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Dauers, sexual plasticity and variant mouth forms: lessons on phenotypic plasticity from phylum nematoda

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Abstract
Organisms develop and evolve in diverse environment. This environment modulates the translation of genotype into phenotype. Phenotypic plasticity or polyphenism is defined as a phenomenon where an organism adopts different phenotypes depending on the environment. Alternatively, a phenotype may be insensitive to a given set of environmental conditions - a phenomenon called as 'environmental robustness'. Nematodes are one of the best models in Kingdom Animalia to study the environment-specific responses of organisms because of specific characteristics, *viz*., species richness, omnipresence, numerical abundance etc. Formation of dauer larvae under unfavorable conditions, alternate life cycles of certain parasitic nematodes, different mouth morphology and environment induced sexual flexibility are classic examples of phenotypic plasticity in nematodes. However, the knowledge of molecular mechanisms underlying phenotypic plasticity is still scant, and a lot more research is required to understand it better and propose a unified theory for phenotypic plasticity in organisms.

Keywords: Phenotypic plasticity, Environmental robustness, Nematodes, Dauer, Sexual plasticity

Introduction
Organisms develop and evolve in a diverse environment by responding to the environmental changes. This environmental heterogeneity modulates the translation of genotype into phenotype, ultimately affecting the biological organization and phenotypic outcome of the organisms. The phenotype of the organism refers to the actual observed properties, such as morphology, development or behaviour of an organism or it is the physical expression or characteristics of the trait. Phenotypic expression is encoded by the genotype i.e., the genetic makeup of a specific organism or set of genes in DNA which are responsible for a particular trait. Organisms with the same genotype should produce similar phenotypes. But variations are there in phenotypes encoded by the same genotype which results from genotype and environmental interactions. It is very important to know the interaction between organism, genotype and phenotype to understand the plasticity induced in phenotypic characters.

Phenotypic plasticity is defined as an ability of a genotype to produce phenotypic variation under varying environmental conditions [1]. If the phenotypic plasticity is induced at the developmental level, it is also referred to as developmental plasticity. Phenotypic plasticity is important in evolutionary biology and ecology and is crucial to the life traits of higher organisms, such as order and duration of critical life happenings such as the development of juveniles, dormant stage formation, and sexual maturity [1].

The dormant stage formation is seen in all domains of life as a survival strategy. For example, the formation of spores in fungi and bacteria, plant seed dormancy, and alternative life cycles in animal kingdoms. These alternative survival tactics are illustrations of phenotypic plasticity. In the insects, the butterfly wing morphs, different hymenopteran castes, the sexually dimorphic beetle horns, and alternating aphid generations exhibiting various physiological and morphological characters are some other examples for phenotypic [2] (Figure 1).

