

Phenotypic plasticity in nematodes

Evolutionary and ecological significance

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Model systems, including *C. elegans*, have been successfully studied to understand the genetic control of development. A genotype's phenotype determines its evolutionary fitness in natural environments, which are typically harsh, heterogeneous and dynamic. Phenotypic plasticity, the process by which one genome can produce different phenotypes in response to the environment, allows genotypes to better match their phenotype to their environment. Phenotypic plasticity is rife among nematodes, seen both as differences among life-cycle stages, perhaps best exemplified by parasitic nematodes, as well as developmental choices, such as shown by the *C. elegans* dauer/non-dauer developmental choice. Understanding the genetic basis of phenotypically plastic traits will probably explain the function of many genes whose function still remains unclear. Understanding the adaptive benefits of phenotypically plastic traits requires that we understand how plasticity differs among genotypes, and the effects of this in diverse, different environments.

Introduction

Modern biology's success in discovering how genes control developmental phenotypes has been due to studying genetically defined organisms in tightly controlled laboratory conditions. The invariant cell lineage of *C. elegans* has particularly facilitated notable progress in understanding the control of body formation and morphogenesis. This progress in *C. elegans* is arguably more than has been achieved for analogous endeavors in model systems which do not have such cell lineage control. More broadly, in all model systems, studying defined wild-type genotypes in constant environments has been the bed-rock of developmental biology. Genetic analytical power comes from consistency and sameness in the object of study.

The real world is much messier, and more interesting, than the laboratory. The real world is where the traits we analyze and genetically un-pick evolve, and where the precise trait characters determine an individual's fitness. An organism's phenotype is the difference between living and reproducing, or evolutionary death. In the real world, the environment is demanding and dynamic. It varies in its quality over space and time, both within and across individual generations. Environmental quality is due

to both abiotic factors and biotic factors, including conspecifics; the competitive environment therefore contains genes and it evolves.¹ This environmental heterogeneity has driven, through natural selection, the evolution of phenotypes and of developmental strategies that maximise the match of a phenotype to the prevailing environment.

There is therefore a potential gap between our current genetic understanding of the control of traits and of how organismal traits contribute to fitness. Evolutionary fitness of an individual comes by surviving and reproducing in its environment and out-competing other individuals, which depends on its phenotype being better matched to this environment. Because the environment is variable, having only fixed, invariant traits is unlikely to maximise fitness. More precisely, such a completely fixed phenotype developmental strategy would be out-competed by a genotype whose phenotype was able to better match the environment, as the environment varies. Better matching of phenotypes to environments can be achieved by phenotypic plasticity. Phenotypic plasticity is the ability of one genotype to produce different phenotypes in response to environmental conditions. These different phenotypes can be discrete (e.g., different morphs) or continuous. Thus, phenotypically plastic traits can allow a genotype to, in some way, match its phenotype to its precise environment, thereby contributing to fitness. At an extreme, a perfectly plastic phenotype (where any phenotype was possible, and the phenotype-to-environment match was perfect) would give eternal, maximal fitness. That such perfect plastic phenotypes do not exist is both because of developmental constraints (some phenotypic options are just not possible) and because of presumed costs of phenotypic plasticity (the costs of the plasticity are greater than the benefit that could have been gained by that plasticity).

While we have a very good understanding of how genes control phenotypes, this is generally only true for phenotypic traits that are not plastic (or that may be plastic, but have been studied in a single, controlled environment, such that any phenotypic plasticity is absent). The time might now be ripe for mature research fields of model organisms to turn their attention to phenotypically plastic traits.

It is remarkable, that despite our success in determining genome sequences for many species, and for understanding the function of many genes, we still actually have very little idea of what most of the genome is doing. Of course, there is some annotation for very many genes in sequenced genomes, but the vast

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majority of these annotations are no more than identification of sequence analogs in other species, when they exist. The difficulties in understanding gene function can be seen, for example, from yeast gene knockout studies, where systematic knockouts of the approximately 5,000 genes resulted in phenotypes for only some 15% of genes.^{2,3} Clearly, 85% of genes could not be functionless. Indeed, deletion of many of these other genes did have phenotypic consequences, but these were only seen in other environmental conditions.² Analogous findings, that phenotypic analyses require environmental space, have now been made more widely.⁴ Similar results are also, in essence, implicit in the genome-wide RNAi screens of *C. elegans* (e.g., ref. 5). Here, RNAi of a majority of genes had no observed effect. If it is the case that the functional effect of many genes/gene products is context-dependent, then assay of these RNAi-treated worms in different, diverse environments would increase the proportion of genes for which a phenotype could be observed, and hence a putative role assigned, which is now being observed in on-going research. Therefore, an organism's phenotypic space can only be known by looking at phenotypes in environmental space.

