Flatt 2006). More recently, the evolutionary consequences of robustness have become of central interest because robustness of a given phenotype to environmental variation may affect its evolvability, i.e. its capacity to evolve (Hansen and Houle 2004; Flatt 2005; Wagner 2005). Environmental robustness of the phenotype is likely to correlate with mutational robustness (insensitivity to mutation) of the phenotype (Houle *et al*. 1996; Ancel and Fontana 2000; Meiklejohn and Hartl 2002). Thus, robustness of the phenotype can have seemingly paradoxical evolutionary consequences. On the one hand, robustness will reduce the capacity to evolve at the phenotypic level, since robustness decreases the expression of phenotypic variation. On the other hand, as the phenotype becomes robust to mutational variation, the underlying genotype accumulates genetic changes; thus, the capacity to evolve increases at the genetic level. Such genetic variation that does not translate into phenotypic variation is often termed hidden or cryptic genetic variation (Gibson and Dworkin 2004). Cryptic genetic variation might be uncovered (i.e. phenotypically expressed) as result of mutation or change in environmental conditions; selection might then act on this variation. Thus, cryptic genetic variation, once released, may potentially play a role in phenotypic evolution.

The degree of environmental sensitivity – ranging from extreme phenotypic plasticity to extreme environmental robustness – may affect the capacity to evolve at genotypic and phenotypic levels. Yet, these inferences mainly stem from theoretical work and very little is understood about how environmental sensitivity is mediated by molecular and cellular mechanisms during development. As a consequence, we have limited insights into how environmental sensitivity of the phenotype evolves and how it affects evolutionary change.

1.3 *Developmental system features contributing to robustness*

Theoretical work shows that many features of developmental systems may lead to their robustness when facing environmental, genetic and stochastic variation: basic genetic mechanisms are epistasis and pleiotropy and may involve systemic properties, such as redundancy, network topology or modularity (von Dassow *et al*. 2000; Meir *et al*. 2002; Siegal and Bergman 2002; Wagner 2005; Azevedo *et al*. 2006). These studies suggest that developmental systems can tolerate extensive variation in diverse parameters. Recent experimental studies have started to uncover specific mechanisms that may contribute to the robustness of developmental systems in a given environment (e.g. Eldar *et al*. 2002; Houchmandzadeh *et al*. 2002; Eldar *et al*. 2003; Surkova *et al*. 2008). These examples show that diverse features at different levels of biological organization may cause developmental robustness, thereby maintaining an invariant and precise phenotypic outcome. Despite these advances in understanding the mechanisms underlying developmental robustness, we still know very little about how developmental systems generate robustness when exposed to variation in ecologically relevant conditions. Hence, the significance of proposed mechanisms conferring robustness to environmental variation – the main source of developmental perturbations – remains unclear.

1.4 *Study system: C. elegans vulval formation*

In this paper, we discuss mechanistic and evolutionary aspects of developmental robustness. We focus on a developmental system underlying the formation of an invariant phenotype: *Caenorhabditis* vulval cell fate patterning. Please refer to our previous reviews concerning more general aspects of robustness and environmental sensitivity of this developmental system (Félix and Wagner 2007; Braendle *et al*. 2008). Here we focus on recent studies, which have aimed to measure the precision of this system and to characterize deviant outputs (section 2); the environmental sensitivity of the vulval cell fate patterning process (section 3); the evolution of *Caenorhabditis* vulval cell fate patterning (section 4) (Félix 2007; Braendle and Félix 2008; Milloz *et al*. 2008).

C. elegans vulval formation has served as a prominent genetic model to elucidate how molecular pathways control intercellular signalling events. As a consequence, the cellular and molecular processes underlying this developmental system are very well understood (Sternberg 2005). The *C. elegans* vulva forms through a subset of ventral epidermal blast cells, the Pn.p cells (posterior descendants of Pn cells) (figure 1). During the first larval stage, six of these cells, P3.p to P8.p, express the Hox gene *lin-39*, which prevents them from fusing to the syncytial epidermis hyp7 and renders them competent to adopt a vulval cell fate (Clark *et al*. 1993; Eisenmann *et al*. 1998; Maloof and Kenyon 1998; Wagmaister *et al*. 2006). P3.p to P8.p represent the vulva precursor cells and form the vulva competence group. Note that P3.p may alternatively behave like P4.p (i.e. it divides once) or fuse with the epidermis at the end of the L2 stage

