Investigation of Nutritional Plasticity and Fitness Consequences of a Polyphenic Trait in *Pristionchus pacificus*

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Table of contents

I. Abstract i

II. Zusammenfassung ii

III. List of publications iii

IV. Introduction 1

1. Phenotypic (developmental) plasticity 1
1.1 What is phenotypic plasticity? 1
1.2 Significance of phenotypic plasticity in biological evolution 1
1.3 Constraints on the evolution of phenotypic plasticity–costs and limits 2
1.4 Polyphenism–a special case of phenotypic plasticity 4
1.5 Impact of nutrition on polyphenism–nutritional plasticity 4

2. *Pristionchus pacificus* 7
2.1 Phenotypic plasticity in *P. pacificus* 8
2.1.1 Life cycle and dauer plasticity 8
2.1.2 Mouth-form plasticity 9
2.2 Ecology of *P. pacificus* 11
2.3 Mouth-form gene regulatory network 12
2.3.1 Developmental switch 12
2.3.2 Downstream components of the switch 14
2.3.3 Epigenetics and environment 15
2.4 What is known about the nutritional plasticity of the mouth form in *P. pacificus*? 16
2.5 Fitness consequences of mouth-form plasticity 17

3. Aims of the thesis 18

V. Results 19

1. Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait 19
1.1 Synopsis 19
1.2 My own contribution 19

2. High nutritional conditions influence feeding plasticity in *Pristionchus pacificus* and render worms non-predatory 20
2.1 Synopsis 20
2.2 My own contribution 20

VI. Discussion 21

VII. References 25

VIII. Appendix 38

I. Abstract

Phenotypic (developmental) plasticity is the ability of an organism to adjust its phenotype in response to changes in its environment. This phenomenon has been suggested to impact the course of biological evolution by conferring an adaptive advantage to organisms in variable or novel environments and facilitating phenotypic novelty and evolutionary diversification. Polyphenism is an extreme case of phenotypic plasticity which results in environment-sensitive alternative phenotypes. *Pristionchus pacificus* is a polyphenic nematode model which exhibits two discrete mouth forms: eurystomatous (Eu) and stenostomatous (St). While the Eu morph facultatively predates on other nematodes, the St morph strictly feeds on microbes in its environment. Over a decade, studies have identified the unique molecular control of the mouth-form plasticity in *P. pacificus*, revealing developmental switch genes coupled to a complex regulatory network. In addition, several environmental factors such as temperature, population density, and culture condition have been found to influence feeding plasticity. However, our understanding of how nutritional conditions affect mouth-form plasticity has been limited, although studies in eusocial insects and scarab beetles have indicated a significant role for nutrition in the regulation of polyphenisms. In my thesis, I studied the effect of nutrition on mouth-form plasticity in *P. pacificus*. I established experimental setups to induce changes in nutritional status of worms by supplementing their growth medium with monosaccharides and fatty acids. I also studied lipid storage in worms as a measure of nutrition through Oil Red O staining and quantification. I found that fat storage-inducing nutritional conditions promote the development of non-predatory worms. Specifically, glucose-supplemented diet renders worms predominantly St. Additionally, I carried out transcriptomic and mutant analyses to elucidate associated molecular mechanisms. Findings revealed that *de novo* fatty acid synthesis and peroxisomal beta-oxidation pathways are essential for nutrition-induced mouth-form plasticity. Furthermore, I investigated fitness costs and benefits associated with mouth-form plasticity in different dietary conditions. In a separate project, I examined cost of plasticity and phenotype in natural strains of *P. pacificus* by studying their life history traits along with their mouth-form frequencies under standard and changing dietary conditions. Results suggest that there are fitness costs associated with plasticity and the production of the Eu morph. Taking a similar approach, I also showed that plasticity benefits *P. pacificus* in a high nutritional condition by facilitating the development of non-predatory worms which provide a fitness advantage over predatory ones. Overall, findings obtained from this work highlight the importance of nutrition in the regulation of mouth-form plasticity and enhance our understanding of fitness costs and benefits of phenotypic plasticity in *P. pacificus*.

II. Zusammenfassung

Phänotypische Plastizität (Entwicklungsplastizität) bezeichnet die Fähigkeit eines Organismus den Phänotyp als Reaktion auf Änderungen seiner Umwelt anzupassen. Es wurde angenommen, dass dieses Phänomen den Verlauf der Evolution beeinflusst, indem es Organismen in sich ändernden oder neuen Umgebungen einen adaptiven Vorteil verschafft und neue Phänotypen sowie evolutionsbiologische Diversifizierung begünstigt. Polyphänismus ist ein Extremfall phänotypischer Plastizität welcher zu umweltabhängigen alternativen Phänotypen führt. *Pristionchus pacificus* ist ein Nematodenmodellorganismus der aufgrund von Polyphänismus zwei eigenständige Mundformen zeigt: eurystomat (Eu) und stenostomat (St). Während die Eu-Morphe fakultativ andere Nematoden jagt, ernährt sich die St-Morphe ausschließlich von Mikroben in ihrer Umgebung. Über ein Jahrzehnt hinweg haben Studien die molekulare Steuerung der Mundformplastizität in *P. pacificus* identifiziert und Gene für Entwicklungsschalter entdeckt, die an ein komplexes regulatorisches Netzwerk gebunden sind. Darüber hinaus wurde festgestellt, dass mehrere Umweltfaktoren wie Temperatur, Populationsdichte und die Bedingungen im Kulturmedium die Ausbildung eines bestimmten Phänotyps beeinflussen. Unser Verständnis darüber, wie Ernährungsbedingungen die Mundformplastizität beeinflussen, war jedoch bisher begrenzt, obwohl Studien an eusozialen Insekten und Blatthornkäfern eine bedeutende Rolle der Ernährung in der Regulation von Polyphänismus nahegelegt haben. In meiner Dissertation habe ich die Auswirkungen der Ernährung auf die Plastizität der Mundform bei *P. pacificus* untersucht. Ich entwickelte experimentelle Ansätze, um den Ernährungszustand der Würmer durch den Zusatz von Monosacchariden und Fettsäuren zum Kulturmedium gezielt zu verändern. Dabei wertete ich mithilfe von „Oil red O“-Färbung und anschließender Quantifizierung die Fettspeicherung als ein Maß für die Nährstoffversorgung aus. Ich konnte zeigen, dass Ernährungsbedingungen, die zu mehr Fettspeicherung führen, die Entwicklung von nichträuberischen Würmern begünstigen. Insbesondere führt eine glukosereiche Ernährungsbedingung dazu, dass die Würmer überwiegend die St-Morphe ausbilden. Darüber hinaus führte ich Transkriptom- und Mutantenanalysen durch, um die zugrunde liegenden molekularen Mechanismen zu entschlüsseln. Die Ergebnisse zeigen, dass die Stoffwechsel- und Signalwege der *de novo*-Fettsäuresynthese und der peroxisomalen beta-Oxidation essenziell für die ernährungsbedingte Mundformplastizität sind. Zudem untersuchte ich, inwiefern Fitnesskosten oder -vorteile mit der Mundformplastizität unter verschiedenen Ernährungsbedingungen einhergehen. In einem separaten Projekt betrachtete ich die Kosten der Plastizität und der Phänotypen an natürlichen Stämmen von *P. pacificus* durch Auswertung von Kennzahlen während des Lebenszyklus („life-history-traits“) zusammen mit der Verteilung der Mundformmorphen unter normalen und sich ändernden Ernährungsbedingungen. Die Ergebnisse deuten darauf hin, dass die Plastizität und die Ausbildung der Eu-Morphe mit Fitnesskosten verbunden sind. Weiterhin konnte ich in einem ähnlichen Ansatz zeigen, dass unter nährstoffreichen Bedingungen die Plastizität für *P. pacificus* vorteilhaft ist, da sich bei dieser Bedingung der nichträuberische Phänotyp ausbilden kann, der Fitnessvorteile gegenüber dem räuberischen Phänotyp zeigt. Insgesamt verdeutlichen die Ergebnisse dieser Arbeit die Bedeutung der Ernährung für die Regulation der Mundformplastizität und verbessern unser Verständnis der Vor- und Nachteile phänotypischer Plastizität für die Fitness bei *P. pacificus*.

