27. S. M. Cohen, G. Bronner, F. Kuttner, G. Jürgens, H. Jäckle, *Nature* **338**, 432 (1989).
28. B. Cohen, A. A. Simcox, S. M. Cohen, *Development* **117**, 597 (1993).
29. S. M. Cohen and G. Jürgens, *EMBO J.* **8**, 2045 (1989).
30. M. Price, M. Lemaistre, R. Ki Lauro, D. Duboule, *Nature* **351**, 748 (1991); P. Dollé, M. Price, D. Duboule, *Differentiation* **49**, 93 (1992).
31. S. M. Cohen, in *Development of* Drosophila, A. Martinez-Arias and M. Bate, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1993), pp. 747–841.
32. Libraries of *P. coenia* cDNA were constructed from polyadenylated mRNA isolated from embryos and wing discs. Clones from cDNA for *ap, dpp,* and *sd* were isolated from the embryo library, and *en/inv* clones were isolated from the disc library by cross-hybridization [D. Klessig and J. O. Berry, *Plant Mol. Biol. Rep.* **1**, 12 (1983)] with *Drosophila* probes, whereas a segment of the *wg (wnt-1)* gene was amplified by polymerase chain reaction (PCR) from genomic DNA [A. Sidow, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5098 (1992)], and the resulting 450-bp fragment was used to probe the embryonic cDNA library. The preparation of DNA and subcloning were performed as by J. Sambrook, E. F. Fritsch, and T. Maniatis [*Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1989)]. Clones from cDNA were sequenced by the method of F. Sanger, S. Nicklen, and A. R. Coulson [*Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)].
33. Photo courtesy of S. Blair.
34. The *P. coenia* butterflies were maintained at 28°C under a 16L:8D photoperiod, and larvae were fed an artificial diet containing *Plantago lanceolata* [R. T. Yamamoto, *J. Econ. Entomol.* **62**I, I427 (1993)]. Fifth-instar larvae were anaesthetized by submersion in distilled water for 30 min, and the imaginal discs were removed under cold phosphate-buffered saline. The preparation of the imaginal discs for in situ hybridization was adapted from J. D. Masucci, R. J. Miltenberger, and F. M. Hoffmann [*Genes Dev.* **4**, 2011 (1990)], the important distinction being the proteinase K treatment. For DNA probes, the discs were treated from 3 to 4 min in proteinase K (100 μg/ml), while for RNA probes, proteinase K (50 μg/ml) was used for 2 to 3 min. Discs were hybridized with 40 ng of digoxigenin-labeled DNA probe (*inv*) or 20 to 50 ng of digoxigenin-labeled RNA probe [J. Jiang, D. Kosman, T. Ip, M. Levine, *Genes Dev.* **5**, 1881 (1991)] in 100 μl of hybridization buffer for 24 hours at 48°C (DNA) or 55°C (RNA). The detection and colorimetric development of digoxigenin-labeled staining was performed as described [J. D. Masucci, R. J. Miltenberger, F. M. Hoffmann (above)] except for the omission of Levamisol. Detailed protocols are available upon request. Localization of *D. melanogaster* gene expression patterns are as follows: The detection of *wg, ap,* and *sd* enhancer trap *lacZ* expression was performed as described (*5*), while *en/inv* gene expression was detected with a monoclonal antibody that recognized both proteins (*25*).
35. We thank F. Nijhout for his encouragement, advice, provision of our colony of *P. coenia,* and for writing a great book; S. Cohen, A. Laughon, F. M. Hoffmann, and S. Campbell for the *Drosophila ap, en, dpp,* and *sd* probes, respectively; T. Hammond and B. Majewski for the help with the cross sections of the wing discs; J. Langeland and A. Laughon for their reviews of the manuscript; L. Olds for help with the illustrations; and J. Wilson for assistance in preparing the text. J.A.W. was supported by a Medical Research Council (Canada) Postdoctoral Fellowship, D.N.K. by an NIH predoctoral traineeship, and G.E.F.P. by an NIH postdoctoral fellowship. Supported by the Shaw Scientists Program of the Milwaukee Foundation and the Howard Hughes Medical Institute.

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Changes of Induction and Competence During the Evolution of Vulva Development in Nematodes

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In *Caenorhabditis*, the vulva is formed in the central body region from three of six equivalent cells and is induced by the gonad. In some nematodes, however, the vulva is located in the posterior body region. Vulval development has been analyzed in three such genera. The same precursor cells give rise to the vulva in *Caenorhabditis* and in the posterior vulva species, but in the latter the cells first migrate posteriorly. In two such species, the vulva is not induced by the gonad, but instead relies on intrinsic properties of precursor cells. Thus, evolution of organ position involves changes in induction and competence.

Morphological change during evolution arises from modification of ontogeny, which implies that an understanding of morphologic evolution will require insight into the evolution of development. To elucidate how developmental processes evolve, it is necessary to have detailed knowledge at the cellular and genetic level of a particular aspect of development, as well as the ability to compare the development of a number of species. The invariant development of free-living nematodes provides a useful experimental system for such an analysis at the single cell level. Formation of the vulva, the egg-laying structure of nematodes, is one tractable aspect of development for an evolutionary developmental analysis.