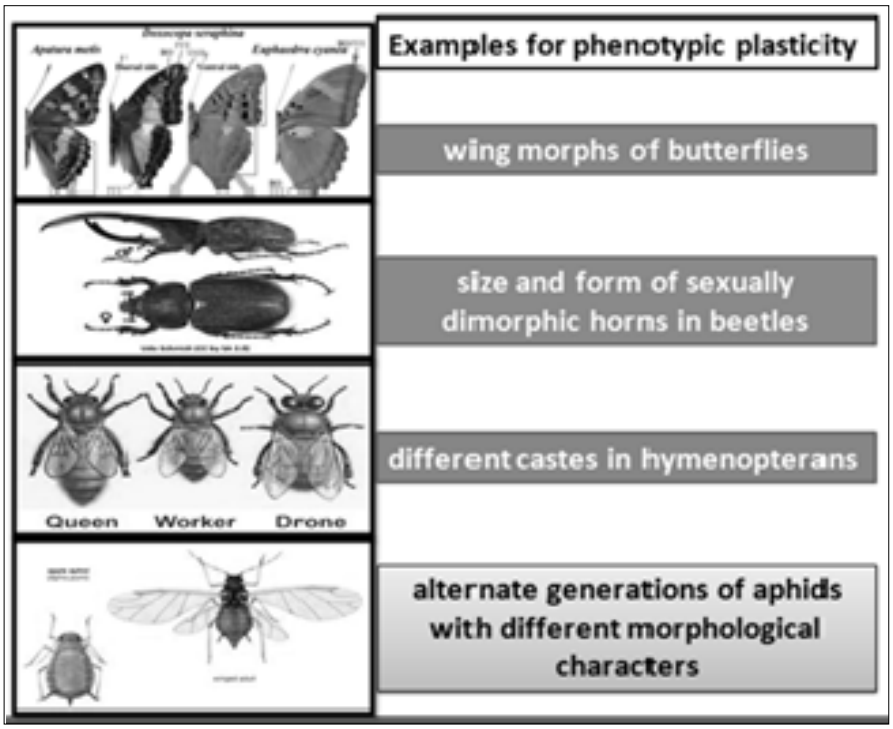

Fig 1: Examples of phenotypic plasticity in insects.

Environmental sensitivity of the phenotype

Biological organization of organisms is shaped by genetic and external environmental factors (both biotic and abiotic factors). These genetic and environmental effects are additive and/or interactive in affecting the phenotype. Environmental effects are in general non-heritable because the environment does not modify DNA sequence. However, the mutation rate of DNA and epigenetic mechanisms like DNA methylation are induced by environmental factors and are transgenerational in nature. Environmental factors also play an important role in natural selection when there is the presence of genetic variation in response to environmental factors [3].

A given phenotype is either sensitive or insensitive to a given range of environmental conditions. Environmental sensitivity of an organism results in phenotypic plasticity. Phenotypic plasticity or polyphenism is an organism's ability to manifest altered phenotypes depending on the environment [2]. In a sensitive response to the environment, a single genotype expresses two or more discretely different phenotypes in response to a given range of environmental conditions. Alternatively, a phenotype may be insensitive to a given set of environmental conditions, resulting in indifferent phenotypes irrespective of the changing environment - a phenomenon called as 'environmental robustness' or 'environmental canalization' [4]. Both can be used to refer adaptive and non-adaptive phenotypic responses.

Phenotypic plasticity and environmental robustness represent opposite ends of phenotypic sensitivity to the environment; however, they are not mutually exclusive [5]; both plastic and robust traits may be observed in same individual phenotype. A given phenotype may exhibit plasticity or robustness of the same trait depending on the range of environmental conditions. 'Reaction norm' is a statistical tool used to explain the environmental sensitivity of the phenotypes, and represents different phenotypes that vary as a continuous function of the environment but produced by a single genotype (Figure 2).

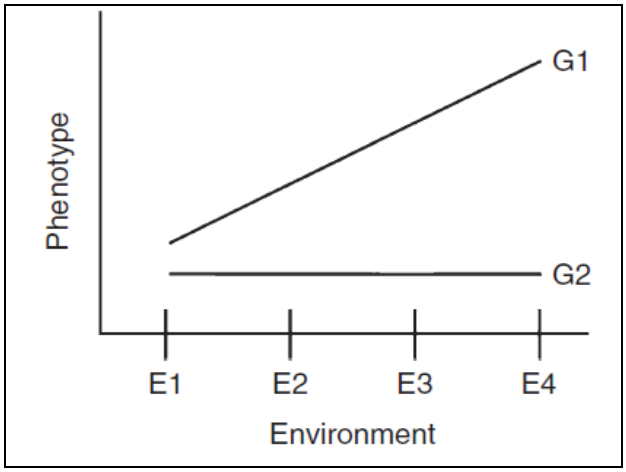

Fig 2: Reaction norm to explain the environmental sensitivity of the phenotype (after [5]). Here, the genotype G1 is environmentally sensitive and produces different phenotypes in different environments, exhibiting phenotypic plasticity. Genotype G2 shows the same phenotype in all environments representing environmental robustness. The slope of the reaction norm shows the degree of phenotypic sensitivity to environmental variation. Genotype-by-environment interaction is represented by reaction norms of the two genotypes, for e.g. a non-parallel reaction norm represents genetic variation in phenotypic plasticity.