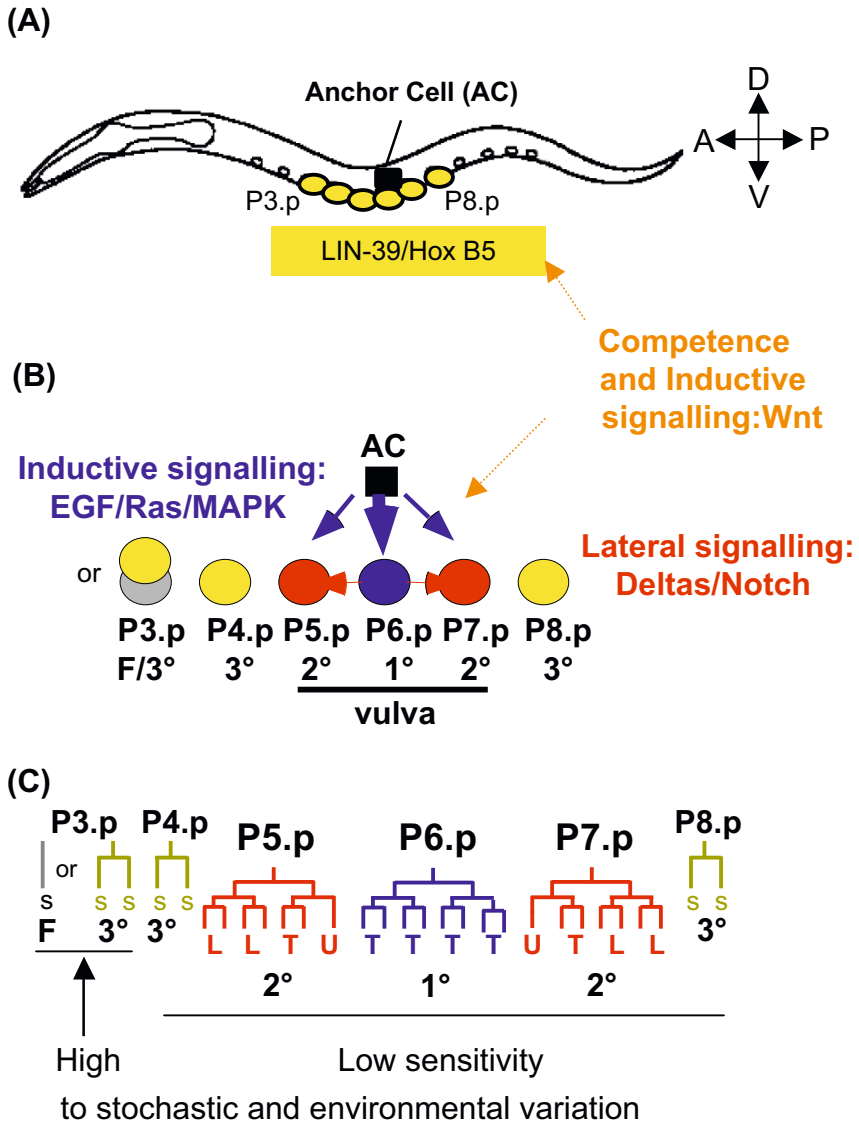

Figure 1. *C. elegans* vulval development**.** Vulval cell fate specification. (**A**) Establishment and maintenance of the vulval competence group in the L1 and L2 stages. (**B**) Specification of the pattern of three vulval precursor cell fates during the early L3 stage. (**C**) Cell lineages. AC, anchor cell; T, transverse (left-right) division; L, longitudinal (antero-posterior) division; U, undivided; S, fusion to the epidermal syncytium in the L3 stage; F, fusion to the syncytium in the L2 stage (S and F, non-vulval fates).

(Sulston and Horvitz 1977; Delattre and Félix 2001). In the L3 stage, the vulval precursor cells adopt alternative cell fates. While all six cells are capable of forming all vulval cell types, only three of them (P5.p to P7.p) will give rise to the vulva (Sulston and White 1980; Sternberg and Horvitz 1986). The central cell, P6.p, adopts the 1° fate while its neighbouring cells, P5.p and P7.p, adopt the 2° fate. P3.p, P4.p and P8.p usually adopt a non-vulval (3° or F) fate, and their progeny fuse to the epidermal syncytium (Horvitz and Sternberg 1991). The pattern of three cell fates is induced via a LIN-3/EGF signal emanating from a specialized cell of the somatic gonad, the anchor cell. This signal activates a Ras/MAPK signalling cascade via the LET-23/EGFR receptor (Aroian *et al*. 1994). P6.p, the cell closest to the anchor cell, receives the highest dose of LIN-3/EGF, causing it to adopt the central 1° vulval fate (Katz *et al*. 1995). Ras/MAPK signalling in P6.p also activates transcription of Delta-like molecules. This lateral signal results in LIN-12/Notch activation, which induces P5.p and P7.p to adopt the 2° fate and prevents them from adopting the 1° vulval fate by inhibiting the Ras pathway (Berset *et al*. 2001; Yoo *et al*. 2004). P3.p, P4.p and P8.p adopt a non-vulval fate because they receive insufficient levels of either signal. In addition to Ras and Notch pathways, components of a

canonical Wnt pathway control *lin-39/HoxB5* expression and maintain competence of vulval precursor cells in the L2 stage (Eisenmann *et al*. 1998). Furthermore, Wnt signalling may also play a role in inducing vulval cell fates and thus shows partial functional redundancy with the Ras pathway (Gleason *et al*. 2002). During the mid to late L3 stage, this patterning process results in invariant fate-specific cell divisions, giving rise to a total of 22 vulval cells (figure 1C). During the L4 stage, vulval morphogenesis takes place and the final organ forms at the final moult.