III. List of publications

Dardiry, M., **Piskobulu, V.*,** Kalirad, A.*, & Sommer, R. J. (2023). Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait. *Evolution Letters*, 7(1), 48–57. doi:10.1093/evlett/qrac001

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<u>Additional papers not included in this thesis</u>

Feldhaus, C., & **Piskobulu, V.** (2024). A low bias method for quantification of Oil Red O staining. *BioRxiv*, 2024.06.18.599611. doi:10.1101/2024.06.18.599611

IV. Introduction

1. Phenotypic (developmental) plasticity

1.1 What is phenotypic plasticity?

Organisms respond to environmental variation in many ways. One particular strategy for organisms to persist and thrive in dynamic environments is to exhibit phenotypic (developmental) plasticity which allows them to alter their phenotype in response to changes in environmental conditions (Pigliucci, 2001; West-Eberhard, 2003). This ability can be observed in an organism's physiology, morphology, behaviour, and life history traits. Phenotypic plasticity is ubiquitous in nature since every organism can respond to its environment through changes in gene expression, adjusting certain aspects of its phenotype. Therefore, plasticity is "*an intrinsic property of organisms*" (Sultan, 2021). Besides predictably varying environments, plasticity can be advantageous in novel or extreme environments which have never been experienced by organisms before (Ghalambor et al., 2007; Price et al., 2003). In this instance, plasticity may generate adaptive or non-adaptive traits. Plasticity is particularly considered to be adaptive in novel environments when it allows organisms to produce optimal phenotypes, which provide fitness advantage and are target of directional selection, facilitating the evolution of adaptive traits (Ghalambor et al., 2007). Although less pronounced, the importance of non-adaptive plasticity for evolution of novel traits has also been argued in the scenarios where organisms are facing stressful environments (Ghalambor et al., 2007, 2015). In line with these assumptions, plasticity has been acknowledged as a source of adaptation for animals colonising new environments such as islands (Aubret & Shine, 2009; Herrel et al., 2008) or novel climatic habitats (Yeh & Price, 2004). Moreover, plasticity can be discrete which results in alternative non-overlapping phenotypes, i.e. polyphenism; or continuous, exhibiting a variation in the expressed phenotype such as height or weight. The former case, polyphenism, is an astonishing form of phenotypic plasticity which is of particular interest in this thesis.

1.2 Significance of phenotypic plasticity in biological evolution

Phenotypic plasticity is often considered to be the facilitator of phenotypic novelty (West-Eberhard, 2003), and it has been argued that plasticity-associated mechanisms such as "genetic accommodation" and "genetic assimilation" (described below) can potentially contribute to evolutionary diversification and speciation (Pfennig et al., 2010; West-Eberhard, 1989).

The “plasticity-first hypothesis” describes a proposal of how phenotypic plasticity, as the origin of novelty, can facilitate adaptive evolution (Levis & Pfennig, 2016; West-Eberhard, 2003). In this hypothesis, plasticity in novel or stressful environments reveals “cryptic genetic variation”, a hidden variation, which is not expressed under normal environmental conditions. This may result in a range of phenotypic variation. Selection can then favour a new phenotypic variant and modify it over time to reach an optimal adaptive phenotype through genetic alterations. This is generally termed as “genetic accommodation”. At this point, a population may become less sensitive to the environment and may not express the ancestral phenotype anymore. A novel polyphenism can also emerge, if the ancestral phenotype is still maintained in the population and the environmental sensitivity persists. In the former case (reduced environmental sensitivity), an initially environment-induced phenotype can be canalised over time, and the population can completely lose its sensitivity to the ancestral environment. This process is called “genetic assimilation”, and as the last step of plasticity-first evolution, it is associated with loss of plasticity and the introduction of a novel phenotype which is constitutively expressed regardless of environmental variation. Thus, plasticity-first hypothesis lays an important framework of how phenotypic plasticity can contribute to the evolution of novel traits, and plasticity-related research has yielded empirical support for the steps involved in this process over the years (Levis & Pfennig, 2019; Suzuki & Nijhout, 2006; Waddington, 1953, 1956). Nonetheless, our understanding of molecular mechanisms of plasticity-associated processes, e.g. genetic accommodation and assimilation, and of the prevalence of plasticity-first evolution in nature remain limited, representing important challenges to be addressed in the field (Levis & Pfennig, 2016; Sommer, 2020).

1.3 Constraints on the evolution of phenotypic plasticity–costs and limits

Evolution of phenotypic plasticity is often positively associated with environmental variability. Adaptive phenotypic plasticity provides an ability for organisms to execute phenotypes which would enhance fitness in variable environments. In this case, selection favours plasticity as it benefits organisms and promotes survival. However, “limits” and “costs” associated with plasticity may hinder its evolution.

“Perfect plasticity”–the limitless ability to respond to environmental cues at any stage of development by producing phenotypes that perfectly match any type of environment–would be rare (if there is any) or completely absent in nature. This argues for the presence of constraints (costs and limits) on the evolution of plasticity (Callahan et al., 2008; DeWitt et al., 1998; Murren et al., 2015). Costs associated with plasticity have been categorised as: cost of plasticity (mainly referred to as ‘cost of being plastic’) and cost of phenotype (‘cost of inducing a plastic trait’) (Callahan et al., 2008; Murren et al., 2015). Plasticity cost is a reduction in fitness a plastic organism incurs as a cost relative to non-plastic or less plastic organisms.

Plasticity is inherently a costly ability; for example, there are costs due to maintenance of environment-sensitive gene regulatory elements which facilitate plastic responses. Phenotype cost mainly describes a fitness trade-off of allocating resources into producing a particular phenotype. While plasticity costs are universal (present in all environments), phenotype costs are dependent on the environment (Murren et al., 2015). For instance, induction of defensive morphs in *Daphnia* may be beneficial in environments where there is a risk of predation, promoting survival (Weiss, 2019). Therefore, expressing these morphs in low- or non-predation environments may incur a fitness cost (phenotype cost) for the organism. As for plasticity costs, plastic *Daphnia* would be expected to incur a cost despite of the environment it finds itself. Additionally, one can hypothetically compare "plastic" and "fixed" (environmentally insensitive, constitutively producing only one phenotype) genotypes in both regimes (predation and non-predation). A fixed genotype, which produces the defensive morph in the non-predation environment, would incur a phenotype cost relative to the plastic genotype. However, in the predation environment, the plastic genotype incurs a plasticity cost because it must produce the phenotype through its plastic ability, unlike the fixed genotype which constitutively exhibits the defensive morph (see Figure 6.1 by Snell-Rood & Ehlman, 2021).

Non-plastic organisms are often considered as "specialists" as they constitutively produce a phenotype. On the contrary, plastic organisms facultatively produce the same phenotype depending on the environment. Nonetheless, the plastic organism may be constrained in its ability to produce the phenotype as good as the specialist, thus the produced phenotype would be considered as suboptimal (Auld et al., 2010; DeWitt et al., 1998). This is termed as the "limit" to plasticity as plastic organisms are often regarded as "jack-of-all-trades" but "a master of none". Several factors can limit the ability of a plastic genotype producing the optimal phenotype and a few of these include reliability of the environmental cue, time lags (duration of environmental changes and of developmental responses), and the time in development when the induction of a phenotype is initiated (producing a phenotype earlier in development is better than later) (DeWitt et al., 1998). Specifically, the reliability of the environmental cue has been emphasised as a significant factor limiting the evolution of plasticity (Snell-Rood & Ehlman, 2021) because a "mismatch" between the executed phenotype and the anticipated environment would decrement fitness (Bateson et al., 2014).

Taken together, both types of costs and limits theoretically constrain the evolution of phenotypic plasticity. However, empirical studies in plants and animals when meta-analysed revealed weak fitness costs associated with plasticity (Van Buskirk & Steiner, 2009). This suggests that detecting costs experimentally is a challenging task and plasticity somehow finds a way to evolve regardless of associated constraints (e.g. through evolution of offsetting mechanisms over time; Murren et al., 2015). In addition, it is important to note that some types

of plasticity can be more costly than others. For instance, it has been argued that “learning” as a mechanism of behavioural plasticity is highly costly and costs associated with this ability are manifested as delays in reproduction and investment in a fewer number of progenies (Snell-Rood, 2012; Snell-Rood et al., 2011, 2018). Nonetheless, identifying the plastic trait and evaluating right fitness parameters is critical for measuring costs of plasticity.

1.4 Polyphenism–a special case of phenotypic plasticity

Polyphenism is a remarkable form of phenotypic plasticity, which enables a single genotype to produce two or more discrete phenotypes in response to environmental cues. This phenomenon is prevalent in plants and animals such as insects, amphibians, reptiles, fish, nematodes, freshwater crustaceans (water fleas), and rotifers (Gilbert, 2017; Simpson et al., 2011; Whitman & Agrawal, 2009; Yang & Pospisilik, 2019). Polyphenism can also be observed in unicellular organisms like ciliates (Kopp & Tollrian, 2003; Ryals et al., 2002), and even considered to exist in viruses (lytic versus lysogenic cycles bacteriophage lambda; Ptashne, 1986). Polyphenic species can produce unique phenotypes whose expression is tightly linked with specific environmental conditions such as temperature, light, pheromones, seasonal changes, population density, and nutrition. Therefore, environmental specificity of polphenisms has resulted in various types displayed by different species. A few of the notable examples of polyphenisms in animals include resource polyphenism (e.g. nematodes; Lightfoot et al., 2021, and spadefoot toad tadpoles; Pfennig, 1992), seasonal polyphenism (e.g. butterflies; Brakefield et al., 1996), temperature-dependent sex determination (e.g. crocodiles; Lang & Andrews, 1994 and turtles; Zaborski et al., 1988), pheromone-induced defence morphs (e.g. *Daphnia*; Weiss, 2019), dispersal polyphenism (e.g. aphids; Braendle et al., 2006), and population density-dependent polyphenism (e.g. locusts; Pener & Simpson, 2009). Besides environmental conditions, stochastic factors can also influence polyphenisms (Susoy & Sommer, 2016).