Nematodes as models to study environmental sensitivity of the phenotype

Nematodes are excellent models to study the evolution and mechanisms of phenotypic plasticity. Nematodes are one of the largest animal group with an estimated 1 to 10 million species [6]. Nematodes are found everywhere - marine, freshwater and terrestrial environments [7]. Ecological omnipresence, the richness of species, numerical abundance, simple morphology and varying adaptations and lifestyles make them ideal for developmental, evolutionary and ecological studies at the molecular (genetic) and organismal (physiological) levels [8]. Nematodes such as *Caenorhabditis*

elegans, Pristionchus pacificus and entomopathogenic nematodes are the key model organisms for eco-evo-devo interrogations. Different environmental factors and their role in nematode development, molecular mechanisms of phenotypic plasticity, and evolutionary and ecological implications have been studied in these nematodes in detail.

For example, both the type of responses of developmental processes to changes in the environment can be well explained by the studies carried out in *C. elegans*. Dauer formation in *C. elegans* illustrates how development responds to variation in the environment to generate complex phenotypic variation. Vulva formation illustrates how development responds to variation in the environment while generating an invariant final phenotype [5].

Phenotypic plasticity in nematodes

Nematodes have been used to study phenotypic plasticity at developmental and genetic level. They have also been used to study the effect of different environmental factors under lab conditions, resulting in molecular insights into the phenotypic plasticity, and its evolutionary and ecological implications. Formation of dauer larvae, alternate life cycles, different mouth morphs and sexual plasticity are the examples of phenotypic plasticity in nematodes. It has been found that these phenotypically plastic traits are linked.

a. Dauer larvae

C. elegans has been extensively used to study dauer larvae, which is the best-studied example of phenotypic plasticity. Under optimum growth conditions, L2 larvae develop into the third stage (L3), and then to the adult stage. Under unfavourable growth conditions, L2 larvae become dauer larvae (DL3), meant for survival and dispersal and are environmentally tough.

A dauer larva exhibits several morphological, physiological and behavioural adaptations as a phenotypically plastic trait (Table 1).

Table 1: Morphological, physiological and behavioural adaptations of dauer larvae.

Morphological adaptations	Metabolic changes	Behavioural adaptations
Dauer larvae go through radial shrinkage of cuticle and appear thinner Surface-to-volume ratio is more: render dauer larvae tolerant to low oxygen conditions Closed mouth and constricted pharynx: non-feeding Thin dark intestine Fat body accumulation Thicker cuticle, specific cuticular pattern with lateral ridges (alae) Remodeling of neurons, foregut, and other structures [9]	Dauer larvae are non-feeding Partial shift to anaerobic fermentation on dauer entry Reduced specific activity of enzymes involved in metabolism: indicates lowered utilization of energy Up-regulation of acyl-CoA synthetases, which are necessary for utilization of energy sources such as lipids Dauer larvae accumulate large quantity of lipids in the intestine and hypodermis [10]	Dauer larvae exhibit lethargy and host-seeking behaviour. Dauer larvae are usually immobile Dauer and dauer like larvae in many host associated nematodes display nictation. Dauer larvae stand on their tail tip and swing their body to enhance the interaction with their hosts. Example: Foraging behaviour seen in entomopathogenic and parasitic nematodes [11]

The entry or exit from the dauer stage is a decisive step in nematode life cycle. Any erroneous choice affects the fitness of organism and may lead to the fewer progeny or premature death. Environmental cues influence the decision of dauer entry or exit. Perception of environmental cues by nematode leads to redirecting nematode development and dauer formation. Following environmental cues are known to regulate dauer development-

1. High dauer pheromone/daumone concentration (e.g., at high population density): Ascarosides such as Ascr-1, Ascr-2, Ascr-3, Ascr-4, Ascr-5 in *C. elegans* act as daumone
2. Low food concentration: Starvation conditions usually enhance the propensity to enter the dauer stage
3. High temperature: The tendency to form dauers generally increases as temperature increases.