The vulva of *Caenorhabditis elegans* is a derivative of the ventral epidermis, which consists of 12 ectoblasts (Fig. 1A) (*1*). These 12 cells are equally distributed between the pharynx and anus and are named according to their anterior-posterior position (P1–P12). The gonadal anchor cell (AC) induces three of six vulval precursor cells (VPCs) to adopt vulval fates (*2*). The VPCs (P3–8.p) are descendants of the central ectoblasts. They form a so-called equivalence group because all cells have the potential to adopt vulval fates. During wild-type development, only the three cells centered around the AC, P(5–7).p, respond to the inductive signal by generating vulval cells. The cell closest to the AC, P6.p, has the 1° cell fate and generates eight progeny, whereas the two more distal cells, P5.p and P7.p, have the 2° cell fate, and each generates seven progeny. The three remaining VPCs (P3.p, P4.p, and P8.p) do not contribute to formation of the vulva; instead, they generate nonspecialized epidermis, the 3° fate. The VPCs that normally give rise to 3° cells can make 1° or 2° cells and thus regenerate the vulval pattern after ablation with laser microbeam irradiation of more AC-proximal VPCs (*3*). Extensive genetic and molecular analysis indicates that three intercellular signals—an inductive signal, a lateral signal, and a negative signal—are involved in the specification of the precise pattern of VPC types (*4*).

In *Caenorhabditis* and in most other nematode species, the vulva forms at approximately 50% body length (*5*). However, species with the vulva in the posterior body region are present in many nematode taxa (*5, 6*). The family *Rhabditidae* contains several evolutionary lines with posterior vulva–forming species (*7*). Here, we have used three different genera of the subfamily *Rhabditinae* to study vulva development in species that form a posterior vulva (*8*). In *Cruznema tripartitum* and *Mesorhabditis* sp. PS1179, the vulva forms at 80% body length; in *Teratorhabditis palmarum*, the vulva forms at 95% body length, in a position immediately anterior to the anus (Figs. 1 and 2).

In *Caenorhabditis* and in other members of the family *Rhabditidae* with the vulva in the central body region, P(5–7).p generate the vulva (*1, 9*). In *Panagrellus redivivus* of the distinct family *Panagrolaimidae*, in which the vulva forms at 60% body length, P(4–9).p have the potential to generate vulval tissue (*10*). Because a more posterior cell, P9.p, has the potential to participate in vulva formation in *Panagrellus*, it might be expected that the more posterior Pn.p cells would form the vulva in species with posterior vulvae. However, our cell lineage analysis revealed that the central Pn.p cells form vulval tissue in all three species examined (Fig. 1) (*11*). In *Mesorhabditis* and *Teratorhabditis* P(4–8).p are VPCs; in *Cruznema* P(3–8).p are VPCs. In the first larval stage (L1) of all three species, the 12 Pn.p ectoblasts are located in order along the anterior-posterior axis as they are in *Caenorhabditis* (Figs. 1 and 2A). The VPCs migrate posteriorly during the second larval stage (L2), adopting a species-specific position. In *Cruznema*, P(3–8).p lie anterior to P(9–11).p (Fig. 1B). In *Mesorhabditis*, the VPCs lie in the same region as P(9,10).p (Fig. 1C). In *Teratorhabditis*, the VPCs move posterior to P(9–11).p (Fig. 1D) (*12*). P(5–7).p have vulval cell fates in all three species, but

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cell lineages generated by the VPCs differ between species (Fig. 1, B to D). Because the same set of precursors assume vulval fates in species with central or posterior vulvae, we suggest a regulatory constraint exists at the cellular level that requires central cells to migrate to the posterior and form the vulva rather than allowing posterior cells to become competent to form the vulva.

In species with posterior vulva formation, contact between the gonadal AC and the VPCs is delayed relative to species with a central vulva (Fig. 1, arrows in lineage diagrams) (*5*, *7*). This delay correlates with different gonad morphology in the central and posterior vulva species. *Caenorhabditis* and most other species with a central vulva have two ovaries located symmetrically about the vulva (didelphic gonad). In contrast, species with a posterior vulva have a single ovary directed anteriorly from the vulva (monodelphic gonad). In *Cruznema*, *Mesorhabditis*, and *Teratorhabditis*, the monodelphic gonad develops from a gonad primordium located in the central body region (Figs. 1, A to D, and 2A). During the L2 and L3 stages, the somatic gonad grows posteriorly, but only contacts the VPCs late in the L3 stage. Thus, in the posterior vulva species there is no contact between the AC and the VPCs in the early L3 stage when vulva induction occurs in *Caenorhabditis*. How does vulval cell fate specification occur in the posterior vulva species?