Phenotypic Plasticity Among Nematodes

Phenotypic plasticity is rife among nematodes, including *C. elegans*. The term phenotypic plasticity has been so widely used (e.g., refs. 6–9) that its utility is weakened; to be useful, the precise trait and the environments that affect it need to be explicit. For nematodes, plasticity of phenotype among life-cycle stages is so obvious that it is rarely commented upon. This form of plasticity is particularly apparent among parasitic nematodes. Here, different life-cycle stages can have very different morphologies, physiologies, lifestyles, etc. Most notably, within a species some life-cycle stages can be parasitic (for example living inside the gut of a vertebrate host) while other life-cycle stages are free-living (e.g., in host faeces, the soil or water) or, depending on species, a transmission stage living inside an arthropod intermediate host.¹⁰ In all these cases, the different phenotypes are products of the same genome. For free-living nematodes, including *C. elegans*, the nearest analog of this within life-cycle plasticity of parasitic nematodes is the dauer larva stage (although clearly there are substantial differences between all life-cycle stages of free-living nematodes, including *C. elegans*).

Considering how parasitic nematodes have different life-cycle stages, which can have different lifestyles etc., and the dauer larva stage of free-living nematodes, makes clear an important distinction. In free-living nematodes, the dauer/non-dauer switch is (1) a facultative developmental choice which is (2) based on environmental conditions (specifically, food availability, con-specific population density and temperature); therefore, the environment controls what developmental choice is made.¹¹ In contrast, in the parasitic nematode examples, where life-cycle stages differ, this is not a developmental choice. In these life-cycles there is progress through larval and adult stages, and each stage has its own different phenotype, physiology and lifestyle. The environment plays no part in the sequence of this life-cycle progression. Instead, the environment cues when the next steps in the sequence are started.

This is very clearly seen with species of parasitic nematodes where there is an infective larva that is free-living whose destiny is to enter a host (often by penetrating the host skin).^{10,12} These free-living infective larvae remain in this stage until they receive cues (usually signaling the presence of a suitable host), which brings about biochemical and behavioral changes that allow them to infect hosts and to recommence their developmental progression to the next stage, which usually also involves a moult.¹³ Thus here, the environment is the cue that controls the timing of events; this is in contrast to the dauer/non-dauer case where the environment controls the nature of the choice that is made, but not its timing.

The developmental choice as exemplified by the dauer/non-dauer choice of free-living nematodes, also occurs in the life-cycle of parasitic nematodes (Fig. 1). Most notably, in Strongyloides and Parastrongyloides (both genera that parasitise vertebrates) the free-living phase of the life-cycle has a phenotypically plastic developmental choice between (1) development as larvae only (leading directly to the development of infective larvae) and (2) development of a free-living dioecious adult generation, whose progeny (following sexual reproduction) develop into infective larvae.^{14–16} The developmental choice between these two routes (so called direct and indirect, respectively) is controlled by environmental conditions. In *S. ratti*, a natural parasite of the rat, these have been well characterized.^{17,18} They are an interaction of out-with-host conditions (especially environmental temperature) and the host immune response (i.e., that of the host from which the relevant transmission stage was passed).^{19–21} For Parastrongyloides spp, a host feces-derived factor affects this developmental choice.²² How Strongyloides and Parastrongyloides sense the host immune response is not known. For example, it could be a parasitic female maternal effect (which then raises the question of how these parasitic females sense the immune response), or an effect acting directly on eggs and larvae in transit in the gut, or on larvae developing outside of the host in faeces.²³ Considering the *C. elegans* and *S. ratti* developmental decisions together shows that they both use multiple cues in making these developmental decision (*C. elegans*: pheromone concentration, food concentration; *S. ratti*: host immune response, temperature). For *S. ratti*, these cues are temporally and spatially separated, which implies that there is a memory of cues, to thereby allow their integration.²⁴ Specifically, the host immune response will affect adult parasites that are in the host gut, and eggs that are passing along the gut to be voided in host faeces, whereas the environmental temperature is sensed later by first and second stage larvae (L1s and L2s) outside of the host, yet together these cues are used by L1s and L2s to make the developmental decision.