The propensity to enter dauer is very sensitive to variation in these conditions and usually depends on a combination of these cues.

Laser-mediated cell ablations and mutant screens determined the role of specific sensory neurons and downstream neuroendocrine signalling cascades involved in dauer formation. Mutant screens yielded two opposite phenotypes with respect to dauer development: dauer-defective (Daf-d) mutants that show no or decreased sensitivity to dauer-inducing conditions, while dauer-constitutive (Daf-c) mutants enter dauer stage in the dearth of such conditions [12]. Laser-mediated cell ablations studies showed that amphid sensory neurons may sense different combinations of chemical cues to regulate dauer entry [13]. In general, the ASI, ADF, and ASG amphid neurons repress dauer entry in good growth environments, while the ASJ neuron is required for dauer entry in unfavourable environments (in addition, the ASJ neuron is also required for exit from dauer). Mutations in the structure and function of sensory neurons cause abnormal dauer formation [14].

Dauer stages have been reported not only in free-living nematodes but also described in parasitic nematodes, for e.g. in insect-parasitic nematodes *Heterorhabditis*, *Steinernema*; in animal-parasitic nematodes *A. duodenale, S. papillosus* and in plant-parasitic nematodes such as *B. cocophilus B. xylophilus.* Dauer larvae exhibit characteristic adaptations which are necessary for parasitism. Evolution of parasitic nematodes demands unique life stages which are capable of searching hosts species, infect, survive and/or reproduce in them. Prominent morphological resemblances of dauer stages of free-living nematodes & infective juveniles support this assumption [15]. The molecular evidence suggests that the evolution of infective juveniles happened from free-living dauer stages. Studies on *Strongyloides papillosus* (parasite of sheep) life cycle [16] show that *S. papillosus* has a homogonic (direct) and a heterogonic (indirect) life cycle. During heterogonic life cycle, free-living males and females arise from the offsprings of the parthenogenetic parasitic females. Free-living females produce infective juveniles by amphimictic reproduction. Infective juveniles attack another host. During homogonic life cycle, there is the direct development of infective juveniles from the parasitic females. Conserved DA/DAF-12 endocrine signalling module has been observed between free-living and parasitic nematodes.

It has been demonstrated that D7-DA is capable of inhibiting IJ formation during both the direct and indirect life cycle of *S. papillosus* [17]. Wang *et al*, 2009 [18] made parallel observations using D7-DA in the allied species *S. stercoralis* and *Ancylostoma caninum* (hookworm). These studies strongly hold up the hypothesis that dauer larvae and an infective juvenile are of common origins and are identical forms of phenotypic plasticity.

b. Dimorphism of nematode mouth form

Dimorphism of mouth form is also an example of phenotypic plasticity, which is exclusively seen in the members of the Diplogastridae family like *Pristionchus* and some related genera [19]. *P. pacificus* worms are omnivores and feed on microbes, nematodes and other decaying organic matter. They exhibit two mouth forms- eurystomatous and stenostomatous form and different mouth form development is phenotypically plastic. Eurystomatous mouth form is characterized by a larger claw-like upper left denticle and an additional lower right denticle, together perform as scissor, the buccal cavity is shallow and broad. Larvae that circumvent dauer develop into adults with eurystomatous mouth form. Stenostomatous (ST) type of mouth form is characterised by small denticles and narrower and deeper buccal cavity. Larvae which go through dauer stage mostly develop narrow mouth (Figure 3). *Pristionchus sycomori* (fig and wasp-associated nematode) displays five mouth morphs. Nematodes which go through dauer stage exhibit a specific mouth morph *i.e.,* morph V. Nematodes not passed through dauer produce remaining four morphs of mouth [20] (Figure 3). Environmental cues regulating dauer formation also influence mouth form decisions. For example, nutrient depletion is involved in regulating dauer formation, as well as mouth form decision. Molecular mechanisms governing dauer formation and mouth formation show similarity. Particularly, mouth formation decision is subjected to mutations in *Ppa-daf- 12* and DA hormone concentrations [21].