Here we first discuss how the environment affects the precision of the vulval phenotype. Second, we ask how the underlying developmental processes respond to the environment. Third, we ask how the vulval developmental system evolves.

2. Precision of the final vulval phenotype in different environments

2.1 *Stabilizing selection on the final vulval phenotype*

The *C. elegans* vulval cell fate pattern is evolutionarily conserved, with all *Caenorhabditis* species showing an identical vulval cell lineage (Félix 2007). Such evolutionary stasis of a trait is indicative of stabilizing selection (Hansen and Houle 2004) as expected for a robust trait.

The vulva of the *C. elegans* hermaphrodite is required for egg laying and mating with males. Defects in this reproductive organ can drastically decrease the number of offspring produced and may complicate mating with males. Thus, vulval defects may have negative fitness effects – at least, under laboratory conditions (Braendle and Félix, 2008). As invariance and precision of the vulval phenotype are linked to fitness, we might expect that factors increasing the robustness of the underlying developmental process be favoured by selection.

We examined experimentally whether the vulval patterning process may be under stabilizing selection by testing whether mutation accumulation lines show a decreased precision in the establishment of the vulval pattern. Independent mutation accumulation lines were derived from the *C. elegans* N2 strain by isolating single random larvae in each of approximately 400 successive generations. This treatment results in relaxed selection as mutations spontaneously arising in each generation were allowed to accumulate (Vassilieva and Lynch 1999). We found that the proportion of lines with developmental deviations in the vulva phenotype was significantly higher in mutation accumulation lines than in control N2 lines (Braendle and Félix 2008). These deviant pattern outputs included the loss of competence of Pn.p cells, vulval centering shifts and hypoinduction. In contrast, such developmental deviations occurred very rarely in natural wild isolates in the same standard environment (Braendle and Félix 2008), despite substantial genetic variation among these isolates (Dolgin *et al*. 2007). These results suggest that spontaneous mutations triggering these developmental deviations are apparently counterselected in the wild. Hence, precision and robustness of vulval development are likely under selection (Braendle and Félix 2008).

2.2 *Quantifying precision of vulval patterning in different environments*

The process of *Caenorhabditis* vulval cell fate patterning seems to be robust to stochastic (within-environment) variation since errors in vulval formation are very rare under standard laboratory conditions. A study by Delattre and Félix (2001) showed that of more than 2000 individuals examined (*C. elegans, C. briggsae* and *C. remanei*) only a single individual had a deviant vulval pattern, likely leading to a non-functional vulva. In addition, about 1% of individuals showed vulval patterns that deviated from the canonical pattern, but without causing non-functionality of the vulva (e.g. a fused P4.p or P8.p cell). These results indicate that the final vulval phenotype of several *Caenorhabditis* isolates is indeed robust to stochastic variation within the standard environment.

We have further examined how the final vulval phenotype responds to variation in several laboratory environments (Braendle and Félix 2008). We assayed the *C. elegans* reference isolate, N2, in multiple environments: standard conditions at different temperatures (15, 20, 25°C), starvation in the L2 stage, liquid culture and conditions in which individuals passed through the dauer stage (an alternative diapausing L3 stage). In all conditions, defects leading to an abnormal vulva (e.g. hypoinduction) were rarely more frequent than in standard conditions. Having assayed more than 10,000 individuals (several isolates of *C. elegans* and *C. briggsae*) in multiple conditions, we estimate that actual errors causing a vulval defect affect $< 0.3\%$ of individuals. Thus, the process of vulval formation appears relatively robust to a range of environmental conditions, including stressful conditions.

Furthermore, we found deviations from the canonical vulval cell fate pattern (P3.p to P8.p) that did not lead to defects (figure 2). The frequency of such deviant patterns differed between environments. For example, individuals of the reference *C. elegans* strain N2 in the starvation environment showed a consistently increased propensity to center their vulva on P5.p instead of P6.p (but this developmental variant was never observed in standard environments). Such an anterior shift in vulval centering does not prevent the formation of a functional vulva because