A different timing of vulva induction solves the problem of the delayed contact between the gonad and the VPCs in *Cruznema*. Cell ablation experiments demonstrate that induction takes place after the AC reaches the VPCs (Fig. 3). In the early L3 stage, the VPCs lie in their final position at 80% body length (Fig. 3A). The gonad has extended posteriorly, but has not yet reached the VPCs. The VPCs do not divide until the AC reaches P6.p late in the L3 stage (*13*) (Fig. 3B). Thirty to forty-five minutes after contact between the AC and P6.p is established, the VPCs start to divide, suggesting that the AC induces vulval fates (five animals followed) (*13*). Indeed, ablation of the somatic gonad precursors Z1 and Z4 in the L1 stage or ablation of the AC in the L3 stage prevents vulva formation; instead, the VPCs form epidermis (Fig. 3, C and D). We conclude that vulva formation in *Cruznema* is gonad-dependent and that in comparison to *Caenorhabditis*, the signaling event takes place later in the L3 stage.

Changing cell-cell interactions involved in vulva induction might be an alternative way to compensate for the delayed contact between gonad and VPCs. In *Mesorhabditis* and *Teratorhabditis*, vulva development is not induced by the AC. In both species, the VPCs undergo two rounds of cell division before the AC contacts the forming vulva (*13*) (Figs. 2C and 4B). Ablation of Z1 and Z4, the potential precursors of the AC, in the early L1 stage does not affect vulva formation (Figs. 2E and 4C and Table 1). We believe it is unlikely that the gonadal precursors induce the vulva during embryogenesis, because we observed regulation of vulval cell fates after ablation of individual VPCs later in development (Table 1). This implies that signals necessary for patterning are still active.

A simple hypothesis is that another single cell could replace the AC and induce the vulva. However, we have been unable to prevent vulva differentiation by ablation of cells other than the VPCs (*14*). Therefore, it is possible that processes autonomous to the VPCs are responsible for vulva formation. We cannot, however, eliminate the possibility that a group of cells induces vulva formation.