Another more recently described example of phenotypic plasticity is of mouth morphology in *Pristionchus pacificus* (a scarab beetle necromenic nematode) and among other groups of diplogastriid nematodes.^{25,26} In *P. pacificus*, there are two mouth morphologies, one wide (containing two denticles), one narrow (with just one denticle) (more formally known as eurystomatous and stenostomatous mouth morphs, respectively). The mouth morphology is a developmental choice that is controlled by starvation in early larval stages (which favors the wide mouth morphology) and by a *P. pacificus* pheromone.

Figure 1. The life-cycle choices of (A) *C. elegans* and other free-living nematodes, (B) *S. ratti* where gray box 1 is a sex-determination event, and gray box 2 is a female larva-only developmental choice between direct and indirect development (larval stages, except the infective L3 stage, have been omitted for clarity) and (C) *P. trichosuri* as for *S. ratti*, except that the *P. trichosuri* life-cycle differs (1) by having a dioecious parasitic generation and (2) the progeny of the free-living adult generation can form multiple, secondary free-living adult generations.

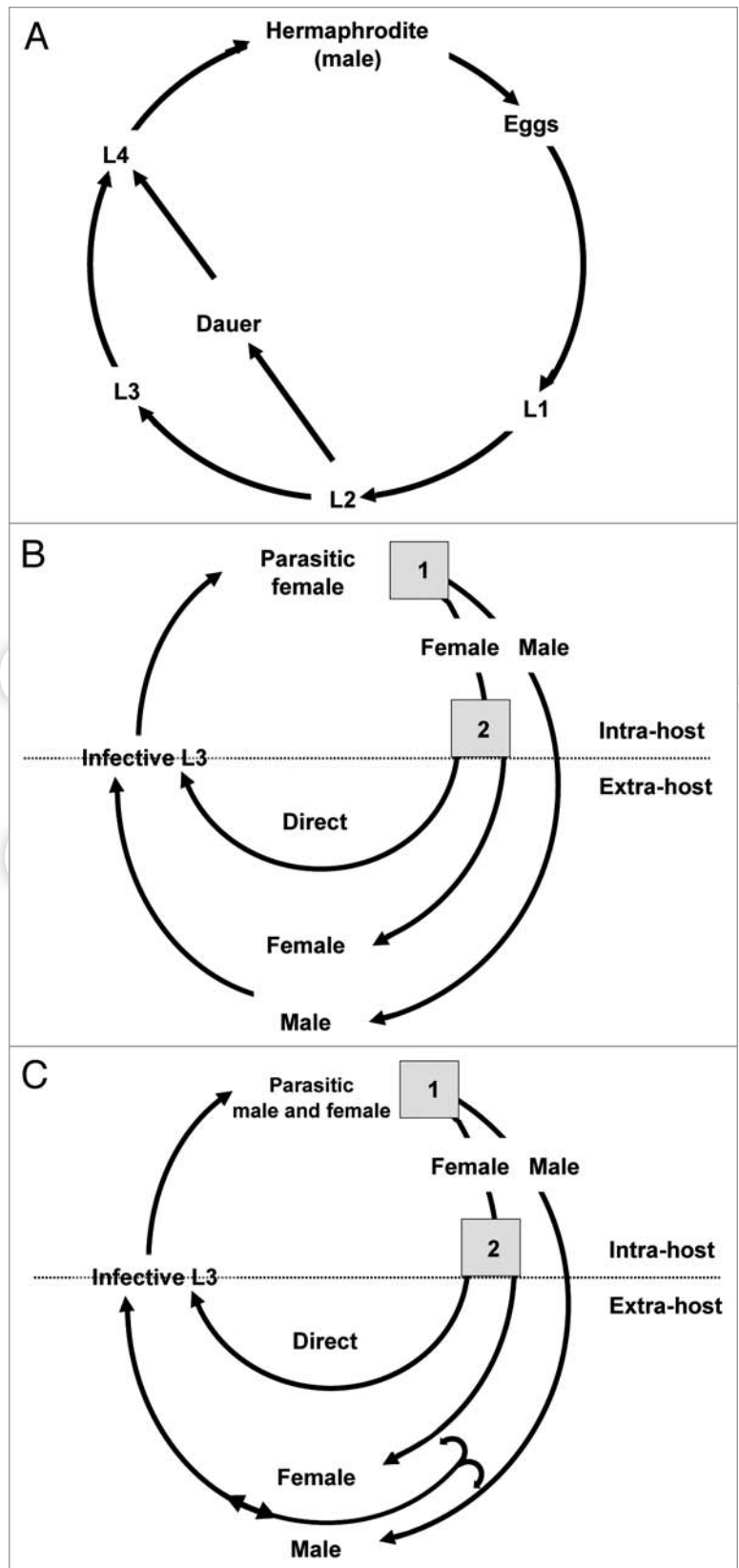
These examples of phenotypically plastic traits are where there are alternative developmental choices, so-called polyphenisms. There are, of course, other examples of plasticity of phenotypes among nematodes too (e.g., ref. 27). For example, the food that is fed (i.e., bacterial species and strain and quantity) to *Caenorhabditis* spp larvae alters adult body and brood size.^{28,29} Here the environment is food, and the plastic trait is body size and fecundity (see Testing for Adaptation). Among parasitic nematodes there are analogous effects. For *S. ratti*, the within-host immune environment affects the size and fecundity of parasitic female worms, such that in immune hosts parasitic adult size is approximately halved, but this is reversible if the host is immune suppressed; analogous effects are seen in other parasitic nematodes too.^{30,31} These may be direct effects of the host immune response that damages the worms, or this may occur via interference with feeding, or a combination of both of these effects.³² In all these examples, there are clearly opportunities for maternal effects more widely, for example, while maternal environment may reduce fecundity, there may be a compensatory change in offspring quality, as seen in other systems.³³