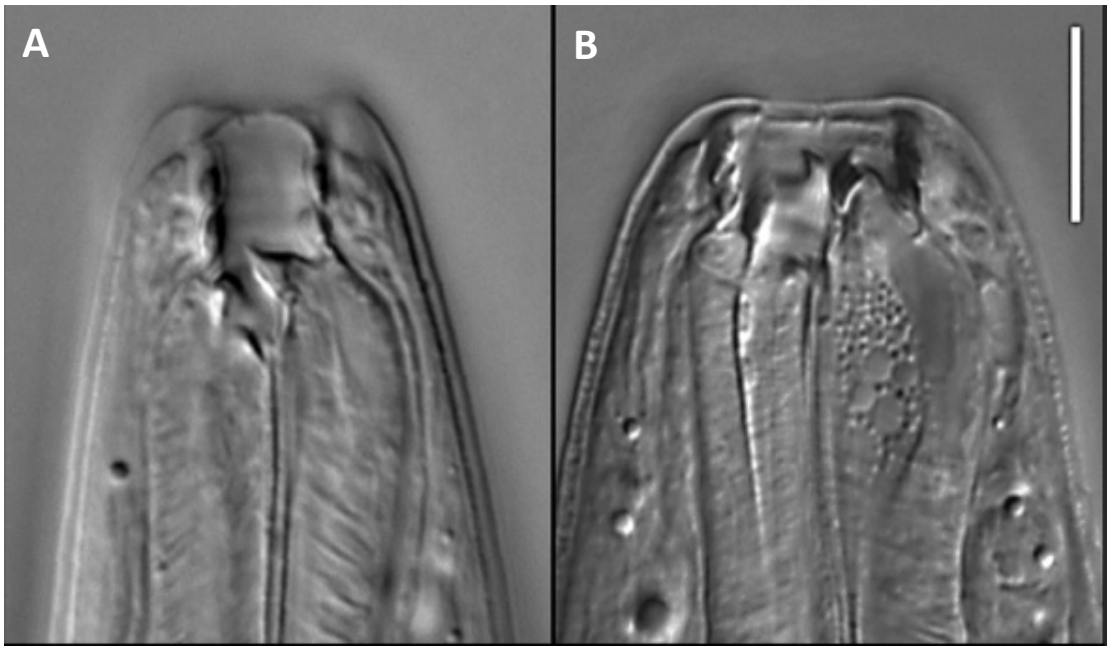

Fig 3: Various mouth form of *P. pacificus* [2]. (A). Sternostomatous and (B). Eurystomatous.

c. Sexual plasticity

Environmental factors influence organisms' sexual fate. A wide range of reproductive modes (parthenogenesis to amphimixis) is seen in the Phylum Nematoda. This makes them ideal for studying the evolution of novel modes of reproduction /mating systems *i.e.,* sexual plasticity in this phylum. Sexual plasticity is well studied in free-living nematode *Rhabditis* sp. SB347. This nematode is sexually polymorphic, comprised of males, females and hermaphrodites. The disparity is seen in larval stages of nematodes which are destined to develop into females or hermaphrodites. Under optimal growth conditions, larvae pass through four larval stages and develop into females. These larval stages possess functional mouthparts and are feeding in nature. But under dauer inducing conditions growth can be re-specified towards hermaphroditic development. Under these circumstances, nematode larvae go through an obligatory non-feeding dauer stage after a second stage larval moult [22]. Differential rates of gonad development can be used to distinguish *Rhabditis* sp. SB347 larvae that develop into hermaphrodites and females at the mid-L1 stage. Larvae possessing bigger gonad primordia grow into females, while those with smaller gonads develop into dauer stage preceding the hermaphrodite formation. Experiments conducted [22] showed that sexual fate of *Rhabditis* sp. SB347 can be influenced by external factors like nutrition, hormones etc.