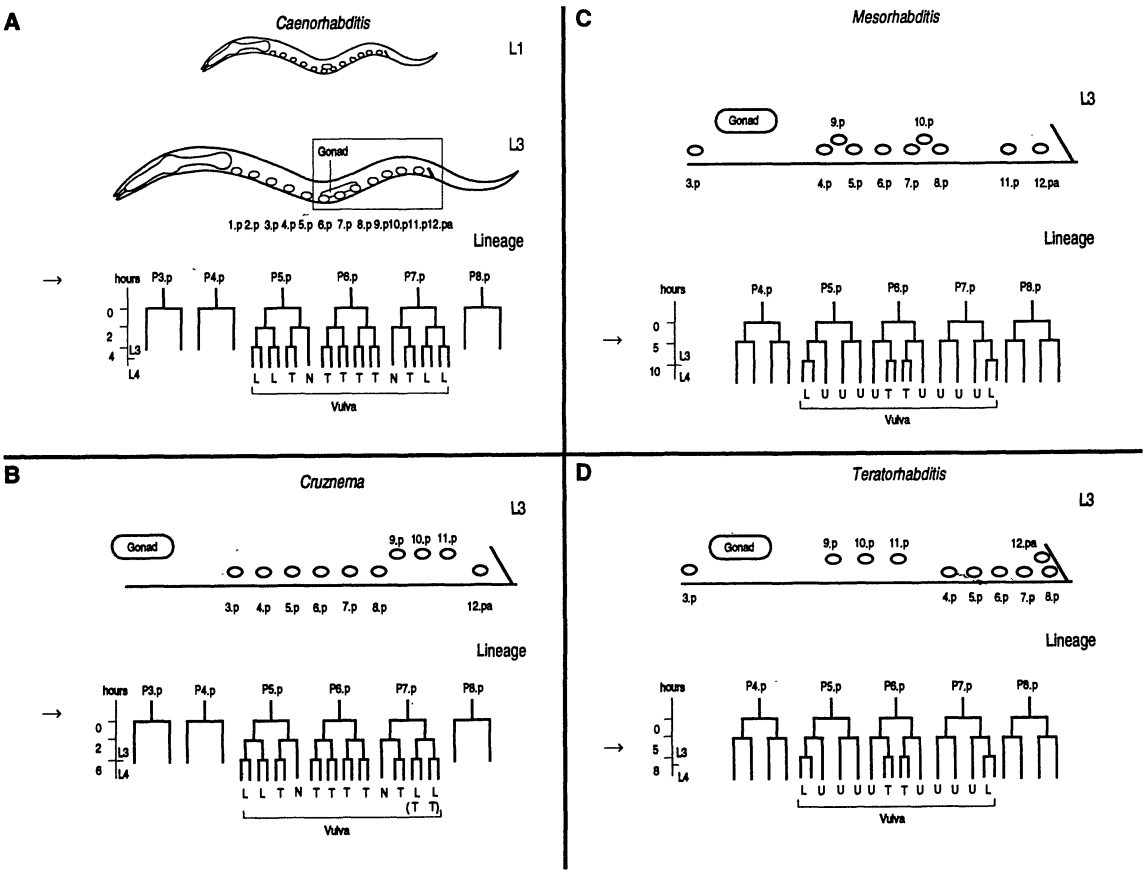


Fig. 1. Schematic summary of the position of the Pn.p ectoblasts in the L1 stage and the early L3 stage and the vulva cell lineage in *Caenorhabditis* (**A**), *Cruznema* (**B**), *Mesorhabditis* (**C**), and *Teratorhabditis* (**D**). In the L1 stage (A), the Pn.p ectoblasts are equally distributed in the region between the pharynx and the anus. This stage is shown for *Caenorhabditis* only, but is true for the other species. P(3–8).p in *Cruznema* and P(4–8).p in *Mesorhabditis* and *Teratorhabditis* migrate to the posterior body region in the L2 stage. In the early L3 stage these cells have species-specific positions relative to the posterior Pn.p ectoblasts (B to D). (B), (C), and (D) show a higher magnification of the corresponding body region indicated by the box in (A). (A to D) Pn.p line- ages for the vulva precursor cells. We refer to the first VPC division as 0 hour; T, transverse division; L, longitudinal division, N, no division according to the definition in *Caenorhabditis* (*3*); U, undivided cell in *Mesorhabditis* and *Teratorhabditis*. We use a different terminology, N and U, because of different cell morphology. The P7.p lineage in *Cruznema* is variable with respect to the division axes as indicated. The arrow in the lineage diagrams indicates the first time of contact between AC and vulval cells. Note that in *Caenorhabditis* this proximity is in principle already present in the L1 stage. For experimental procedures and generation times, see (*11*).

In previous work on *Caenorhabditis*, the isolation of subsets of VPCs by the ablation of their neighbors helped to define the intercellular signals involved in vulva formation (*1*, *15*). To investigate the cell interactions responsible for pattern formation, we carried out a series of experiments in *Mesorhabditis* in which we ablated all but one, two, or three VPCs and observed the fates of the remaining VPCs (Table 1). These experiments suggest that, unlike in *Caenorhabditis*, the VPCs have differential intrinsic properties in *Mesorhabditis*. Only P5.p or P6.p can adopt the 1° cell fate in *Mesorhabditis*. After ablation of four of five VPCs, single P5.p or P6.p cells assume the 1° cell fate. In contrast, single P7.p or P8.p cells generate intermediate lineages with abnormal AC contact and abnormal invagination (*16*). P4.p assumes only the 3° fate as an isolated cell. In contrast, in *Caenorhabditis* all cells of the equivalence group have the potential to adopt the 1° cell fate. Further experiments in *Mesorhabditis*, summarized in Table 1, indicate the nonequivalence of the VPCs. After ablation of one, two, or three VPCs, only P5.p or P6.p can become 1° at the expense of other cells (*17*). In contrast, after the ablation of both P5.p and P6.p, no correct 1° cell fate was observed; P(4,7,8).p had 2°, 3°, or intermediate cell fates. In 21 animals in which P6.p was ablated, P5.p had the 1° cell fate. In *Caenorhabditis*, by comparison, either P5.p or P7.p can assume the 1° fate after the ablation of P6.p (*1*, *3*).

Similar ablation experiments in *Teratorhabditis* give similar results (Table 1). An isolated P7.p or the isolated pair P(7,8).p did not assume the 1° fate, whereas an isolated P5.p did. Taken together, these data provide evidence for the nonequivalence of the VPCs (*18*).

Models for vulva formation in *Mesorhabditis* involve three steps. (i) An early specification process distinguishes at least some of the VPCs from one another in competence to assume vulval cell fates. In particular, only P5.p or P6.p are able to adopt the 1° cell fate. (ii) The decision to distinguish

Table 1. Ablation experiments in *Mesorhabditis* and *Teratorhabditis*. Cells were ablated in the Pn or Pn.p stage in L1 animals. No difference was observed when the ablation was performed at these two stages. In intact animals and after ablation of VPCs, some variability in the anterior-posterior position of the VPCs was seen with respect to P(9,10).p. There is no correlation between the position of cells and the observed pattern formation. In two experiments only, P5.p stopped migration at approximately 70% body length (*) and assumed a 3° or 4° cell fate. We define 4° cell fate as a VPC that did not divide. Cell lineage of the ablated animals was followed by observation at 20-min intervals during the late L3 stage. When the third round of cell divisions began, animals were observed continuously until final vulval progeny were generated. We only counted animals in which the last round of cell divisions was observed.