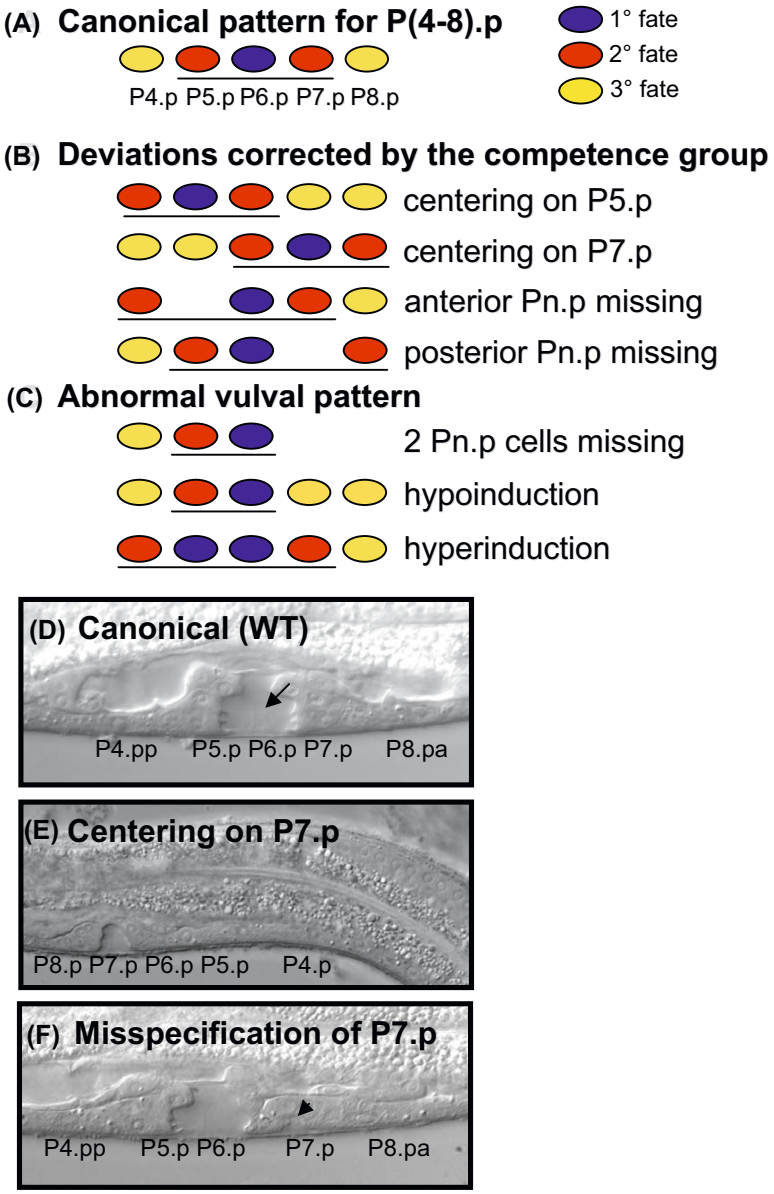

Figure 2. Canonical and deviant vulval cell fate patterns. (**A**) Canonical (wild-type) vulval cell fate pattern of P4.p to P8.p. P3.p is not represented. To each fate corresponds a specific cell lineage in the L2 to L3 stages (see figure 1). Cells adopting vulval fates are underlined. (**B**) Variant patterns that deviate from the canonical pattern but still allow establishment of a functional vulva with a complete 2°-1°-2° pattern, because of the presence of additional competent cells. (**C**) Strongly disrupted deviant patterns leading to a non-functional vulva. (**D–F**) Nomarski images of wild-type vulva and two developmental deviations of the *C. elegans* isolate N2. (**D**) Wild-type vulva (mid L4 stage). (**E**) Vulval centering on P7.p (early L4 stage). (**F**) Hypoinduction and additional vulval invagination of some P7.p progeny (arrowhead) (mid L4 stage), likely leading to a non-functional vulva.

P4.p is competent to form vulval tissue. These results indicate that certain environmental conditions may cause specific developmental changes while maintaining the production of a correct phenotypic outcome.

Overall, the quantitative analysis of vulval phenotypes in different environments demonstrates that the frequency spectrum of variant outputs depends on both environment and genetic background.

3. Environmental sensitivity of the vulval cell fate patterning process

Although the final vulval phenotype appears robust and shows little environmental variability, circumstantial evidence indicates that the underlying molecular processes may be sensitive to environmental changes. Ferguson and Horvitz (1985) reported variation in the penetrance of