Phenotypic Plasticity and Adaptation

These developmental choices of *C. elegans*, Strongyloides, Parastrongyloides, *P. pacificus* can be intuitively understood as an adaptation to environmental conditions in which worms find themselves. As is oft repeated for *C. elegans* dauer larvae, it is obvious that assessment of environmental conditions (how much food and how many other con-specifics there are to eat it), is key information for this developmental decision.³⁴ The choice is between (1) continuing developmental growth to adulthood and reproduction, both of which require food or (2) altering one's metabolism to store food, to arrest development as a dauer larvae, which is therefore adaptive when there is insufficient food available to allow choice (1). More precisely, therefore, the assessment of environmental conditions is also a prediction (presumably with some error) of future conditions too. Failure to make the correct, optimal decision will lose evolutionary fitness.

Understanding the analogous developmental choice of Strongyloides and Parastongyloides is not quite so straightforward. At the core of the developmental choice in these genera is a

choice between sexual reproduction (i.e., developmental via free-living adults, which reproduce sexually) and direct development (which is asexual, because there is no further reproduction).^{14,35}



(A further consequence is that development of infective larvae via the sexual route takes longer, compared with direct development). Development via the sexual reproduction route has two effects: sexual reproduction and an increase in the number of worms (because two mating adults produce ca. 40 offspring).^{36,37} It is likely that sexual reproduction is the particular advantage that is acquired by an indirect developmental route. The reason for believing this is that given the diversity of reproductive mechanisms among nematodes it is clear that nematodes could evolve an asexual method of adult reproduction that generates more offspring (i.e., sexual reproduction is not needed for adults to produce progeny). That *Strongyloides* and *Parastrongyloides* did not do this suggests that sexual reproduction is the key functional aspect of their developmental choice. In this respect, the pattern of the induction of facultative sexual reproduction is similar to that seen in other species, for example cyclical parthenogens (*Daphnia* spp, aphids), where sexual reproduction occurs at times of environmental stress. For *Strongyloides*, the stress is presumably some aspect of the within-host conditions (signaled by the host immune response), perhaps also signaling some wider aspect of the status of the host biology and population.

The presumed adaptive value of the *P. pacificus* developmental choice is that the different mouth morphologies give access to different food sources. The narrow (stenostomatous) form allows worms to feed on bacteria, whereas the wide (eurystomatous) form allows worms to slice open fungal hyphae and other nematodes to use as a food source, which may be the next best food source in the absence of bacteria. Because evolution has found this solution to *P. pacificus* feeding, it is presumed that developing alternative, specialized mouth morphologies gives greater fitness compared with having a generalist, all-purpose mouth morphology.

The sensitivity of a genotype's switch to environmental conditions can be an evolved trait, which will have evolved in the environment in which the genotype in question is living. This has the consequence that genotypes that have evolved in different micro-niches may have different sensitivities (measured as the phenotypic response) to the same environmental signals. This is now well known for both *C. elegans* and *Strongyloides*. Specifically, different genotypes of *C. elegans* have quantitatively different responses to the same dauer pheromone or food concentration signals.^{38,39} Of parenthetical interest, the *C. elegans* wild type, N2, routinely seems to have the greatest sensitivity to dauer larva formation, which is presumably a result of artificial laboratory selection for survival on temporarily neglected laboratory agar plates where the formation of dauer larvae results in survival. *C. elegans* genotypes differ in other traits too (e.g., refs. 40–42), as does *P. pacificus*.⁴³