Ablation	P4.p	P5.p	P6.p	P7.p	P8.p	Obs./total
			Mesorhabditis			
Intact	3°	2°	1°	2°	3°	
Z(1,4)⁻	3°	2°	1°	2°	3°	17/17
P6.p⁻	2°	1°	X	2°	3°	21/21
P(6,7,8).p⁻	2°	1°	X	X	X	7/7
P(4,5,6).p⁻	X	X	X	Hybrid	Hybrid	3/4
	X	X	X	Hybrid	3°	1/4
P(4,5,8).p⁻	X	X	1°	2°	X	6/6
P(4,6,8).p⁻	X	1°	X	2°	X	7/9
	X	4°	X	Hybrid	X	1/9*
	X	2°	X	1°	X	1/9
P(4,7,8).p⁻	X	2°	1°	X	X	8/9
	X	Hybrid	Hybrid	X	X	1/9
P(4,6,7).p⁻	X	1°	X	X	2°	7/9
	X	2°	X	X	1°	1/9
	X	3°	X	X	1°	1/9*
P(4,5).p⁻	X	X	1°	2°	3°	3/3
P(7,8).p⁻	3°	2°	1°	X	X	3/3
P(4,6).p⁻	X	1°	X	2°	3°	7/7
P(6,8).p⁻	2°	1°	X	2°	X	6/6
P(5,6).p⁻	3°	X	X	Hybrid	3°	3/9
	Hybrid	X	X	2°	2°	1/9
	Hybrid	X	X	Hybrid	3°	1/9
	3°	X	X	Hybrid	Hybrid	2/9
	3°	X	X	Hybrid	2°	2/9
P(5,6,7,8).p⁻	3°	X	X	X	X	4/4
P(4,6,7,8).p⁻	X	1°	X	X	X	3/5
	X	3°	X	X	X	1/5
	X	Hybrid	X	X	X	1/5
P(4,5,7,8).p⁻	X	X	1°	X	X	3/4
	X	X	Hybrid	X	X	1/4
P(4,5,6,8).p⁻	X	X	X	Hybrid	X	6/6
P(4,5,6,7).p⁻	X	X	X	X	Hybrid	8/8
			Teratorhabditis			
Z(1,4)⁻	3°	2°	1°	2°	3°	6/6
P(4,5,6).p⁻	X	X	X	Hybrid	Hybrid	1/4
	X	X	X	Hybrid	3°	3/4
P(4,6,7,8).p⁻	X	1°	X	X	X	2/3
	X	Hybrid	X	X	X	1/3
P(4,5,6,8).p⁻	X	X	X	Hybrid	X	4/6
	X	X	X	3°	X	2/6

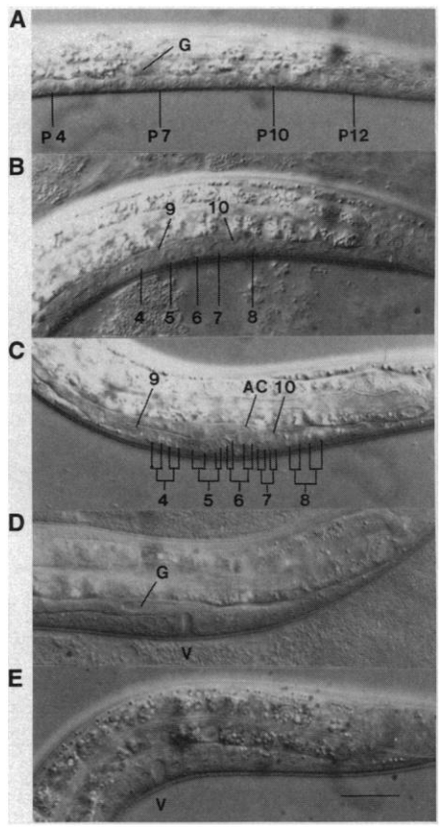
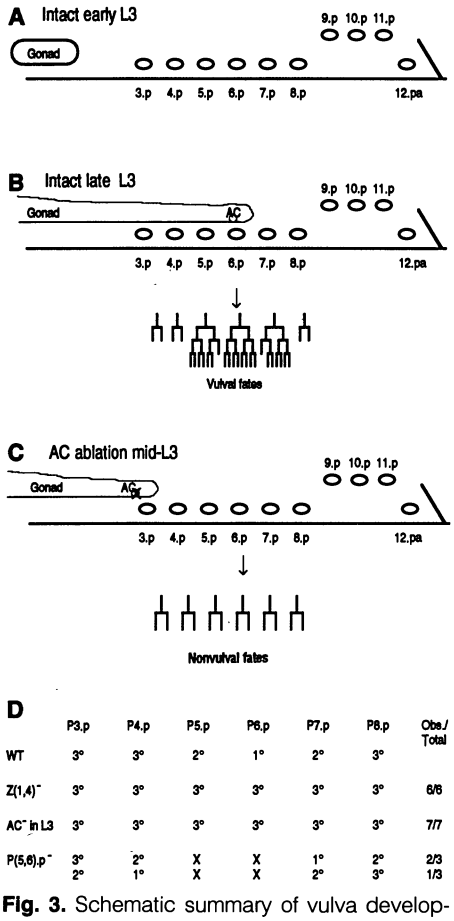


Fig. 2. Nomarski photomicrographs of lateral views of *Mesorhabditis* hermaphrodites at various stages of vulval development. Anterior is to the left. (**A**) Early L1 stage. Distribution of P(3–12) in the ventral cord. The gonad (G) is in a different focal plan, between P6 and P7. (**B**) Early L3 stage. P(4–8).p are located in the region of P9.p and P10.p. P(11,12).p are not visible in this plane of focus. The gonad is still in a more anterior position. (**C**) L3 molt. P9.p is anterior to the progeny of P4.p. All VPCs have divided twice. The P(7,8).p progeny are only partially visible in this plane of focus. AC, anchor cell. (**D**) Mid-L4 stage showing the invagination of the vulva (V) connected to the uterus. (**E**) Mid-L4 stage of a gonad-ablated worm. No uterus is formed; the vulva is still present. Scale bar, 20 μm.