several loss-of-function mutations in genes of the EGF/Ras/MAPK signalling cascade (Ferguson and Horvitz 1985). Such mutations (e.g. in lin-3/egf) result in vulval defects due to an induction of fewer than three vulval precursor cells (hypoinduction). However, individuals that were starved or underwent dauer formation showed considerable suppression of some of these mutant phenotypes relative to normal growth conditions. How such environmental and physiological inputs may be transduced to modify mechanisms underlying vulval cell fate patterning has been elucidated in more detail by two studies. The first study (Moghal et al. 2003) showed how the environment may cause physiological changes to modify pathway activities during vulval patterning. Culture in liquid medium led to suppression of loss-of-function mutants of the EGF/Ras/MAPK cascade. This result suggests that liquid growth may increase levels of vulval inductive signalling. The observed suppression requires the heterotrimeric Gq protein EGL-30 (expressed in neurons) and the voltage-gated calcium channel EGL-19 (expressed in muscle cells). Thus, environmental modification of vulval induction could be mediated by neuronal and muscle cells. The environmental effect transduced by EGL-30 acts in parallel or downstream of the EGF/Ras/MAPK pathway. As the effect requires Wnt signalling via BAR-1/β-catenin, the signals involved may act through the Wnt pathway. This is consistent with the hypothesis that partial redundancy of Wnt and EGF/Ras/MAPK pathways during vulval induction may be important to maintain robustness of the final cell fate output pattern in varying environments. The second study (Battu et al. 2003) showed that starvation conditions might modulate vulval induction via the G-coupled receptor SRA-13 and the G protein GPA-5, which are expressed in body wall muscles and sensory neurons. In their experimental assay, starvation stimuli caused a decrease in vulval induction as inferred from mutant analyses, indicating that the EGF/Ras/MAPK cascade may be modified by environmental inputs. However, where this input acts to affect vulval induction is not known.

To systematically explore how the environment affects the vulval signalling network, we measured the penetrance of approximately 50 vulval mutations in six different environmental conditions. The penetrance of most mutations affecting vulval development varied significantly among environments. For example, a strong hypomorphic mutation in the EGF inducing signal, *lin-3(n378)/egf*, was strongly suppressed upon starvation, whereas a null *bar-1/β-catenin* (regulated by a canonical Wnt pathway) mutation was enhanced (Braendle and Félix 2008).

Taken together, these studies indicate that the molecular mechanisms underlying vulval formation are sensitive to the environment, but it remained unclear to what extent

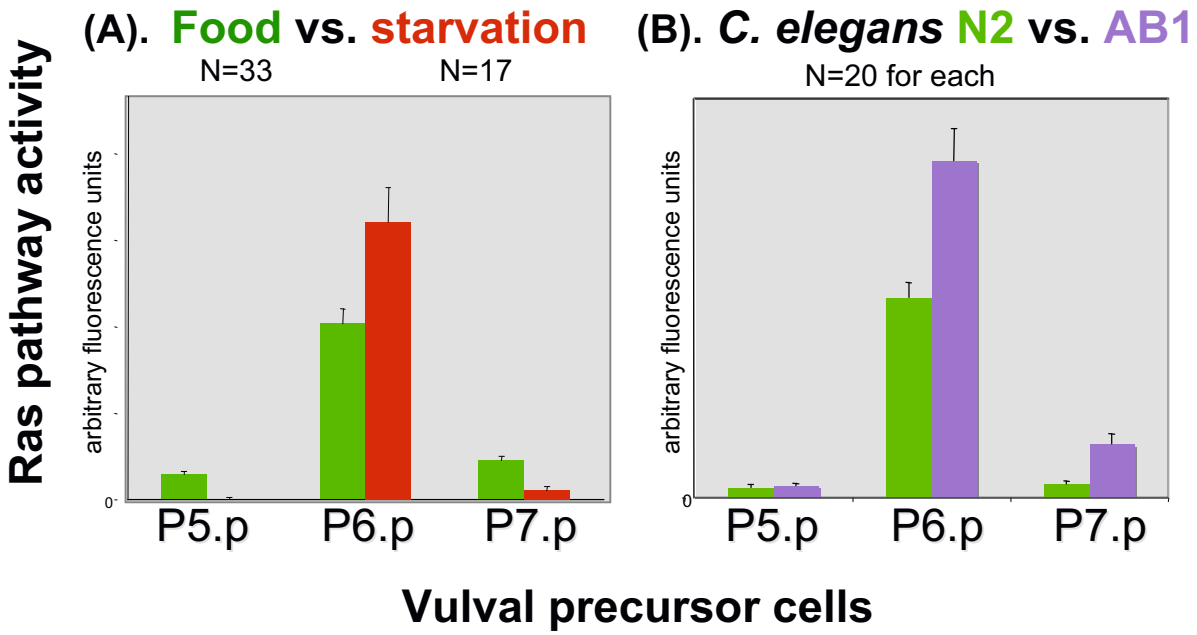

Figure 3. Variation in an intermediate developmental phenotype in different environments or wild genetic backgrounds. The level of a Ras transcriptional reporter, *egl-17::CFP*, is plotted on the y-axis for the P5.p, P6.p and P7.p cells. Quantification was performed using fluorescence microscopy on L2 lethargus worms and expressed in arbitrary units on a linear scale. (**A**) Reporter activity level varies significantly in each cell depending on the environment (single *C. elegans* isolate N2) (modified from Braendle and Félix 2008). (**B**) Reporter activity level in P6.p varies significantly depending on the *C. elegans* wild genetic background (assayed in the standard laboratory environment) (modified from Milloz *et al* 2008).