For *S. ratti*, different genotypes also make quantitatively different responses to the same within- and, or without-host environmental conditions, such that some genotypes have almost complete asexual development, whereas others have extensive sexual development.^{17,44} It is likely that these differences reflect genotypes' different sensitivities to the host immune response, perhaps therefore reflecting different local within-host environments experienced by those genotypes during their evolution.⁴⁵

For *S. ratti*, there appears to be a geographic effect too. Genotypes isolated from the UK (but assayed in defined laboratory conditions) have predominately asexual reproduction, whereas development via the sexual route is more common among genotypes from other geographical locations.¹⁷ The degree of sexual/indirect vs. asexual/direct developmental can be selected for, as can aspects of within-host development, which is likely to be due in part to how within-host stages interact with the host immune response.^{44,46}

That there are genotype-specific differences in plastic phenotypes is then a further challenge for developmental biology. Not only do environmentally plastic phenotypes need to be considered, but also that the nature of that plasticity may be genotype-dependent. In fact this is an important, positive factor because this genotype-diversity can be used to facilitate analyses of such traits, particularly using quantitative genetic approaches.

While our understanding of these facultative traits can give an idea (though untested) of their likely adaptive value, it is rather more difficult to understand and investigate why these trait values differ among genotypes. In part this difficulty is because we do not really understand the precise niche from which these genotypes came. While a genotype's geographical location may be known, the functionally relevant niche is likely to exist on a much smaller scale, thus a micro-niche. Further, when trying to understand the adaptive value of a trait, it is likely to be inappropriate to consider one trait in isolation, because different traits will trade-off with one another. Consider the *C. elegans* dauer example and imagine two genotypes that have different dauer larva formation responses: one that forms a high proportion of dauer larva, and one a low proportion, under the same environmental conditions. These different phenotypes are unlikely to be the only evolved differences between the genotypes. There will be other differences too, very possibly in key life-history traits (for example, brood size or schedules of reproduction).³⁸ The way to understand this is that these genotypes have evolved in their own micro-niches, where a life-history strategy has evolved, manifest in more than one trait. There may be common patterns of how different traits are related among genotypes, such that these can be characterized as trade-offs, be they positive or negative. Trade-offs can result from selection within a niche that independently affects different genetic pathways and hence traits, or they can occur directly from pleiotropic effects, for example within a genetic pathway. The term "trait" is useful for biologists, but it is not something with which an organism, or evolution, is concerned. The challenge for us seeking to understand the adaptive value and evolution of traits, is therefore to consider suites of traits and how they differ among genotypes.

Measuring Phenotypic Plasticity

That phenotypically plastic traits are environmentally determined obviously requires that different environments are used to induce and assay the different phenotypes. For species in which genetic clones are available (thus individuals of inbred, homozygous lines of *C. elegans* or *P. pacificus* etc.), different individuals

of the same genotype can be placed in the different environments, such that environmental-dependent effects can be seen. Other systems also have this experimental advantage. For taxa that are parthenogenetic, clones can be readily produced, such as for *Strongyloides* spp as well as, for example, *Daphnia* spp. For dioecious sexually reproducing species, lines can be inbred to achieve homozygosity, therefore producing functional clones. If this is not possible (either because the inbreeding would take so long, or because of deleterious effects of inbreeding), then the alternative approach is to use various sib-ship experimental designs, where genetic effects among sib-ships have to be accounted for.

Using clones, while straightforward, allows greater resolution of phenotypically binary traits. Consider the *C. elegans* dauer/non-dauer developmental choice. Each individual developing larva has to make a binary choice between these two developmental possibilities. However, among genetically identical individuals not every individual will make the identical choice, for example 60% of the larvae may form dauer larvae, 40% non-dauer larvae. This means that the probability of an individual forming a dauer larva under the given conditions is 60%. Therefore, an apparently binary trait, is actually a continuous trait, and can be thought of as a threshold trait.⁴⁷ This conceptual approach also applies to both the *Strongyloides* and *Parastrongyloides* developmental choices as well as to the alternative mouth morphologies of *P. pacificus*. Of course, for continuous traits (e.g., brood size, length), the value of the trait in the different environments can be directly measured. Therefore, there is no conceptual difference between phenotypically discrete traits and phenotypically continuous traits.