1.5 Impact of nutrition on polyphenism–nutritional plasticity

Across all taxonomic groups, insects have a striking diversity of polyphenisms (Simpson et al., 2011). Nutrition, as an environmental factor, plays an important role in the regulation of caste polyphenism in certain species of eusocial insects (bees, ants, vespine wasps, termites) (Scharf et al., 2007; Trible & Kronauer, 2017; Wheeler, 1986); and in the development of exaggerated traits (male weapons) in dung beetles (Emlen, 1994, 1997b; Moczek, 1998) and stag beetles (Gotoh et al., 2014). In this section, I will introduce two comprehensively studied cases of nutrition-regulated insect polyphenisms: horn polyphenism in dung beetles, and caste determination in honeybee.

Dung beetles provision their young in brood balls made up of manure available in their surroundings. Females lay a single egg on each brood ball which provides a nutritional environment for the development of the larva. Unlike most holometabolous insects, in which certain developmental stages and thresholds must be reached for metamorphosis, dung beetle adult transformation through pupation in *Onthophagus* species (Coleoptera) starts once the larva completes consuming the provided food source (Shafiei et al., 2001). Therefore, quality and quantity of the brood ball affect the body size of the larva which conditionally dictates horn development (Emlen, 1994, 1997b; Moczek, 1998, 2002). Generally, in *Ontophagus* species, a high nutritional condition results in larger body size, promoting the development of larger horns. In contrary, a low nutritional condition leads to smaller adults which exhibit restricted or inhibited horn development. Therefore, the horn length is dependent on the body size. The scaling relationship between the body size and horn length indicates a sigmoidal distribution which suggests that body size acts as a threshold switch mechanism (Moczek & Nijhout, 2003). However, this scaling relationship can differ across *Onthophagus* species. Some species show a linear or disrupted continuity in the scaling relationship of body size-horn length (Moczek, 2010). The mating experiments also provided that the genotype of fathers does not affect the horn sizes of the progeny, further indicating that this trait is regulated by the environment that is the larval nutritional condition (Moczek & Emlen, 1999). The horn polyphenism also modulates behaviour of males. While horned males guard and defend the entry of burrows to prevent other males from entering and mating with the female, hornless males avoid conflict and display a “sneaky” behaviour to achieve mating via digging alternative routes to reach females (Emlen, 1997a; Moczek & Emlen, 2000).

Studies aimed at identifying molecular mechanisms of this developmental switch have revealed that several factors play a vital role in horn polyphenism. For instance, juvenile hormone is an important regulator of horn expression in *Ontophagus taurus* (Emlen & Nijhout, 1999), and its significance as a developmental regulator has been implicated in the evolutionary diversification of thresholds between two different and far apart populations of *Ontophagus taurus* (North Carolina and Australia). It has been argued that adjustments to the sensitivity to juvenile hormone, in terms of developmental timing and magnitude, may have contributed to differences in the threshold response observed between these two populations (Moczek & Nijhout, 2002). Molecular studies in *O. taurus* have revealed that various pathways, such as Doublesex, Hedgehog, and insulin/insulin-like signalling (specifically Forkhead Box O (*Foxo*) transcription factor), are involved in the regulation of horn polyphenism (Casasa & Moczek, 2018; Kijimoto et al., 2012; Kijimoto & Moczek, 2016). Doublesex and insulin/insulin-like pathways have also been recognised in other insects as an important mediator of nutrition-induced plasticity (Chandra et al., 2018; Emlen et al., 2012; Gotoh et al., 2014). Taken

together, these findings suggest that conserved developmental pathways can be repurposed for novel functions (Casasa, 2024).

In honeybee (*Apis mellifera*), female castes are determined through larval nutrition. Workers and queens have the same genotype but the nutrition they receive during larval stages alter their caste fate. Studies in honeybee have revealed that nurse bees can differentially feed larvae for caste differentiation (Haydak, 1970). In this case, the quality of the food provided for the developing larva regulates the caste differentiation. The larva destined to be a queen is fed with “royal jelly”, whereas the larva destined to be a worker is fed with “worker jelly”. Royal jelly differs from worker jelly in terms of composition, and it is more nutritious (Guo et al., 2021). Research suggests that specific components of the royal jelly contribute to the caste differentiation. For instance, royalactin, a protein found in royal jelly, induces queen development in *A. mellifera* (Kamakura, 2011). Royalactin reduces developmental time and increases ovary size and adult weight, which all correspond to changes obtained by royal jelly feeding. This protein also increases the level of juvenile hormone which is associated with queen development as it peaks at the 4th instar larvae which are queen destined. Further, royalactin have similar phenotypic effects in *Drosophila melanogaster*, and its effect on queen development is mediated via epidermal growth factor receptor signalling pathway.

Caste determination in honeybee is also heavily influenced by epigenetic factors. An investigation on the functional significance of DNA methyltransferase 3 (*Dnmt3*), which establishes DNA methylation *de novo*, revealed that DNA methylation plays a crucial role in the regulation of caste development (Kucharski et al., 2008). Silencing *Dnmt3* via small-interfering RNA in newly hatched larvae leads to queen development, mimicking the effect of royal jelly feeding. This suggests that royal jelly has an inhibitory effect on *Dnmt3*. The Honeybee genome is predominantly methylated in gene bodies, particularly at exons in queen and worker brain tissues, with differences in DNA methylation patterns observed in over 550 genes between these female castes (Lyko et al., 2010). Occurrence of DNA methylation at splicing associated sites suggested that DNA methylation may direct alternative splicing in this species. In fact, RNA interference knockdown of *Dnmt3* results in a decrease in genomic DNA methylation levels and changes alternative splicing in *A. mellifera* (Li-Byarlay et al., 2013). Therefore, in this case, gene body methylation is often associated with facilitation of gene expression. Additionally, several genes have been found to be differentially expressed between queen and worker bees during larval development (Barchuk et al., 2007). Based on these transcriptomic analyses, it has also been proposed that the nutritional effect of royal jelly is mediated via Tor pathway (target of rapamycin, a regulator of nutritional status-induced growth) to promote juvenile hormone synthesis, inducing queen development. The significance of the Tor pathway in queen development has been confirmed by a study in which

the knockdown of *amTOR* (Tor-encoding gene) in larvae fed with royal jelly led to the development of workers (Patel et al., 2007).

Besides DNA methylation, several other epigenetic mechanisms such as histone modifications, microRNAs, and RNA modification, have also been associated with honeybee caste polyphenism (Ashby et al., 2016; Dickman et al., 2013; Jin et al., 2023; Wang et al., 2021; Wojciechowski et al., 2018). Overall, caste determination in honeybee is a remarkable example of nutritional plasticity, which is regulated by complicated genetic and epigenetic regulatory mechanisms. Apart from honeybees, research on several other hymenopteran species has provided substantial evidence that nutritional regulation of polyphenisms is a widespread phenomenon in this order, contributing to reproductive and behavioural division of labour among castes.

Taken together, studies in beetles and eusocial insects have indicated how important nutrition is for the regulation of polyphenisms. Besides insects, nematodes also exhibit polyphenisms which are sensitive to food availability, such as the formation of “dauer” larva, a stress-resistant alternative larval stage in many nematode species (Golden & Riddle, 1984; Grant & Viney, 2011), and mouth-form polyphenism in certain species of diplogastrid nematodes like *Pristionchus pacificus* (Susoy et al., 2015; von Lieven & Sudhaus, 2000). In the rest of this introduction section, I will introduce *P. pacificus* as a model for studying phenotypic plasticity by highlighting its unique features, such as mouth-form polyphenism.

2. *Pristionchus pacificus*

Pristionchus pacificus is a hermaphroditic, self-fertilising nematode (roundworm) in the Diplogastridae family. Initially, it was utilised as a comparative model to *Caenorhabditis elegans* for the studies of vulval development (Sommer & Sternberg, 1994, 1995, 1996), and was officially described in 1996 (Sommer et al., 1996). Since then, as a model system, *P. pacificus* has become a subject of intense research in the field of evolutionary biology with an integrative approach, combining various other fields, such as developmental biology, developmental genetics, ecology, population genetics, genomics, and behavioural genetics (Sommer, 2009, 2015, 2020; Sommer & McGaughran, 2013). Over the years, various molecular and genetic tools and experimental methods have been established to study the unique biology of *P. pacificus* (Han et al., 2020; Igreja et al., 2022; Schlager et al., 2009; Sun et al., 2021; Tian et al., 2008; Witte et al., 2015). The genome of *P. pacificus* has been sequenced and it is well-annotated (Dieterich et al., 2008; Rödelsperger et al., 2017). Genomic and phylogenetic analyses have also estimated that *C. elegans* and *P. pacificus* diverged from each other over 200 million years ago (Howard et al., 2022; Qing et al., 2024 preprint). However, despite this divergence, both species still exhibit similarities. Like *C. elegans*, *P. pacificus* can be easily cultured in the lab, using *Escherichia coli* as a food source, and it has

a short, 4-day, generation time when incubated at 20ºC. *Pristionchus pacificus's* remarkable ecology, namely the necromenic lifestyle with beetles in nature, and mouth-form plasticity make it an interesting model system.