such modifications occur in the wild-type situation. We therefore directly measured intermediate phenotypes of the developmental system. First, we observed that the position of the anchor cell relative to P5.p, P6.p and P7.p varied stochastically between individuals. Second, the level of activity of the Ras and Notch pathways (as measured using downstream transcriptional reporters) varied between environments. We measured Ras and Notch pathway activities using downstream transcriptional reporters (*egl-17p::CFP* and *lip-1p::GFP* or *YFP*, respectively) and found higher Ras reporter expression after L2 starvation (Braendle and Félix 2008) (figure 3A). In conclusion, the developmental system is sensitive to stochastic and environmental variation, but this sensitivity does not translate into variation in the system's output. Thus, *C. elegans* vulval formation is buffered against a range of relevant developmental perturbations.

Possible mechanistic features of the vulval developmental system causing robustness to environmental variation are reviewed in Félix and Wagner (2008) and Braendle *et al*. (2008). In addition, the characterization of deviant output phenotypes in different environmental conditions revealed specific features that allow for a functional vulva despite variation in the developmental process. In particular, functional redundancy among vulval precursor cells allows formation of a complete vulva when one competent Pn.p cell is missing or when the pattern is abnormally centered.

4. Robustness and evolution of *Caenorhabditis* vulval cell fate patterning

The vulval phenotype (the fate pattern and the corresponding cell division patterns) of *C. elegans* is conserved in all known *Caenorhabditis* species. Recent experiments show that this morphological stasis underlies considerable evolutionary divergence in underlying developmental mechanisms. To reveal such cryptic evolution, we used several experimental approaches to debuffer the system (anchor cell ablations, EGF overexpression, mutations) and by measuring intermediate developmental phenotypes (Ras and Notch pathway reporters).

In different *Caenorhabditis* species, anchor cell ablations experiments at different developmental time points and mild EGF overexpression revealed many differences in the regulation of vulval cell fate patterning (Félix 2007). When the anchor cell had been ablated at different times during the induction process, P6.p was directly induced to adopt a 1° fate in a small group of *Caenorhabditis* species while an intermediate 2° fate was found in most others. Phylogenetic comparison of nematode vulval development shows that the mode of direct induction of P6.p appeared within the *Caenorhabditis* genus, but was secondarily lost in *C. briggsae*, *C. remanei* and *C. sp. 5* (Félix 2007; Kiontke *et al*. 2007). Surprisingly, in *C. remanei*, anchor cell ablation experiments generated an intermediate pattern of P(5-7).p with the cell fates 2°-3°-2°. This pattern could result from evolution in the relative weights of the two events downstream of Ras in P6.p, with a higher threshold for 1° fate induction and a lower one for lateral signalling in *C. remanei*. Such hypotheses suggesting quantitative changes in the molecular network underlying vulval fate patterning were successfully tested using mutants, transgenesis and RNAi on Ras, Wnt and Notch pathway components (Félix 2007).

To examine intraspecific cryptic variation of *C. elegans* vulval development, Milloz *et al*. (2008) introgressed vulval mutations (isolated in the reference strain N2) into six different wild isolates by repeated backcrossing. The effect of a given mutation was highly variable depending on the genetic background. Of the 12 mutations affecting the three signalling pathways (Ras, Notch and Wnt), the penetrance of nearly every mutation varied significantly among backgrounds. Strikingly, the induction index of Ras pathway mutations was always higher in the AB1 genetic background while a *bar-1(0)*/β-*catenin* (Wnt pathway) mutation was enhanced in other backgrounds than N2.

The anchor cell was ablated at different time points during vulval induction in three *C. elegans* isolates: mild but significant differences among the N2, CB4856 and AB1 isolates were found in the timing and intermediate cell fate pattern. The final cell fate pattern was reached earlier in AB1 than in N2. Finally, Ras and Notch pathway activities were measured using downstream transcriptional reporters and Ras reporter expression was found to be higher in AB1 than in N2 (figure 3B). Cryptic variation between these two strains is thus reflected in Ras pathway activity differences. Consequently, as for environmental variation, intra-specific genetic variation modulates Ras pathway activity in vulval precursor cells (figure 3), yet such variation does not translate into variation in the cell fate pattern output.

The intra- and interspecific analyses of *Caenorhabditis* vulval development indicate that its underlying genetic and developmental architecture evolves. These observations are therefore consistent with the hypothesis that robustness of the phenotype may allow for evolution of its underlying mechanisms (True and Haag 2001; Gibson and Dworkin 2004; Flatt 2005; Wagner 2005). Such a scenario would imply that primarily non-adaptive forces cause such "developmental system drift" (True and Haag 2001). Whether and how evolutionary divergence of vulval developmental mechanisms is further influenced by selection is currently unclear. However, the current data suggest that different environments likely impose different selective pressures on this developmental system, which may also shape its evolution.