Among the phenotypic plasticity literature, the concept of reaction norms is used, particularly to compare plastic traits among different genotypes (Fig. 2).⁴⁸ This is useful because it clarifies different aspects of the plastic trait, each of which may separately be relevant and subject to both statistical and genetic analyses. The different aspects of the trait are the trait value in each environment and the difference in trait value between environments; this latter value is a measure of how plastic (or sensitive) a trait is to a change in the environment. From Figure 2, it can be seen that different genotypes can have different trait values (i.e., the absolute elevation of the line), but the same plasticity (i.e., the slope of the line between environments). Different genotypes may also have different plasticities, thus where the slopes of the reaction norms differ. This can be summarized as the difference in trait value between environments, though there are difficulties (Fig. 2). For traits measured on a continuous scale there are little further difficulties; however, for traits measured as

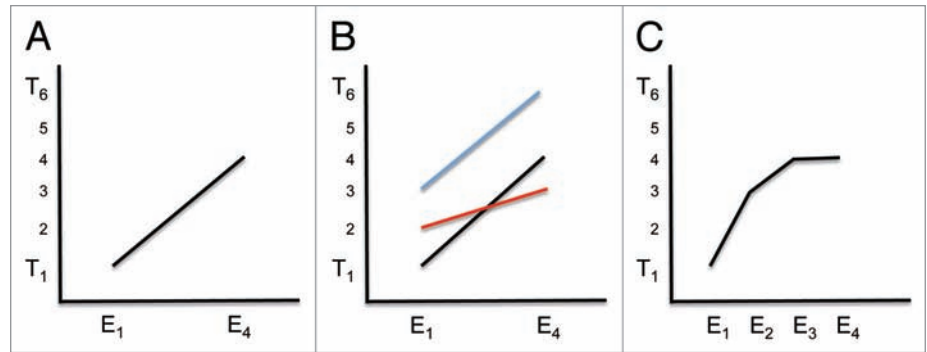


Figure 2. A reaction norm displays the value of a trait in a minimum of two environments. Thus, in (A), as the environment (e.g., temperature, or quantity of food) changes from E_1 to E_4 , then the value of the trait changes from T_1 to T_4 . The difference in the trait value, which is the slope of the line, is therefore the norm of reaction, more easily thought of as the sensitivity of the phenotype to the environment. This can be summarized numerically as $T_4 - T_1$. Different genotypes may have different reaction norms. In (B) a second genotype (blue line) has different trait values (T_3 and T_6), though the sensitivity is the same, $(T_4 - T_1) = (T_6 - T_3)$. A third genotype (red line) has different trait values (T_2 and T_3), and a different sensitivity, in this case less sensitive, $(T_3 - T_2) < (T_4 - T_1)$. For traits measured in two environments (A and B), only a linear reaction norm can be interpolated. Measurement in more environments, may reveal nonlinear reaction norms (C). For binary traits measured as proportions (e.g., for *C. elegans* the proportion of developing larvae that form dauer larvae, and for analogous traits in *Strongyloides* and *Parastrongyloides*) the trait values have 0 and 1 minima and maxima, respectively. As a consequence, the trait value in E_1 limits the maximum trait sensitivity, because the trait has a maximum of 1 in E_2 (for the case where the slope is positive, in A or B). Such proportion data also have a non-normal error structure (errors cannot lie outside of the 0–1 range) which requires a different error structure.

proportions, that are therefore bounded by a 0 and 100% (or 0 and 1) minima and maxima, the slope of the reaction norm can become confounded with the absolute trait value (Fig. 2).

Of course, there can be more than two environments, and then reaction norms need to be described over this more fine-grained environmental range. If only two environments are considered, one can only ever consider a linear reaction norm across those environments (Fig. 2). Measurement of phenotypes in more than one environment, will therefore show whether a reaction norms are, indeed, linear or not. Understanding different shapes of reaction norms is also likely to be important in considering the adaptive value of phenotypically plastic traits.⁴⁹

Testing for Adaptation

There are two meanings to the question of whether a trait is adaptive. The first is whether the trait per se is adaptive. The *P. pacificus* mouth morphologies is an example of this; here, is having alternative mouth morphologies adaptive compared with not having this (thus instead, presumably, a single mouth morphology)? This is a deep evolutionary question, which asks why the trait that is observed has evolved. The second version of this question is to ask when and where the extant trait value is adaptive. Continuing the *P. pacificus* example, this would ask under what conditions does a narrow mouth morphology give greater fitness compared with the wide mouth morphology. When we considered this before (Phenotypic Plasticity and Adaptation section) these two questions were asked together. This second question can be extended by asking why specific trait values are adaptive,

which comes from observing that there are genotypic differences in trait values. Both of these questions (and their answers) are context-dependent.