2.1 Phenotypic plasticity in *P. pacificus*

2.1.1 Life cycle and dauer plasticity

Pristionchus pacificus exhibits plasticity in the form of an alternative life cycle. In the lab, when grown on *E. coli* OP50, *P. pacificus* goes through a direct life cycle which includes four juvenile stages (J1, J2, J3, J4; in this respective order) before reaching adulthood (Figure 1). Unlike *C. elegans*, it completes its first juvenile stage (J1) in the egg, and moults to J2 before hatching (Fürst von Lieven, 2005). Under unfavourable environmental conditions (e.g. low food availability and crowding), *P. pacificus*, like many other nematodes including *C. elegans*, can go through a developmentally arrested and non-feeding stage called "dauer" (Mayer & Sommer, 2011; Ogawa et al., 2009; Ogawa & Brown, 2015). Generally, in this stage, the metabolic activity of nematodes is greatly reduced (Burnell et al., 2005; O'Riordan & Burnell, 1989; Van Voorhies & Ward, 1999) and they are morphologically distinct (Albert & Riddle, 1988; Cassada & Russell, 1975). Dauer worms can be induced at the early stages of development and bypass the J3 stage in the direct life cycle. In the lab, *P. pacificus* has been observed to survive in the dauer stage for up to a year (Mayer & Sommer, 2011). When the environmental conditions are favourable again, the worm exits dauer and continues its development through the J4 stage and later becomes an adult. In addition, *P. pacificus* is androdioecious, which is a mating system that consists of hermaphrodites and males. While hermaphrodites dominate the population, males can be spontaneously generated at low rates. *Pristionchus pacificus* has an XO sex determination system, in which hermaphrodites have two copies of the sex chromosome (XX) and males have one (XO) (Pires-daSilva & Sommer, 2004). Occurrence of males in this system allows researchers to carry out genetic experiments via outcrossing thus, exchanging genetic material between different strains. Moreover, research suggests that outcrossing with males can potentially facilitate the generation of new allelic combinations, promoting adaptation under changing environmental conditions in nature (Morgan et al., 2017). This suggests that maintenance of males in the population may have an adaptive value, even though hermaphrodites can successfully reproduce via self-fertilisation.

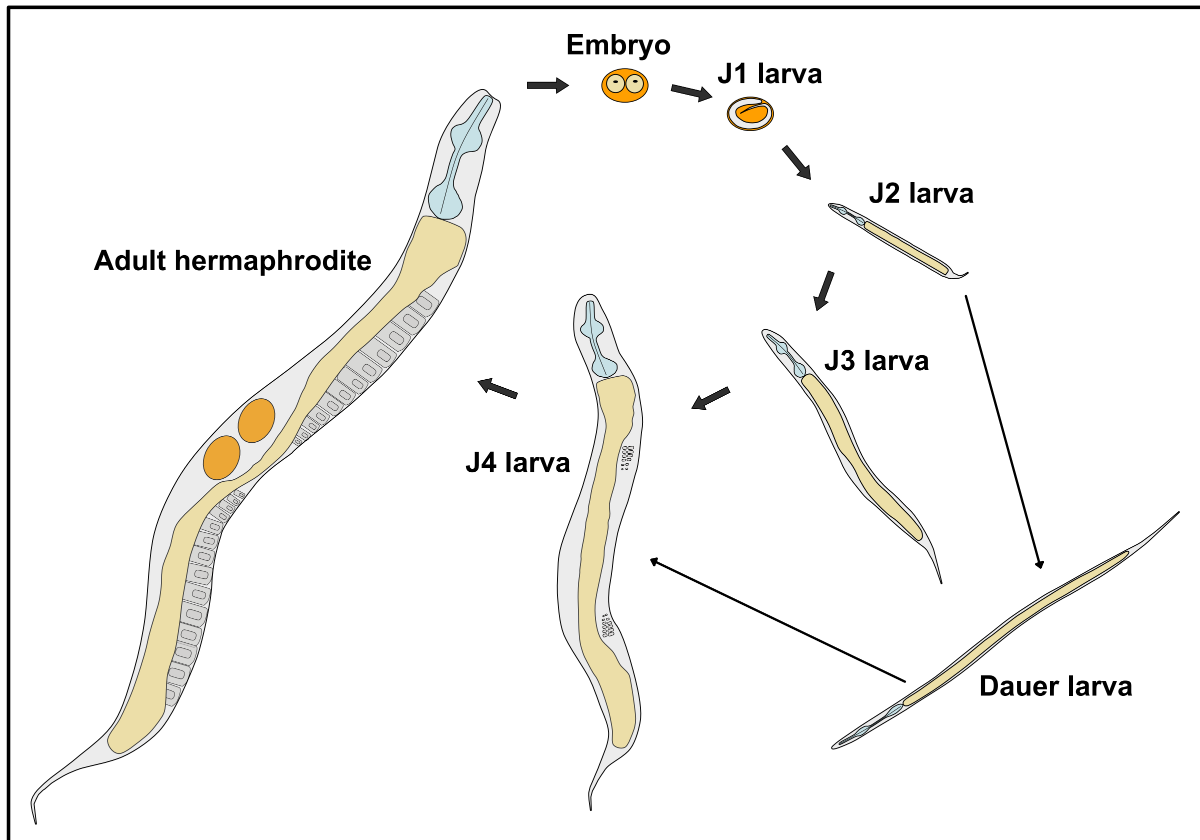

Figure 1: Schematic representation of the life cycle of *P. pacificus*.

2.1.2 Mouth-form plasticity

Besides dauer plasticity, *P. pacificus* also exhibits mouth-form dimorphism. Adults of *P. pacificus* can express either one of two distinct mouth forms (Figure 2). An adult worm has either a "eurystomatous" (Eu) mouth or a "stenostomatous" (St) mouth (Bento et al., 2010; Ragsdale et al., 2013). The Eu worm has a wider mouth and two hook-like "teeth": a dorsal tooth and a right subventral tooth on the opposite side. On the contrary, the St worm has a narrow mouth with only one flint-shaped dorsal tooth. Therefore, the right subventral tooth is absent in the St morph. In *P. pacificus*, the mouth-form decision is made at juvenile stages during development and is irreversible, meaning adults can only express one morph throughout their lifetime. The critical period for the mouth-form decision is between 36 and 60 hours after starting cultures with age-synchronised eggs (Werner et al., 2023). Therefore, the mouth-form decision is made at relatively earlier stages of development, and it is challenging to alter the mouth-form ratio of a population of juvenile worms at later stages.

In *P. pacificus*, both sexes can express either one of the mouth forms. However, most of the males develop the St morph, whereas hermaphrodites predominantly develop the Eu morph (95% Eu), at least in the reference strain (PS312) (Serobyan et al., 2013). Mouth-form plasticity in *P. pacificus* is predominantly studied in hermaphrodites, and it is heavily influenced by biotic and abiotic factors, such as food availability, population-density, pheromones,

temperature, bacterial diet, and culture condition (Bento et al., 2010; Bose et al., 2012; Dardiry, Piskobulu, et al., 2023; Lenuzzi et al., 2023; Serobyan et al., 2013; Werner et al., 2017, 2018). Under standard laboratory conditions, where all abiotic factors are kept constant, a population of *P. pacificus* does not constitutively produce only one morph, it rather expresses both mouth-forms even though the ratio may be biased towards only one. This suggests that there is also stochasticity in the regulation of mouth-form plasticity (Susoy & Sommer, 2016).

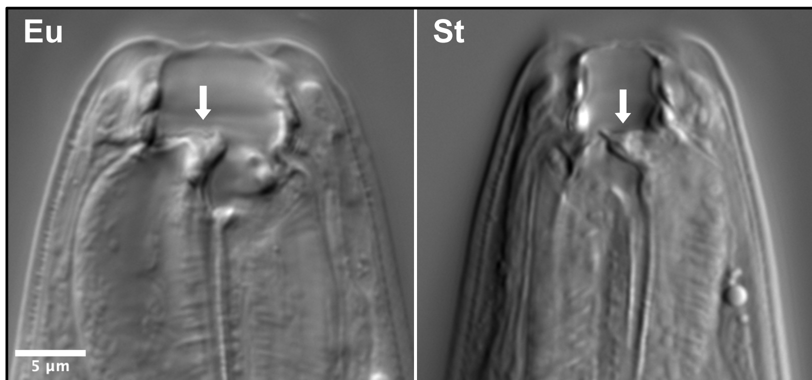

Figure 2: Mouth-form polyphenism in *P. pacificus*. The eurystomatous (Eu) morph exhibits a hook-like dorsal tooth (indicated with an arrow) and a right subventral tooth. The stenostomatous (St) morph has only one flint-shaped dorsal tooth (indicated with an arrow).