P5.p from P6.p is made. In experiments in which all but P5.p and P6.p were ablated in *Mesorhabditis*, P6.p always had the 1° fate (Table 1). Two different factors could be responsible for this result. An as yet unidentified inductive signal might specify P6.p to be 1° rather than P5.p. We expect such a signal would be produced by more than one cell in the posterior body region. Another possibility is that P6.p is different from P5.p, which allows it to become 1° at the expense of P5.p. (iii) Intercellular signaling events specify the final cell fate pattern among the VPCs (3°-2°-1°-2°-3°). These lateral signaling interactions may well be similar to the signaling in *Caenorhabditis* (*15*). In addition, there could be a permissive inductive signal necessary for vulva differentiation. The signal that promotes P6.p to become 1°, if it exists, could serve this permissive function.

Our analysis of the development of the posterior vulva in *Cruznema*, *Mesorhabditis*, and *Teratorhabditis* demonstrates that in all three species the central Pn.p cells still form the vulva. The possibility that the vulva is formed by the posterior Pn.p cells, which are in the appropriate position to make the posterior vulva (Fig. 1), was not found, indicating that a constraint has restricted what changes can occur in this taxon. Because phenotypic changes of vulva formation (central as opposed to posterior; at the level of adult morphology) do not require coincident changes at the level of the precursor cells (P3.p–P8.p), we call this phenomenon a regulatory constraint at the cellular level (*19*). This regulatory constraint might be created by the underlying morphogenetic mechanism. In *Caenorhabditis*, the specification of the vulval equivalence group is controlled, in part, by the Hox gene *lin-39*, which is required for the central Pn.p cells (P3.p–P8.p) to be VPCs rather than epidermis (*20*). The Hox cluster is conserved in the animal kingdom and appears to provide most of the positional cues along the anterior-posterior body axis (*21*). This property could create regulatory constraints at the single cell level. The position of a structure controlled by this multifunctional cluster might be unable to change without the disruption of other processes controlled by the Hox genes. The observed VPC migration might be the simplest way to bypass this regulatory constraint (*22*).

We have documented at a cellular level evolutionary changes of induction and competence. A major task is to understand the genetic changes that underlie such alterations in these fundamental features of intercellular signaling during metazoan development. In molecular terms, different positional values within the VPCs might result from an earlier pattern formation process, for example, by the Hox gene cluster (*20*). In the *Caenorhabditis* male, the different combination of the Hox genes *lin-39* and *mab-5* establishes different positional values along the anterior-posterior axis (*20*). *lin-39* and *mab-5* are coexpressed in the region of P(7,8).p. This overlap in expression makes this region different from P(5,6).p, in which only *lin-39* is expressed. A corresponding difference in Hox gene expression might create the difference between P(5,6).p and P(7,8).p in *Mesorhabditis*. This positional information could result in a differential response by the VPCs to intercellular signals.



	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	Obs./ Total
WT	3°	3°	2°	1°	2°	3°	
Z(1,4)⁻	3°	3°	3°	3°	3°	3°	6/6
AC⁻ in L3	3°	3°	3°	3°	3°	3°	7/7
P(5,6).p⁻	3°	2°	X	X	1°	2°	2/3
	2°	1°	X	X	2°	3°	1/3

Fig. 3. Schematic summary of vulva development in *Cruznema* in intact and ablated animals. (**A**) Early L3 stage. The VPCs are located at 80% body length, far posterior to the gonad primordium. (**B**) Late L3 stage. The gonadal AC contacts the VPCs. After the AC reaches P6.p, vulval cell divisions occur. (**C**) AC ablation in mid-L3 gives rise to nonvulval cell fate in all the VPCs. (**D**) Summary of ablation experiments. Each line represents a different type of experiment. X represents the ablated cells.

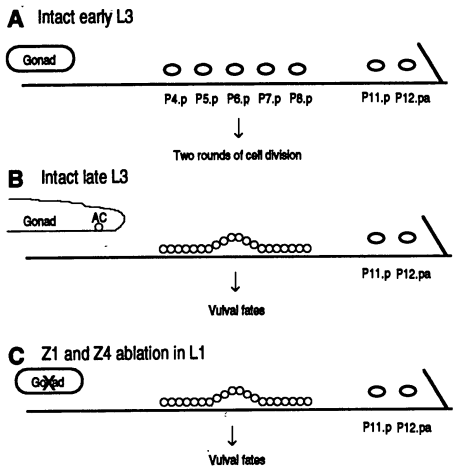


Fig. 4. Schematic summary of vulva development in *Mesorhabditis* in intact and ablated animals. (**A**) Early L3 stage. The VPCs are located at 80% body length, far posterior to the gonad primordium. (**B**) Late L3 stage. The gonadal AC has not contacted the vulval cells, but the VPCs have already divided twice. (**C**) Z(1,4) ablation in the L1 stage. The vulva is still formed. P9.p and P10.p are not shown in these diagrams.