Rigorously testing and investigating adaptation of phenotypically plastic traits is key to understanding how suites of trait values contribute to fitness, especially when the trait values differ among genotypes. Studies with plants have been very powerful in this respect. Here, with the key perspective that a genotype's traits only make adaptive sense in the niche in which they evolved, reciprocal transplant, common-garden experiments have been used. In these, the genotype in question is grown both in its original environment (ideally in the wild) and in a different environment (and this is repeated for many genotypes, with a reciprocal experimental design) (e.g., ref. 50). Evidence in support of a genotype's trait value being adaptive, is that it will have greater fitness in its home environment compared with the other environments; specifically, this would be observed if there has been local adaptation of the genotype to its environment. More generally though, each genotype should have an environment where it has the greatest fitness. In these studies the question of adaptiveness is asked by testing this among different environmental contexts. These style of studies are relatively straight forward for plants, but much more challenging for animals especially because mark-release-recapture approaches are essentially impossible for small invertebrates. The next best approach is therefore to use microcosms, which would test different environments (especially harsh and dynamic environments), and within which different genotypes are allowed to compete.

Experimentally testing for evidence of trait adaptation to an environment is key; not testing for this only leaves 'just so' stories. This is especially important when considering plastic traits, to try and separate whether a plastic phenotype is adaptive or an inevitable physiological limit or constraint, and thus not adaptive. To think about this further, consider the example of how the feeding environment of *C. elegans* changes adult worm size and reproduction, i.e., less food results in smaller, less fecund worms.²⁹ This is clearly phenotypically plasticity (the food environment changes the phenotype), but is it adaptive or is it an inherent physiological constraint of being a *C. elegans* worm (or any other invertebrate or animal), or it is both? This is a difficult question; it might actually be an uninteresting question of semantics. Notwithstanding, taking the examples above of testing for adaptiveness, key to the approach is to make comparisons: for poorly fed, and so small, low fecundity worms, then what is the appropriate comparison? The correct environment in which to test them is the environment in which they developed (i.e., a poor food environment). In such an environment in nature there are likely to only be other *C. elegans* genotypes, similarly poorly fed, and thus small and with a low fecundity. Therefore, among-genotype comparisons within the same environment would be the appropriate comparison. Clearly, well fed (and so large and highly fecund) worms will outcompete small poorly fed worms, but this is an uninformative comparison; it can tell us nothing about adaptation, because it is comparing what natural selection does not compare during evolution. This therefore makes clear that the

relevant comparisons are context dependent, in this example it being within a poor food environment. The key point is that a plastic phenotype is recognized, indeed defined, by changing as the environment changes, but with respect to questions of adaptation, the across-environment comparison might be uninformative, and rather an among-genotype comparison within one environment may be informative. It is important to remember that in this example of the *C. elegans* food environment, larval exposure to food conditions effects a lifelong change to size and fecundity. There are other phenotypes which can continually change through an individual's life. In these cases the relevant comparisons for questions of adaptation may well be other comparisons, such as among genotypes within and between environments. This makes the important, general point that the best test of the adaptiveness of a plastic trait depends on the nature of that traits plasticity.

Phenotypic Plasticity and Noise

A challenge for natural selection driving the evolution of phenotypically plastic traits is the accuracy with which an environmental cue can be used to measure the environment (and possibly a future environment) of an individual where its fitness will be determined. There are two different problems: (1) the actual accuracy of the cue and (2) whether the relevant environment is in fact knowable (i.e., the environment may not be knowable via any cue, because stochasticity determines the environment, and there is no cue for stochasticity). One approach to addressing these difficulties that may evolve is the evolution of variation in trait(s) among individuals of a single genotype. This type of trait variation will therefore generate some phenotypes that better match the actual environment in which individuals find themselves. In effect, within-genotype trait variance widens the phenotypic space of a genotype. With this perspective, such variance in a trait could be an adaptive. Empirically it would be observed as phenotypic variation among genetically identical individuals. This trait variance is distinct from phenotypic plasticity, because the trait variance is independent of the environment.

Clearly, for any trait measured among genetically identical individuals in a constant environment, there is some variance due to stochastic processes of development (as well as experimental and measurement error). Adaptive within-genotype trait variation would be variation that is beyond this background level. Characteristics of adaptive trait variation are likely to be that the degree of trait variance will be subject to selection, such that different genotypes may differ in their trait variance (and that trait variance can be artificially selected). Variance among genetically identical *C. elegans* individuals has been noted before. In fact, the very extensive genetic analyses of *C. elegans* implicitly show this too. For many mutations, those mutations result in incomplete phenotypic penetrance, which means that genetically identical individuals are phenotypically different, thus there is within-genotype trait variance.^{51,52} A common way of thinking about this is that a trait is genetically tightly controlled, such that a reduction or loss of function mutation of a gene that contributes to that trait, relaxes the control of the trait, seen as incomplete

penetrance. While this isn't necessarily wrong, these observations show that (1) within-genotype trait variance can be genetically controlled and then (2) raise the more relevant question of whether such trait variance can be adaptive, and if so, when and how is it adaptive?