Mouth-form plasticity does not only induce differences in morphology but also in behaviour between two morphs. While the Eu worm allows facultative predation on other nematodes, the St morph results in strict microbial feeding. It is important to note that both morphs can feed on microbes like bacteria; therefore, *P. pacificus* strains, regardless of their mouth-form ratio, can easily be maintained in cultures with *E. coli* as food source. In addition to predation, it has also been observed that the Eu worms in *P. pacificus* exhibit territorial behaviour by biting adult *C. elegans* worms and expelling them from the competition for a bacterial resource (Quach & Chalasani, 2022). Furthermore, *P. pacificus* recognises its kin and does not predate on them (Lightfoot et al., 2019). Its aggression towards other strains is also dependent on the genetic relatedness. In this case, the likelihood of predation increases as the genetic relatedness decreases (Lightfoot et al., 2021). Predatory behaviour of *P. pacificus* can also be modulated via certain types of naturally isolated bacterial diets, particularly vitamin B12-producing ones, which enhance predation (Akduman et al., 2020). Thus, *P. pacificus* exhibits behavioural plasticity as well.

Predatory behaviour aided by moveable teeth is a unique property of diplogastrid nematodes. The occurrence of teeth is considered a morphological novelty in Diplogastridae, which has been facilitated by phenotypic plasticity in the form of mouth-form polyphenism (Ragsdale, 2015; Sommer, 2020; Susoy et al., 2016). Phylogenetic constructions and morphological analyses in diplogastrid nematodes suggest that a common ancestor had

acquired mouth-form dimorphism, which led to the diversification of mouth morphologies and their associated regulatory mechanisms observed across different species in Diplogastridae (Susoy et al., 2015).

2.2 Ecology of *P. pacificus*

In nature, *P. pacificus* can be found in the soil as a free-living nematode or on insect hosts. Generally, nematodes in the genus *Pristionchus* are highly associated with various species of insects and their related habitats (Ragsdale et al., 2015). For instance, scarab beetles, such as chafers, dung beetles, and stag beetles, commonly host numerous species of *Pristionchus* nematodes (Herrmann et al., 2006b, 2006a; Ragsdale et al., 2015). Specifically, *P. pacificus* has been consistently isolated from the Oriental beetle (*Exomola orientalis*) which suggests a close association with this beetle in nature (Herrmann et al., 2007). In fact, it has been found that the sex pheromone produced by this beetle attracts *P. pacificus* (Herrmann et al., 2007; Hong et al., 2008). Beetles facilitate the dispersal of *Pristionchus* nematodes to new environments. Research suggests that *P. pacificus* has an Asian origin and a cosmopolitan distribution (Herrmann et al., 2007, 2010; Kanzaki et al., 2012). The beetle-nematode association has led to collection of nematodes from around the world, allowing isolations of not only *P. pacificus* strains but also many other *Pristionchus* species over the years. These isolates are cryopreserved in liquid nitrogen, which allows the formation of an extensive catalogue of worms to study in the lab.

La Réunion, a Mascarene Island in the Indian Ocean, had been one of the destinations for field work to discover new *Pristionchus* nematodes. This investigation led to an intriguing discovery. *Pristionchus pacificus* was found to be highly abundant and diverse on La Réunion Island, and associated with several scarab beetles, particularly with the endemic rhinoceros beetle *Oryctes borbonicus* (Herrmann et al., 2010). Mitochondrial haplotype analysis categorised isolated strains into four major clades found around the world, suggesting beetle host-associated establishment of *P. pacificus* on the island from various independent origins (Herrmann et al., 2010). This study pioneered follow up ecological and population genetic studies on isolates of *P. pacificus* obtained from La Réunion and the neighbouring islands, which have enhanced our understanding of this nematode's evolutionary ecology (McGaughran et al., 2013, 2016; Morgan et al., 2012, 2014).

Why does *P. pacificus* use beetles as hosts? *Pristionchus pacificus* has a necromenic relationship with its beetle host. The nematode lives on the beetle as a non-feeding dauer larva (Herrmann et al., 2010; Ragsdale et al., 2015). When the beetle dies, the cadaver starts decomposing with the proliferation of bacteria and fungi. The nematode then senses these changes, exits the dauer stage and resumes its development (Meyer et al., 2017). The cadaver of the insect provides a nutritious environment for the worm to grow and populate this

habitat. Moreover, dauer nematodes display a dispersal behaviour, leaving the beetle carcass and looking for new hosts. Carefully designed lab and field experiments, investigating succession of *P. pacificus* and bacteria at several time points (series of weeks) after the death of the beetle host (*O. borbonicus*), have shown that *P. pacificus* display a “boom and bust” strategy for dispersal (Renahan et al., 2021). Dauers are very abundant after 4 weeks post-death of the host. When the bacterial load peaks at the 6th week, worms of mixed stages increase, and the number of dauers plummet. When the food becomes limited at the 8th week, dauers take over again. In addition, dauers disperse rapidly as they emerge, probably right after beetle death and after a generation when new dauers emerge. Interestingly, adult worms derived from dauers obtained directly from beetle carcasses strictly develop the Eu morph. This is a unique pattern as a dauer-derived further generation of worms (F1) collected from beetle carcasses and laboratory-induced dauers do not show this extreme trend. In addition, *P. pacificus* shares the beetle host with other nematode species which also creates a competitive environment for resources (Renahan & Sommer, 2022).

Taken together, in nature, *P. pacificus* experiences dynamic and multifaceted environments, and exhibit unique dispersal and succession strategies coupled to its phenotypic plasticity and its association with beetles.

2.3 Mouth-form gene regulatory network

For over a decade, *P. pacificus* has been intensively studied for elucidating the regulatory mechanisms underlining its mouth-form plasticity. These studies have provided ample amount of evidence that the regulation of mouth-form development is a complex and unique process that involves several components: environment, epigenetic mechanisms, developmental switch genes, downstream regulatory transcription factors, and structural genes which constitute mouth morphology and teeth.

2.3.1 Developmental switch

How does phenotypic plasticity achieve environmental sensitivity to execute alternative phenotypes? It has long been argued that polyphenisms require a developmental switch mechanism which integrates the environmental cues with the execution of distinct phenotypes (West-Eberhard, 2003, 2005). However, developmental switch genes are often accompanied by complex molecular networks which can influence the induction of plastic traits (Levis & Ragsdale, 2022; Sommer, 2020). Nonetheless, developmental switch mechanisms promote environmental sensitivity, potentially playing a significant role in plasticity-led evolution. Understanding the molecular mechanisms of such switch modules and their associated regulatory networks can enhance our understanding of the significance of phenotypic plasticity in the evolution of novel traits (Sommer, 2020).

In *P. pacificus*, a developmental switch was first discovered by Ragsdale and co-workers (Ragsdale et al., 2013). A sulfatase, *eud-1,* functions as a developmental switch gene. Inactivation of this gene leads to an all-St phenotype, therefore '*eud'* refers to *eu*rystomatous form *d*efective. Overexpression of this gene in a *eud-1* mutant background redirects mouth-form development to all-Eu. Since *eud-1* is located on the X chromosome, introducing an extra copy of this gene into *P. pacificus* males leads to complete Eu morph expression, which indicates a dosage-dependent effect, as males normally have only one copy of the X chromosome and they are predominantly St. Later, *eud-1* was found to be part of a multigene locus, containing two pairs of inverted duplicate genes in tandem arrangement (Sieriebriennikov et al., 2018). The centre of the multigene locus houses the sulfatase pair, *eud-1* and its paralog *sult-2.2.1*, which is surrounded by two alpha-N-acetylglucosaminidase genes, *nag-1* and *nag-2*. Sieriebriennikov and colleagues functionally characterised these genes for their role in mouth-form plasticity. Findings revealed that simultaneous inactivation of *nag-1* and *nag-2* has an opposite effect on the mouth-form phenotype compared to *eud-1* mutants, rendering all worms Eu, whereas inactivation of *sult-2.2.1* had no significant effect. Therefore, *eud-1*, *nag-1*, and *nag-2* all regulate mouth-form plasticity. While functional *eud-1* promotes the development of the Eu phenotype, *nag-1* and *nag-2* facilitate the development of the St phenotype. The inactivation of the entire multigene locus leads to all-St phenotype, indicating an epistatic effect of *eud-1* over *nag-1* and *nag-2*. Moreover, the expression of these genes overall localises in the sensory neurons in a non-overlapping fashion, further indicating a role in environmental sensing and mouth-form regulation (Sieriebriennikov et al., 2018).

Besides the reference strain, mouth-form plasticity has also been studied in natural isolates of *P. pacificus* and generally in *Pristionchus* species (Dardiry, Eberhardt, et al., 2023; Lightfoot et al., 2021; Ragsdale et al., 2013). Specifically, various natural strains of *P. pacificus* exhibit differing mouth-form ratios ranging from highly St to highly Eu, and intermediates in between, under standard laboratory conditions (Ragsdale et al., 2013). The multigene locus, as in its tandem arrangement in *P. pacificus* reference strain, is conserved in the *Pristionchus* genus (Sieriebriennikov et al., 2018). In particular, *eud-1* does not only regulate the mouth-form plasticity in natural isolates of *P. pacificus* but also other species in the genus *Pristionchus* (Ragsdale et al., 2013). It has also been shown that the natural variation in the cis-regulatory elements within the multigene locus significantly contributes to differences in the degree of plasticity observed in natural isolates (Dardiry, Eberhardt, et al., 2023).

In *P. pacificus*, the sulfatase gene, *eud-1*, is not the only sulfation-related gene that regulates mouth-form plasticity. The sulfotransferase gene, *seud-1/sult-1*, also controls mouth-form plasticity in a switch-like manner (Bui et al., 2018; Namdeo et al., 2018). Inactivation of this gene drives the development of the Eu morph, and it normally promotes St development.