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References

Ancel L W and Fontana W 2000. Plasticity, evolvability, and modularity in RNA; *J. Exp. Zool*. **288** 242–283

Aroian R V, Lesa G M and Sternberg P W 1994 Mutations in the *Caenorhabditis elegans let-23* EGFR-like gene define elements important for cell-type specificity and function; *EMBO J.* **13** 360–366

Azevedo R B, Lohaus R, Srinivasan S, Dang K K and Burch C L 2006 Sexual reproduction selects for robustness and negative epistasis in artificial gene networks; *Nature (London)* **440** 87–90

Battu G, Hoier E F and Hajnal A 2003 The *C. elegans* G-protein-coupled receptor SRA-13 inhibits RAS/MAPK signalling during olfaction and vulval development; *Development* **130** 2657–2577

Berset T, Hoier E F, Battu G, Canevascini S and Hajnal A 2001 Notch inhibition of RAS signaling through MAP kinase phosphatase LIP-1 during *C. elegans* vulval development; *Science* **291** 1055–1058

Bradshaw A D 1965 Evolutionary significance of phenotypic plasticity in plants; *Adv. Genet.* **13** 115–154

Braendle C, and Félix M-A 2008 Plasticity and errors of a robust developmental system in different environments; *Dev. Cell* (in press)

Braendle C and Flatt T 2006. A role for genetic accommodation in evolution?; *BioEssays* **28** 868–873

Braendle C, Milloz J, and Félix M-A 2008 Mechanisms and evolution of environmental responses in *Caenorhabditis elegans*; *Curr. Topics Dev. Biol.* **80** 171–207

Clark S G, Chisholm A D and Horvitz H R 1993 Control of cell fates in the central body region of *C. elegans* by the homeobox gene *lin-39*; *Cell* **74** 43–55

de Visser J A, Hermisson J, Wagner G P, Ancel Meyers L, Bagheri-Chaichian H, Blanchard J L, Chao L, Cheverud J M, *et al*. 2003 Perspective: Evolution and detection of genetic robustness; *Evolution* **57** 1959–1972

Debat V and David P 2001 Mapping phenotypes: canalization, plasticity and developmental stability; *TREE* **16** 555–561

Delattre M and Félix M-A 2001 Polymorphism and evoluton of vulval precursor cell lineages within two nematode genera, Caenorhabditis and Oscheius; *Curr. Biol.* **11** 631–643

Dolgin E S, Félix M-A and Cutter A D 2007 Hakuna Nematoda: Genetic and phenotypic diversity in African isolates of *Caenorhabditis elegans* and *C. briggsae*; *Heredity* **100** 304–315

Eisenmann D M, Maloof J N, Simske J S, Kenyon C and Kim S K 1998 The β-catenin homolog BAR-1 and LET-60 Ras coordinately regulate the Hox gene *lin-39* during *Caenorhabditis elegans* vulval development; *Development* **125** 3667–3680

Eldar A, Dorfman R, Weiss D, Ashe H, Shilo B Z and Barkai N 2002 Robustness of the BMP morphogen gradient in *Drosophila* embryonic patterning; *Nature (London)* **419** 304–308

Eldar A, Rosin D, Shilo B-Z, and Barkai N 2003 Self-enhanced ligand degradation underlies robustness of morphogen gradients; *Dev. Cell* **5** 635–646

Félix M-A 2007 Cryptic quantitative evolution of the vulva intercellular signaling network in *Caenorhabditis*; *Curr. Biol.* **17** 103–114

Félix M-A and Wagner A 2007 Robustness and evolution: concepts, insights and challenges from a developmental model system; *Heredity* **100** 132–140

Ferguson E and Horvitz H R 1985 Identification and characterization of 22 genes that affect the vulval cell lineages of *Caenorhabditis elegans*; *Genetics* **110** 17–72

Flatt T 2005 The evolutionary genetics of canalization; *Q. Rev. Biol.* **80** 287–316

Gibson G and Dworkin I 2004 Uncovering cryptic genetic variation; *Nat. Rev. Genet.* **5** 681–690

Gleason J E, Korswagen H C and Eisenmann D M 2002 Activation of Wnt signaling bypasses the requirement for RTK/Ras signaling during *C. elegans* vulval induction; *Genes Dev.* **16** 1281–1290

Goldschmidt 1938 *Physiological genetics* (New York: McGraw-Hill)

Hansen T F and Houle D 2004 Evolvability, stabilizing selection, and the problem of stasis; in *Phenotypic integration: Studying the ecology and evolution of complex phenotypes* (eds) M Pigliucci and K Preston (Oxford: University Press, Oxford) pp 130–150

Horvitz H and Sternberg P 1991 Multiple intercellular signaling systems control the development of the Caenorhabditis elegans vulva; *Nature (London)* **351** 535–541

Houchmandzadeh B, Wieschaus E and Leibler S 2002 Establishment of developmental precision and proportions in the early *Drosophila* embryo. *Nature (London)* **415** 798–802