REFERENCES AND NOTES

1. J. Sulston and H. R. Horvitz, *Dev. Biol.* **56**, 110 (1977).
2. J. Kimble, *ibid.* **87**, 286 (1981).
3. P. W. Sternberg and H. R. Horvitz, *Cell* **44**, 761 (1986), *ibid.* **58**, 679 (1989); H. R. Horvitz and P. W. Sternberg, *Nature* **351**, 535 (1991).
4. P. W. Sternberg, *Annu. Rev. Genet.* **27**, 497 (1993).
5. B. G. Chitwood and M. B. Chitwood, *Introduction to Nematology* (University Park Press, Baltimore, 1950).
6. The central position of the vulva is considered to be the ancestral character in the *Rhabditidae* (*5*).
7. W. Sudhaus, *Zoologica* **43** (no. 125) (1976).
8. In the text we use the name of the genus to describe a specific species. The full scientific names of the species used in this study according to the nomenclature of Sudhaus [(*7*), modified] are as follows: *Rhabditis (Cruznema) tripartitum* (von Linstow, 1906) Zullini, 1982; *Rhabditis (Teratorhabditis) palmarum* Gerber and Giblin-Davis, 1990; *Rhabditis (Mesorhabditis)* n. sp. PS1179. *Cruznema* was obtained from D. Fitch; *Teratorhabditis* was obtained from the describer, and *Mesorhabditis* is a new species collected and described by L. Carta (personal communication). *Cruznema* and *Teratorhabditis* are male-female species, and *Mesorhabditis* is hermaphroditic. All species except *Teratorhabditis* were grown on lawns of *Escherichia coli* OP50 at 20°C as described for *C. elegans* [S. Brenner, *Genetics* **77**, 71 (1974)]. *Teratorhabditis* grows best at 25°C. On the basis of morphological studies, the phylogenetic relationship between *Caenorhabditis*, *Cruznema*, and *Mesorhabditis* cannot be resolved (*7*), but a molecular analysis is under way (K. Thomas *et al.*, personal communication).
9. V. Ambros and W. Fixsen, in *Development as an Evolutionary Process*, R. A. Raff and E. C. Raff, Eds. (Liss, New York, 1987); R. J. Sommer and P. W. Sternberg, unpublished observations.
10. P. W. Sternberg and H. R. Horvitz, *Dev. Biol.* **93**, 181 (1982); T. Clandinin and P. W. Sternberg, unpublished observations.
11. Nematodes were observed with Nomarski interference contrast optics. Cell lineages were determined by continuous observation of nuclei as they divided (*3*). Between cell divisions, individuals were cultured on petri dishes with OP50 and were observed with Nomarski optics at 15- to 20-min intervals to observe entry into mitosis. At least 10 animals were observed per species, and every division was seen in at least 3 animals. Vulva cell divisions start after 80 hours of larval development in *Cruznema* (20°C), 66 hours of larval development in *Mesorhabditis* (20°C), and 50 hours of larval development in *Teratorhabditis* (25°C). The vulva cell divisions occur over a time period of 8 to 12 hours in these species. We refer to the time point of the first division as 0 hour.

The migration of the VPCs during the L2 and L3 stage was observed at 2-hour intervals. Animals were kept at 10°C overnight. Cell migrations took place over 3 to 4 days under these conditions and were observed in two animals in each species.
12. Because the VPCs had to pass more posterior Pn.p cells during migration, the latter moved to a more dorsal position in the ventral cord.
13. We define the gonadal cell making the contact between vulva and uterus as the AC. As in *Caenorhabditis*, this cell is morphologically distinct from surrounding gonadal cells.
14. After ablation of M (five animals), V5 (two animals), P(9,10) (four animals), P(11,12) (three animals), B, F, U, and Y (three animals each), KL and KR (three animals), and F and U together (two animals), the vulva developed normally. Ablation of other combinations of these cells was always lethal.
15. P. W. Sternberg, *Nature* **335**, 551 (1988).
16. Criteria for the definition of cell fates were as follows. 1° cell fate: invagination at the four-cell stage; AC attachment between P6.pap and P6.ppa; final division pattern UTTU (U, undivided cell; T, transverse division). 2° cell fate: no invagination in the four-cell stage; only the outer cell undergoes a longitudinal third division (LUUU or UUUL; L, longitudinal division); no AC contact. Intermediate cell fate: no invagination in the four-cell stage; if the AC contacts the cells, the attachment is random; the lineage is variable. We observed LUUU, LLUU, and LLLU lineages; thus, five to seven cells are generated. The LLUU patterns occur most frequently. An invagination occurs after cell divisions are completed, whereas in the 1° fate, invagination occurs in the four-cell stage. In the intermediate lineage the distribution of the cells is random with respect to the invagination. Oblique division axis were observed with low frequency.
17. In 91 ablated animals (Table 1), there were only three cases in which P7.p or P8.p assumed the 1° cell fate.
18. In contrast, after ablation of P(5,6).p in *Cruznema*, P4.p or P7.p assumed the 1° fate (Fig. 3D). In these ablation experiments, the AC stops migration in the region of P4.p or P7.p. Because P4.p and P7.p are able to adopt the 1° cell fate, we conclude that VPCs have equivalent potentials in *Cruznema*. Thus, nonequivalence of VPCs is not required for posterior vulva formation in general. Whereas nonequivalent VPCs and gonad-independent vulva development in *Mesorhabditis* and *Teratorhabditis* are coincident, we have no evidence that they must coevolve.
19. A related phenomenon at the organismal level, called developmental constraint, is discussed in J. M. Smith *et al.* [*Q. Rev. Biol.* **60**, 265 (1985)] and S. J. Gould and R. C. Lewontin [*Proc. R. Soc. London Ser. B* **205**, 581 (1979)].
20. H. M. Ellis, thesis, Massachusetts Institute of Technology (1985); B. B. Wang *et al.*, *Cell* **74**, 29 (1993); S. G. Clark *et al.*, *ibid.*, p. 43; S. J. Salser *et al.*, *Genes Dev.* **7**, 1714 (1993).
21. J. M. W. Slack, P. W. H. Holland, C. F. Graham, *Nature* **361**, 490 (1993).
22. We hypothesize that the positional information the VPCs receive early in development allows them to migrate to the more posterior position.
23. We thank L. Carta and D. Fitch for strains and E. Davidson, S. Fraser, E. Meyerowitz, L. Zimmer, and members of our laboratory for detailed discussion. P.W.S. is an investigator of the Howard Hughes Medical Institute. Supported by a Presidential Young Investigator Program award from the NSF (to P.W.S.). R.J.S. is supported by an EMBO long-term fellowship.