Its expression localises in pharyngeal muscle cells and does not overlap with *eud-1* (Namdeo et al., 2018).

2.3.2 Downstream components of the switch

Identification of the switch genes eventually raised the question: What are the downstream components mediating morph specification? Suppressor screens in mutant worms have revealed two nuclear hormone receptors, NHR-40 and NHR-1, as the downstream players of the developmental switch (Kieninger et al., 2016; Sieriebriennikov et al., 2020). While *loss-of-function* mutation in *nhr-40* renders all worms St, *gain-of-function* mutations induce the opposite morph, Eu (Sieriebriennikov et al., 2020). The *loss-of-function* mutation in *nhr-1* also leads to all-St expression; however, the mouth morphology appears as an intermediate, which is an St that displays reminiscent features of the Eu. This suggests that *nhr-1* plays a crucial role in the differentiation process of the mouth form. Nonetheless, both genes functionally facilitate the development of the Eu morph in wildtype *P. pacificus*. Furthermore, both genes are co-expressed in the head, particularly in the pharyngeal muscle cells, where mouth-form differentiation is operated (Sieriebriennikov et al., 2020). Transcriptomic analyses of both mutants revealed common downstream target genes which produce extracellular proteins, such as astacins and chitinases, that are predicted to be involved in mouth-form differentiation. Several of these target genes are co-expressed with *nhr-1* in the dorsal pharyngeal gland cell. While both nuclear receptors, *nhr-40* and *nhr-1*, are conserved in *P. pacificus* and *C. elegans* with one-to-one orthology, their downstream targets in *P. pacificus* show high degree of diversification, suggesting that these genes are evolving fast to serve their novel functions (Sieriebriennikov et al., 2020).

Besides nuclear hormone receptors, a suppressor screen in the *eud-1* mutant background revealed another downstream player in mouth-form plasticity: a homolog of the Mediator subunit MDT-15, which is a conserved transcriptional coregulator (Casasa et al., 2023). *Pristionchus pacificus* has two duplicate homologs of *mdt-15*: *mdt-15.1* and *mdt-15.2*. Like the *C. elegance mdt-15*, both *P. pacificus* homologs control metabolic processes, particularly lipid storage. However, only *mdt-15.1* is involved in the regulation of mouth-form plasticity. Inactivation of *mdt-15.1* results in a fixed intermediate phenotype described as “aberrant Eu”. Like the *C. elegans* homolog, *mdt-15.1* regulates delta-9 desaturases, which are essential components of *de novo* fatty acid synthesis, regulating lipid storage. Intriguingly, transcriptomic analyses aided by the RNA-tomography data of *P. pacificus* revealed that *mdt-15.1*-related genes and transcriptional targets of the switch genes (polyphenism-associated) are commonly enriched in the gut, brain and mouth regions of the worm. Taken together, these findings indicate that *mdt-15.1*, *nhr-40*, and *nhr-1* all function to maintain discreteness of the

mouth forms, as their inactivation, specifically *nhr-1* and *mdt-15.1*, also breaks the discontinuity of the morphs (Casasa et al., 2023).

It is also important to note that further mutant analyses have revealed even more downstream molecular elements involved in mouth and teeth morphogenesis in *P. pacificus*: (i) DPY-6, a mucin-type protein, is involved in the morphogenesis of the mouth and cuticle (Sun & Theska et al., 2022); (ii) NAS-6, an astacin metalloprotease, is required for the structure and movement of tooth, and thus, for predation (Ishita et al., 2023); (iii) chitin is an essential component of the teeth, as the inhibition of chitin synthase, *chs-2*, leads to teethless worms unable to predate (Sun et al., 2023).

2.3.3 Epigenetics and environment

How do switch genes achieve environmental sensitivity? Is there a role of epigenetics in mouth-form plasticity? At early stages of development in *P. pacificus*, histone 4 lysine 5/12 acetylation (H4K5/12ac) promotes chromatin accessibility, exposing the switch gene, *eud-1*, to the environmental cues thus, promoting its expression (Werner et al., 2023). Termination of the critical period for the mouth-form decision is then achieved by deacetylation which ceases switch gene expression. Hence, H4K5/12ac as a chromatin modification regulates mouth-form plasticity. Other chromatin modifications have also been found to be associated with mouth-form plasticity, such as H3K27 acetylation, and H3K4 di- and trimethylation (Serobyan et al., 2016). Specifically, H3K4me1/2 (H3-di/monomethyl-lysine-4) demethylase gene, *spr-5*, is involved in the regulation of plasticity and morphology of the mouth form (Levis & Ragsdale, 2023). Inactivation of *spr-5* affects mouth-form ratios and introduces an intermediate morph besides Eu and St; and reduces plastic responses to environmental influences. In this mutant background, propagating worms by separately selecting Eu and St morphs for several generations results in changes in morphology and mouth-form ratios between selection regimes (e.g. Eu selection leads to higher Eu frequency over generations). These findings suggest that epigenetic inheritance can contribute to the evolutionary diversification of plastic traits (Levis & Ragsdale, 2023).

Studies on environmental regulation of mouth-form plasticity have also identified associated molecular factors. For instance, temperatures either colder or warmer than 20ºC affect mouth-form plasticity, rendering worms St (Lenuzzi et al., 2023). This effect is mediated via cyclic guanosine monophosphate (cGMP) signalling (DAF-11/DAF-25 module) and the cyclic nucleotide-gated channel (TAX-2), which are involved in environmental sensing. Mouth-form plasticity is also affected by population density. Crowding by adults prompts juvenile worms to express the Eu phenotype (Werner et al., 2018). This effect is associated by the secretion of an adult pheromone, di-ascaroside#1 (dasc#1), which induces Eu morph in *P. pacificus* (Bose et al., 2012). This suggests that worms use this pheromone as a messenger

for juveniles informing them of the increase in population size. Besides crowding, food availability also affects mouth-form ratios in *P. pacificus*. Starvation increases the frequency of Eu worms, and dauer-associated pathway, dafachronic acid-DAF-12 module, has been found to contribute to this process, highlighting the potential importance of hormone signalling in mouth-form plasticity (Bento et al., 2010).

2.4 What is known about the nutritional plasticity of the mouth form in *P. pacificus*?

Having summarised all the molecular and environmental factors that regulate mouth-form plasticity in *P. pacificus*, I want to bring the topic back to nutrition, and the importance of nutrition as an environmental factor regulating polyphenisms. I have introduced cases in insects, which demonstrate how nutrition-induced physiological changes affect the expression of polyphenic traits, providing mechanistic insights into the regulation of polyphenisms. In *P. pacificus*, our understanding of how nutrition impacts mouth-form plasticity has been limited. Previous studies have shown that food scarcity (Bento et al., 2010), as well as natural isolates of bacteria provided as food source can be stimulating factors for plasticity in *P. pacificus* (Akduman et al., 2020). However, these studies were not particularly designed to study the effect of nutrition on mouth-form plasticity to draw concrete conclusions. In addition, a gap remains between diet and the plastic response: the organism's nutritional status. It is unclear whether nutritional status can predict the phenotypic outcome of the mouth form in *P. pacificus*, an open question I address in this thesis.

Nematodes, such as *C. elegans* and *P. pacificus*, store a great deal of their energy as lipids mainly in the intestine, germline and hypodermis (Figure 3). Lipid storage is therefore an important indicator of nutritional status. It can easily be visualised via staining methods and quantified through imaging and analysis, providing a measure of nutritional status. Specifically, Oil Red O (ORO) staining visualises neutral lipids, such as triacylglycerols, which are distinct from lysosome-related organelles, and thus represent fat storage (O'Rourke et al., 2009). In addition, lipid storage of nematode models can easily be manipulated via genetic (e.g. CRISPR/Cas9 mutagenesis) and dietary alterations (e.g. via addition of supplementations into the nematode growth medium). In my doctoral study, I utilised these methods to understand how nutrition influences mouth-form plasticity and to uncover associated molecular mechanisms behind this process in *P. pacificus*.