Houle D, Morikawa B and Lynch M 1996 Comparing mutational variabilities; *Genetics* **143** 1467–1483

Katz, W S, Hill R J, Clandinin T R and Sternberg P W 1995 Different levels of the *C. elegans* growth factor LIN-3 promote distinct vulval precursor fates; *Cell* **82** 297–307

Kiontke K, Barriere A, Kolotuev I, Podbilewicz B, Sommer R J, Fitch D and Félix M-A 2007 Trends, Stasis, and Drift in the Evolution of Nematode Vulva Development; *Curr. Biol.* **17** 1925–1937

Kitano H 2004 Biological robustness; *Nat. Rev. Genet.* **5** 826–837

Maloof J N and Kenyon C 1998 The Hox gene *lin-39* is required during *C. elegans* vulval induction to select the outcome of Ras signaling; *Development* **125** 181–190

Meiklejohn C D and Hartl D L 2002 A single mode of canalization; *Trends Ecol. Evol.* **17** 468–473

Meir E, von Dassow G, Munro E and Odell G M 2002 Robustness, flexibility, and the role of lateral inhibition in the neurogenic network; *Curr. Biol.* **12** 778–786

Milloz J, Duveau F and Félix M-A 2008. Intraspecific evolution of the intercellular signaling network underlying a robust developmental system; *Genes Dev.* (in press)

Moghal N, Garcia L R, Khan L A, Iwasaki K and Sternberg P W 2003 Modulation of EGF receptor-mediated vulva development by the heterotrimeric G-protein Gαq and excitable cells in *C. elegans*; *Development* **130** 4553–4566

Nijhout H F 1999 Control mechanisms of polyphenic development; *Bioscience* **49** 181–192

Nijhout H F 2002 The nature of robustness in development; *Bioessays* **24** 553–563

Pigliucci M 2001 *Phenotypic plasticity: beyond nature and nurture* (Baltimore: Johns Hopkins University Press)

Scheiner S 1993 Genetics and evolution of phenotypic plasticity; *Annu. Rev. Ecol. Syst.* **24** 35–68

Schmalhausen I I 1949 *Factors of evolution* (Philadelphia, PA: Blakiston)

Siegal M L and Bergman A 2002 Waddington's canalization revisited: developmental stability and evolution; *Proc. Natl Acad. Sci. USA* **99** 10528–10532

Stearns S C 1989 The evolutionary significance of phenotypic plasticity; *BioScience* **39** 436–445

Sternberg P W 2005 Vulval development, *Wormbook (ed. The C. elegans Research Community)*, pp. doi/10.1895/wormbook.1.6.1

Sternberg P W and Horvitz H R 1986 Pattern formation during vulval development in *Caenorhabditis elegans*; *Cell* **44** 761–772

Sulston J and Horvitz H R 1977 Postembryonic cell lineages of the nematode *Caenorhabditis elegans*; *Dev. Biol.* **56** 110–156

Sulston J and White J 1980 Regulation and cell autonomy during postembryonic development of *C. elegans*; *Dev. Biol.* **78** 577–597

Sultan S E 1992 Phenotypic plasticity and the neo-darwinian legacy; *Evol. Trends Plants* **6** 61–71

Surkova S, Kosman D, Kozlov K, Manu, Myasnikova E, Samsonova A A,. Spirov A, Vanario-Alonso C E, Samsonova M and Reinitz J 2008 Characterization of the Drosophila segment determination morphome; *Dev. Biol*. **313** 844–862

True J R and Haag E S 2001 Developmental system drift and flexibility in evolutionary trajectories; *Evol. Dev.* **3** 109–119

Vassilieva L L and Lynch M 1999 The rate of spontaneous mutation for life-history traits in Caenorhabditis elegans; *Genetics* **151** 119–129

von Dassow G, Meir E, Munro E M and Odell G M 2000 The segment polarity network is a robust developmental module; *Nature (London)* **406** 188–192

Waddington C H 1942 Canalization of development and the inheritance of acquired characters; *Nature (London)* **150** 563–565

Wagmaister J A, Gleason J E and Eisenmann D M 2006 Transcriptional upregulation of the *C. elegans* Hox gene *lin-39* during vulval cell fate specification; *Mech. Dev.* **123** 135–150

Wagner A 2005 *Robustness and evolvability in living systems* (Princeton, Oxford: Princeton University Press)

West-Eberhard M J 1989 Phenotypic plasticity and the origins of diversity; *Annu. Rev. Ecol. Syst.* **20** 249–278

West-Eberhard M J 2003 *Developmental plasticity and evolution* (Oxford: Oxford University Press)

Yoo A S, Bais C and Greenwald I 2004 Crosstalk between the EGFR and LIN-12/Notch pathways in *C. elegans* vulval development; *Science* **303** 663–666

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