Sex reversal in *Meloidogyne* is another example of sexual plasticity in nematodes. Under overcrowding and food scarcity conditions, J2 destined to become female turned into a male [23]. This provides the greater advantage of survival in food-poor condition as males have a low nutrient requirement in comparison to females and sex reversal enhances amphimixis type of reproduction enhancing genetic variability. Sex of juveniles can be distinguished during late J2 stage based on genital primordial shape. J2s which are destined to be males show 'I' shaped genital primordia. J2s which are destined to be females show 'V' shaped genital primordia. Sex reversal at early stage results in males possessing single testis, which are difficult to differentiate from true males. When sex reversal occurs at the mid-L2 stage, produces an atrophied and a well-developed testis in males. Two testes of almost equal size are seen in male nematodes which are developed after delayed sex reversal. Hormonal regulations influence sex differentiation.

Environmental factors play a vital role in sex differentiation via affecting the gene expression and in turn hormonal balance [23].

Reprogramming in germline tissues of *C. elegans* adults cause reproductive arrest [24]; L1 arrest in *C. elegans* [25] are some other examples of phenotypic plasticity in nematodes.

Importance of Phenotypic plasticity

Phenotypic plasticity is useful in many ways, such as-

1. Survival: Direct consequences on survival and fitness of an individual is seen in phenotypic plasticity. Dauer stage enables nematodes to live in hostile situations for a long time.
2. Phenotypic plasticity enables boom and-bust lifestyle. Under food rich conditions, nematodes reproduce as much as possible, and under food poor conditions nematodes undergo arrested dauer larval stage.
3. Phenotypic plasticity allows nematodes to tolerate various environmental stresses, e.g., starvation, desiccation, extreme temperatures, toxins, osmotic stress, and oxygen deprivation.
4. Dispersal and transportation between habitats increase the probabilities of discovering a new source of food.
5. Phenotypic plasticity is a facilitator of phenotypic evolution. For e.g., morphological variation in mouth forms facilitates the evolution of different feeding habits like microbivorous, omnivorous, carnivorous and parasitism. Evolution of more complex parasitic lifestyles is also facilitated by alternate parasitic and free-living lifestyles in Strongylidae and related parasitic nematodes. Development of reproducing systems, for example, trieocy, *i.e.* presence of 3 sexual forms, transitional evolutionary step amongst gonochorism and hermaphroditism was facilitated by phenotypic plasticity.
6. Population divergence and speciation-when phenotypic plasticity are genetically fixed, it may lead to speciation [8].
7. Higher reproductive output, such as post-dauer *C. elegans* hermaphrodites have an advanced propagative output when linked to hermaphrodites that did not go through the dauer stage. A connection amongst expression of reproductive genes and dauer formation has been recognized in *C. elegans.* Epigenetic changes are found in post-dauer animals, which results in higher sperm production.

Conclusions

Phenotypic plasticity is a crucial principle in evolutionary biology and ecology. It permits the organism to respond to the varying conditions of the environment. Phenotypic traits are connected to each other at ecological and molecular level. Early life experiences have a major impact on adult phenotypes. Although conservation and co-option of DA/DAF-12 hormone signalling module indicate its vital role in facilitating phenotypic evolution, information on other pathways and cross-talking of pathways are limited. How the traits are linked and their ecological, evolutionary significance remains unclear. This will need sampling of many nematode species, their ecological characterization and employment of both techniques including molecular biology and classical genetics as well. It can help in understanding the role of polyphenisms in studying evolutionary forces on plastic traits and effects of plasticity on evolution.

Phenotypic plasticity in nematodes may create confusion in the application of morphology-based taxonomic approaches, where it can lead to misidentification of a species. A selective process termed as genetic assimilation can be used to genetically fix environmentally induced phenotypic variations. It leads to the evolution of new races and speciation. Therefore it is very important to know about phenotypic plasticity and resultant population divergence in breeding for resistant crop varieties. In summary, phenotypic plasticity enables organisms to adapt and survive in the diverse environment and facilitates evolution when it gets fixed in organisms. The information related to the mechanisms underlying phenotypic plasticity is still scant except for few examples such as dauer juveniles in nematodes. Therefore, a lot more research is required to understand phenotypic plasticity better and propose some kind of unified theory for phenotypic plasticity in organisms.

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