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Suppression of Odorant Responses by Odorants in Olfactory Receptor Cells

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Odorants activate an inward current in vertebrate olfactory receptor cells. Here it is shown, in receptor cells from the newt, that odorants can also suppress this current, by a mechanism that is distinct from inhibition and adaptation. Suppression provides a simple explanation for two seemingly unrelated phenomena: the anomalously long latency of olfactory transduction and the existence of an "off response" at the end of a prolonged stimulus. Suppression may influence the perception of odorants by masking odorant responses and by sharpening the odorant specificities of single cells.

Odorants activate an inward (depolarizing) membrane current, termed the transduction current, in vertebrate olfactory receptor cells (*1–3*). This current is carried by second messenger–gated ion channels (*4, 5*) that are activated by enzymatic cascades consisting of receptor proteins (*6*), heterotrimeric guanosine triphosphate–binding proteins (G proteins) (*7*), and effector enzymes (*8*). However, these mechanisms cannot explain two prominent characteristics of olfactory responses: their long latencies, which can exceed 500 ms in amphibians and are only weakly dependent on odorant concentration (*9*), and the transient increase in membrane current after the end of a prolonged stimulus, termed an "off response" (*10–12*). Models of the activation kinetics of G protein cascades predict minimum latencies of less than 50 ms and a strong dependence of latency on odorant concentration (*13*). Off responses are not predicted by the G protein cascade model, nor are they observed in other signal-transducing cells. Consequently, the existence of these phenomena suggests fundamental differences between olfactory and other signal transduction mechanisms. Here we report that odorants can suppress the inward transduction current in solitary newt olfactory receptor cells, an effect that provides a simple explanation for these seemingly unrelated phenomena.

Odorant suppression of the transduction current was shown by the application of a brief odorant pulse during a cell's response to an earlier pulse of the same odorant (Fig. 1A) (*14*). A single pulse of the odorant amyl acetate caused a transient inward current that began with a latency of 280 ms (trace 1) (*15*). Such a response is typical of whole-cell recordings from vertebrate olfactory receptor cells (*1–3*). However, a second odorant pulse that was timed to begin at the peak of the response to the first pulse caused an immediate decrease in membrane current (trace 2; latency, 20 ms). This

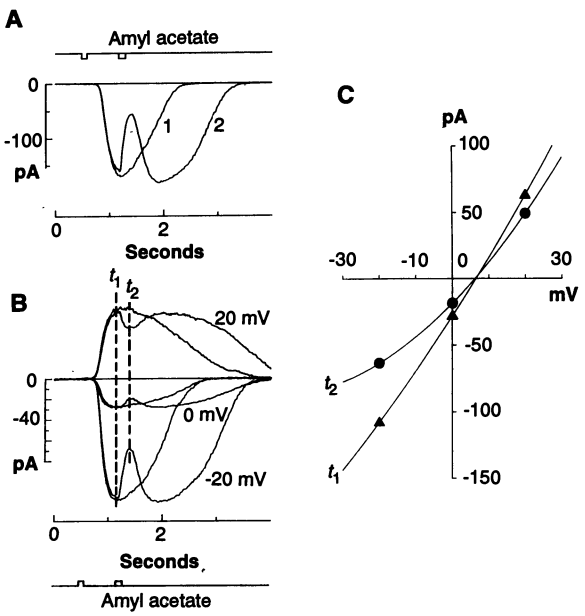

Fig. 1. Suppression of an odorant response by a pulse of odorant. (**A**) Trace 1, response to a single pulse of amyl acetate (10 psi; 100 ms in duration, starting at 0.5 s); trace 2, response to two pulses of amyl acetate, the first pulse identical to the pulse in trace 1 and the second starting at 1.2 s (10 psi, 120 ms in duration). Holding potential, −40 mV. (**B**) The same experiment as in (A), repeated at the holding potentials indicated; t_1 (1.1 s) was the time at the peak of the current indiced by the first pulse; t_2 (1.4 s) was the time at maximal suppression. (**C**) Current-voltage relations for the traces in (B), measured at t_1 (triangles) and t_2 (circles). The curves are second-order regression.

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