Studies in microorganisms may show the way here. There have now been many studies that have looked at heterogeneity in gene expression in bacteria and yeast. There are two sources of the heterogeneity that have been considered. One is so-called intrinsic noise, which is the difference in expression of two genes within an individual cell (measured by the expression of identical, introduced genes, which have distinguishable reporters). The second, referred to as extrinsic noise, is how expression of these pair of genes differs among otherwise identical cells.⁵³ It is this latter, extrinsic noise, which may be adaptive. The fitness effects of different degrees of heterogeneity have been measured, and have shown that this can have different advantages under different environmental conditions. For example, in *Bacillus subtilis* the heterogeneity in gene expression that arises from a small circuit, causes an environmental dose-dependent (i.e., environmental DNA concentration) effect on cellular competence.⁵⁴ Comparatively noisy and non-noisy circuit phenotypes performed similarly in some environments, but the more noisy circuit phenotype had a superior phenotype in other environments. This, in principle, shows that heterogeneity has fitness effects. Analogously, strains of *Saccharomyces cerevisiae* were constructed that differed only in the variability of expression of a gene among individual cells.⁵⁵ Comparing strains with high or low variation in gene expression showed that each was advantageous, but under different environmental conditions. This therefore also shows that cellular heterogeneity can have environmentally-dependent fitness effects.

Gene expression heterogeneity has been shown to underlie incomplete phenotypic penetrance in *C. elegans*.⁵² Specifically, in the molecular circuit which controls the fate of cells forming part of the *C. elegans* intestine, variation in gene expression near a key molecular threshold can alter this intestinal phenotype, such that there is phenotypic variance among genetically identical worms. Inter-individual variation in the expression of reporter genes in early *C. elegans* larval stages can predict a substantial proportion of the variation in adult lifespan among individuals.⁵⁶ Other studies have shown that phenotypic penetrance of a mutation is also dependent on the activity of other genes, including the chaperone Hsp90.^{57,58} This more broadly shows that gene networks control phenotypic variance. Interestingly, chaperone expression, which reduces the penetrance of mutations can have a cost, because it also reduces brood size.⁵⁹ Together, what this work shows is that there is heterogeneity among genetically identical individuals in gene expression, that this can be modified by multiple loci/genetic networks, and

that this heterogeneity can have phenotypic effects whose success differs in different environments. Importantly, this makes the point that “noise”, seen as inter-individual variation in traits may have an adaptive role in organismal life in demanding, heterogeneous environments. Our challenge is to study this, with this perspective.

Conclusions

There has been extensive theoretical study of phenotypic plasticity which has, for example, considered the fitness consequences of plasticity as well as the genetics, and hence evolution, of plastic phenotypes.⁶⁰ The theoretical genetics studies have centered on the question of whether “plasticity” is a trait that is separate from the trait itself.⁶¹ The difficulties of this debate were largely due to a lack of clarity of terminology, including theoretical concepts of genetics beyond empirical knowledge. That we are now in the genomic age means that understanding the control of phenotypically plastic traits is more empirically tractable (though still difficult) and so this can inform these theoretical studies. Moreover, by combing such studies with studies of adaptation, means that the genetic basis of important components of fitness can be dissected, which is a central challenge of modern biology. It is likely that phenotypically plastic traits will have multi-locus control and so understanding the structure and function of gene networks will be key. Considering, as an example, the *C. elegans* dauer/non-dauer developmental choice, the genetic pathways that control dauer larva development are well known, and clearly constitute a large complex network.¹¹ Given that different *C. elegans* genotypes have quantitatively different responses to the same environmental conditions, a pressing question is where are these differences encoded in this genetic network?^{38,39} Conceptually, the network can be considered to have three functional components: sensing the environment, transducing that environmentally-derived information and executing the dauer larva program. Changes in any of these three functional components could alter a genotype's phenotypic response to an environmental signal. Apparently similar dauer formation phenotypes could be achieved by different genetic means. The answer to the question is unlikely to be straightforward (e.g., ref. 62). However, the answer to this question, and analogous plastic phenotypes in other systems, are examples of the next big step for organismal genetics and genomics, in which nematode studies may lead the way.

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