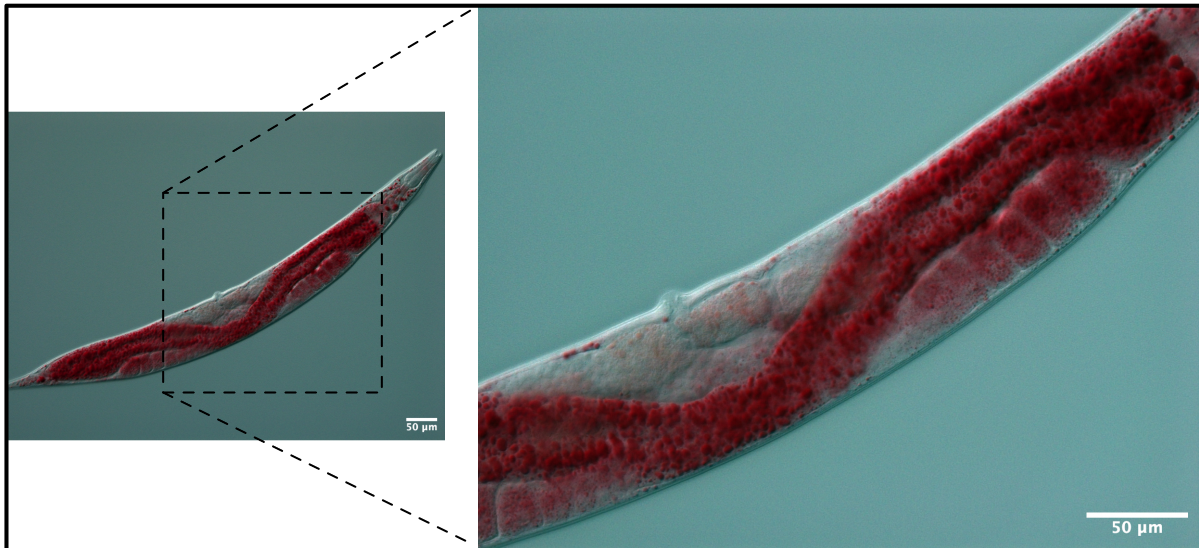

Figure 3: Image of the wild type *P. pacificus* which was grown in the standard laboratory conditions and stained with Oil Red O to visualise storage lipids. Lipid droplets appear red, and they are largely concentrated in the intestine.

2.5 Fitness consequences of mouth-form plasticity

Fitness trade-offs associated with predatory and non-predatory morphs in *P. pacificus* have only been studied almost a decade ago (Serobyan et al., 2013, 2014). These studies evaluated the performance of Eu and St worms separately across varying and standard conditions, revealing fitness advantages associated with each morph. While the Eu morph benefits from its predatory ability and exploits more resources; the St morph claims an advantage due to its faster development. Over the years, the number of *P. pacificus* natural isolates, available in the lab, has drastically increased, and thus allowed more ecologically relevant studies to be conducted. As mentioned before, these *P. pacificus* natural isolates exhibit varying degrees of mouth-form plasticity (Ragsdale et al., 2013). This has motivated a collaborative project to study, for the first time, the cost of phenotype and cost of plasticity in natural strains of *P. pacificus* (Dardiry, Piskobulu, et al., 2023). I also integrated a similar approach into my main project to study fitness consequences of mouth morphs under changing nutritional conditions. Overall, findings obtained from both projects furthered our understanding of costs and benefits of plasticity in *P. pacificus*.

3. Aims of the thesis

The main aim of my thesis was to enhance our understanding of nutritional plasticity in *P. pacificus* by studying dietary-induced changes in its physiology, morphology, and life history traits. Additionally, I aimed to explore fitness costs and benefits associated with plasticity and particular mouth morphs under standard and changing dietary conditions. To achieve these aims, I:

1. Optimised a robust ORO staining protocol for *P. pacificus* to visualise lipid storage in the whole body of the worm as an indicator of nutritional status.
2. Applied a novel ORO quantification method via microscopic imaging and analysis.
3. Established experimental setups to simulate a "high nutrition" environment for the worms via supplementing the nematode growth medium with monosaccharides and fatty acids, and track changes in mouth-form ratios along with worms' nutritional status via ORO staining, imaging, and analysis.
4. Carried out transcriptomic and consequent mutant analyses to reveal the role of lipid metabolism genes in nutritional plasticity of the mouth form.
5. Examined the cost of plasticity and phenotype in natural strains of *P. pacificus* by studying worms' life history traits such as fecundity and developmental speed under standard and changing dietary conditions.
6. Investigated fitness consequences of a high nutritional condition in predatory and non-predatory worms.

First, the findings suggest that nutrition is an important environmental factor regulating mouth-form plasticity in *P. pacificus*. Specifically, I introduce glucose-supplemented diet as a novel environmental condition to induce lipid storage and the development of non-predatory worms. Transcriptomic and mutant analyses reveal that lipid metabolism genes are essential components in mediating plasticity in high nutrition condition. Second, the study of natural isolates of *P. pacificus* provides support for the cost of plasticity and phenotype in this system, also highlighting a fitness advantage for non-predatory worms under standard laboratory conditions. The evaluation of fitness consequences of morphs obtained from high nutritional condition also suggests that *P. pacificus* benefits from its plasticity, as inducing St worms under such environmental condition promotes higher fecundity relative to Eu worms.

V. Results

1. Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait

Dardiry, M., **Piskobulu, V.***, Kalirad, A.*, & Sommer, R. J. (2023). *Evolution Letters*, *7*(1), 48–57. doi:10.1093/evlett/qrac001. *see Appendix*
* indicates equal contribution

1.1 Synopsis

Adaptive phenotypic plasticity can provide organisms with an advantage in variable environments by facilitating the execution of suitable phenotypes. However, it has been argued that there must be fitness costs associated with this ability since “perfect plasticity”, which describes an ability to phenotypically match any type of environment at any stage of development, would ultimately be a rare ability in nature. In addition, inducing a plastic trait can also be costly for the organism due to allocating resources into the production of a phenotype. The endeavour of detecting such fitness costs has been a challenge for plasticity research over the years. In this paper, we examined the cost of plasticity and phenotype in *P. pacificus*. We studied the mouth-form plasticity and life history traits of natural isolates of *P. pacificus* in standard and changing dietary conditions. First, we showed that there is a cost of phenotype for the development of the predatory morph. Predatory-biased development was associated with both reduced fecundity and rate of development under standard laboratory conditions. Second, assessing the degree of plasticity (mouth form) and fitness parameters of two different strains (plastic vs. fixed) under changing dietary conditions revealed a support for the cost of plasticity in this system. The plastic strain, which is predominantly non-predatory under standard conditions, exhibited reduced fecundity and developmental speed in the dietary condition that induces predatory morph. In contrast, the same predatory morph-inducing dietary condition enhances the fitness parameters of the fixed strain that remains predatory between the conditions. Therefore, the fixed strain only incurs a phenotype cost in the non-inducing (standard) condition. Finally, the empirical data allowed execution of computational simulations to examine the population dynamics together with the interaction of plasticity and phenotype costs in different ecological scenarios. Findings suggest that the biotic and abiotic factors can dictate the outcome of the fitness costs associated with plasticity.

1.2 My own contribution

I was mostly involved in the conceptualisation, data generation and analysis. My contribution is 35%.

2. High nutritional conditions influence feeding plasticity in *Pristionchus pacificus* and render worms non-predatory

Piskobulu, V., Athanasouli, M., Witte, H., Feldhaus C., Streit, A., & Sommer, R. J. *Submitted*. doi:10.1101/2024.08.27.609904. *see Appendix*

2.1 Synopsis

Nutrition is one of the most important environmental factors that affect phenotypic plasticity. Nutrition-induced polyphenisms represent some of the extreme cases of phenotypic plasticity, such as caste determination in eusocial insects and the development of exaggerated traits in beetles. Although studies in insects revealed a prominent role for nutrition in the regulation of polphenisms, our understanding of how nutritional conditions affect polyphenic traits in other taxonomic groups is still limited. Nematodes, as important model organisms, have also been studied for their unique polyphenic features that can be influenced by food availability. *Pristionchus pacificus*, a polyphenic nematode, has been used as a model for the studies of phenotypic plasticity, which revealed molecular and environmental mechanisms involved in its astonishing mouth-form polyphenism. However, the influence of nutrition on mouth-form polyphenism had not yet been systematically studied in *P. pacificus* before. In this manuscript, we study the influence of nutritional status on feeding plasticity through dietary and genetic alterations. First, we introduce monosaccharides and fatty acids into the nematode growth medium of worms to induce fat storage and examine changes in mouth-form ratios. Results indicate that fat storage-promoting conditions, in particular glucose-supplemented diet, facilitate the development of the non-predatory morph. Further, we carry out transcriptomic and mutant analyses to reveal associated molecular mechanisms. Results indicate that lipid metabolism pathways involved in the *de novo* fatty acid synthesis and peroxisomal beta-oxidation play a significant role in this mouth-form response. Lastly, we study the fecundity of both morphs (predatory and non-predatory) obtained from glucose-supplemented condition to explore fitness consequences. Findings indicate that non-predatory animals exhibit a greater progeny production relative to predatory ones. Overall, this study highlights the importance of nutrition in the regulation of mouth-form polyphenism and signifies the benefit of this plastic trait in *P. pacificus*.

2.2 My own contribution

I conceptualised the study, performed and analysed most of the experiments. I also wrote the manuscript with revisions from my supervisor and inputs from other co-authors. My contribution is 85%.

VI. Discussion

Nutrition is an important environmental factor that influences phenotypic plasticity. Specifically, the nutritional control of polyphenisms in eusocial insects and scarab beetles represents some of the extreme cases of phenotypic plasticity (Emlen, 1994, 1997b; Gotoh et al., 2014; Moczek, 1998; Wheeler, 1986). Several studies have revealed conserved molecular factors which are involved in the regulation of polyphenisms in honeybee, dung beetles, and stag beetles (Casasa et al., 2020; Casasa & Moczek, 2018; Gotoh et al., 2014; Kamakura, 2011; Kucharski et al., 2008). Besides insects, our knowledge is still limited as to how this important environmental factor affects other polyphenic animals. For over a decade, research on the regulation of the mouth-form polyphenism in the nematode model *P. pacificus* has uncovered several associated environmental and molecular mechanisms which can now be framed as a complex network of interacting components, predominantly governed by developmental switch genes (Sommer, 2020; Theska & Sommer, 2024). However, among all the known environmental factors that affect feeding plasticity, the influence of nutrition has not been extensively studied in *P. pacificus*. An important question in particular has remained unanswered: How do changes in nutritional status affect mouth-form plasticity?

To further our understanding of how nutrition impacts phenotypic plasticity in *P. pacificus*, I established an experimental method to manipulate the nutritional status of the worms via supplementations of monosaccharides and fatty acids into the nematode growth medium (Piskobulu et al., 2024 preprint). I also used ORO staining to visualise and quantify lipid storage as a measure of nutrition in worms across dietary conditions. In addition, I utilised a novel filter-based imaging method that allowed me to effectively quantify ORO, which is a proxy for lipid storage (Feldhaus & Piskobulu, 2024 preprint). Therefore, this methodology aided in detecting changes at various levels in the worm: physiology (lipid storage), morphology (mouth form), and an important life history trait, fecundity.

First, I showed that fat storage-promoting conditions, oleic acid- and glucose-supplemented diets, facilitate the development of the St morph. However, glucose-supplemented dietary effect was more consistent throughout the study, bringing the mouth-form frequency of the reference strain from highly Eu (95% Eu) to highly St (below 20% Eu). In addition, the effect of these fat storage-promoting diets did not induce a transgenerational memory of the mouth form in the reference strain. Together, these findings indicate that nutritional conditions inducing an “overly satiated” state in *P. pacificus* lead to non-predatory feeding and suggest that such conditions must persist during development to obtain this mouth-form response. Integrating these results with previous findings allows the following conclusions to be made: low nutritional condition, i.e. starvation, promotes predatory feeding (Bento et al., 2010), and even cannibalism in another diplogastrid nematode (Wighard et al.,

2024); well-fed condition houses a mixture of morphs depending on the strain and the diet (Dardiry, Piskobulu, et al., 2023; Ragsdale et al., 2013); and beyond well-fed condition, i.e. high nutritional condition that facilitates fat storage, induces non-predatory feeding (Piskobulu et al., 2024 preprint). Therefore, we now have a better understanding of how nutritional conditions affect mouth-form plasticity in *P. pacificus*.

Second, transcriptomic and mutant analyses revealed that both lipogenesis and lipolysis contribute to mouth-form plasticity in a high nutrition environment. Specifically, I studied delta-9 desaturase genes and peroxisomal beta-oxidation genes. Although the functional significance of *Ppa-daf-22.1* and *Ppa-daf-22.2*, which are the most downstream components of the peroxisomal beta-oxidation pathway (Markov et al., 2016), have been characterised for dauer plasticity in *P. pacificus*, our results represent the first time that this pathway is also strongly associated with nutrition-induced mouth-form plasticity. Similarly, delta-9 desaturases, which exhibit a pivotal role for *de novo* fatty acid synthesis, have not been functionally characterised in *P. pacificus* before, not only for lipid storage but also for mouth-form plasticity. The fact that inactivation of delta-9 desaturase and peroxisomal beta-oxidation genes simultaneously causes misregulation of storage and utilisation of lipids, and disruption of mouth-form response to glucose-supplemented diet further points out a strong association between nutritional status and mouth-form plasticity. Furthermore, I found that the dietary effect of glucose supplementation on mouth-form plasticity requires developmental switch genes, *seud-1/sult-1*, *nag-1*, and *nag-2*. However, understanding how this nutritional effect interacts with mouth-form gene regulatory network to execute mouth-form expression requires further investigation. It has been proposed that a metabolic product, which acts as a satiety signal, may suppress the expression of Eu morph by repressing the NHR-40 activity through its activation by the *seud-1/sult-1* (Bui & Ragsdale, 2019). In line with this hypothesis, the peroxisomal beta-oxidation and *de novo* fatty acid synthesis pathways, studied here, are known to facilitate lipid-mediated signalling. For instance, *de novo* fatty acid synthesis pathway processes polyunsaturated fatty acids and produces eicosanoids which function as signalling molecules (Watts, 2016). Similarly, peroxisomal beta-oxidation pathway produces extracellular signalling molecules, ascarosides, which are involved in dauer and mouth-form plasticity (Artyukhin et al., 2018; Bose et al., 2012; Werner et al., 2018). Therefore, this opens up new avenues for further research with a more focused approach to address the functional significance of these signalling components and to explore whether and how they contribute to nutrition-induced mouth-form plasticity. Nonetheless, the challenging aspect of studying lipid metabolism pathways will be their multifunctionality.

The second aim of my thesis was to study the adaptive value of mouth morphs, including fitness costs associated with them, and to generally explore costs and benefits of plasticity under changing dietary conditions in *P. pacificus*. To explore costs of plasticity and

phenotype in *P. pacificus*, we studied the natural isolates in particular, which were collected from various localities in nature (Dardiry, Piskobulu, et al., 2023). We generally selected fecundity and developmental speed as fitness parameters to evaluate costs based on mouth forms obtained in each strain. Effectively, we showed that the Eu morph generally incurs a phenotype cost in within-strain (intermediate mouth-form ratios; separation of morphs) and between-strain (related pairs of highly St and highly Eu strains) comparisons under standard laboratory conditions. Findings revealed that St-biased strains particularly develop faster and produce more progeny relative to Eu-biased strains in each compared pair of strains, suggesting that producing the Eu phenotype is costly for *P. pacificus*. Under changing dietary conditions (*E. coli* to *Novosphingobium*), we also detected plasticity cost by comparing fitness parameters of a plastic and a fixed strain. While the fixed strain constitutively produces the Eu phenotype in both dietary conditions, the plastic strain predominantly produces the St morph in *E. coli* diet and Eu morph in *Novosphingobium* diet. We found that the plastic strain shoulder a plasticity cost, manifested as reduction in fecundity and rate of development, due to changes in mouth-form ratios between these dietary conditions. Computational models based on experimentally obtained life history data also indicated how cost of phenotype and cost of plasticity interact in various ecologically relevant scenarios and affect population dynamics. Generally, the plasticity cost was found to be context dependent (Dardiry, Piskobulu, et al., 2023).

Next, establishing glucose-supplemented diet as a nutritional condition that induces the St morph raised two questions: Is there an adaptive value of facilitating the St morph in a high nutrition environment? Does *P. pacificus* benefit from this plastic response? To answer these questions, I grew worms in this dietary condition and isolated them based on their mouth form in the regular condition to evaluate their fecundity as a measure of fitness. I found that the St worms had higher fecundity relative to Eu worms. This suggests that plasticity benefits *P. pacificus* in this high nutrition environment through the development of the St morph thus, providing a fitness advantage (Piskobulu et al., 2024 preprint).

Taken together, even though plasticity is costly, *P. pacificus* benefits from this ability to produce two alternative mouth morphs with distinct dietary niches, behaviour, and adaptive values. Importantly, findings reveal a significant adaptive role for the non-predatory worm and highlight that it is more than just a microbial feeder. This morph may provide a fitness advantage in extreme environments by better alleviating any potential adverse effects that are imposed. In nature, *P. pacificus* lives in dynamic environments where conditions can rapidly change and therefore, plasticity whether dauer or mouth form can provide this nematode with the ability to thrive in its habitat.

In conclusion, this work provided new perspectives on the regulation of mouth-form polyphenism and furthered our understanding of fitness costs and benefits associated with

phenotypic plasticity in *P. pacificus*. Findings revealed that nutrition is an important regulator of mouth-form plasticity. Specifically, I introduced a novel environmental condition, glucose-supplemented diet, which greatly stimulates mouth-form plasticity in *P. pacificus*, rendering worms non-predatory. Transcriptomic and mutant analyses revealed that lipid metabolism pathways, which are involved in the regulation of lipid storage in worms, are essential for nutrition-induced mouth-form plasticity. This highlights the significance of metabolic processes carried out by these pathways for the mediation of mouth-form plasticity. However, two important questions remain to be answered: How is the satiety of the animal communicated to the mouth-form gene regulatory network? And are these pathways involved in this communication due to their roles in lipid-mediated signalling? Findings obtained via this work represent a promising starting point for answering these questions. Finally, we showed that plasticity and the execution of the predatory morph are costly processes in *P. pacificus*. However, costs associated with plasticity are usually context dependent, as *P. pacificus* can benefit from its plasticity for instance, by facilitating the development of the non-predatory morph, which provides this nematode with a fitness advantage in a high nutrition environment. Overall, *P. pacificus* tunes its mouth-form polyphenism to its nutritional condition, which allows this nematode to favour the development of either one of two alternative mouth morphs to enhance different fitness parameters and exploit different resources.

VII. References

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VIII. Appendix

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Letter

Experimental and theoretical support for costs of plasticity and phenotype in a nematode cannibalistic trait

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Developmental plasticity is the ability of a genotype to express multiple phenotypes under different environmental conditions and has been shown to facilitate the evolution of novel traits. However, while the associated cost of plasticity, i.e., the loss in fitness due to the ability to express plasticity in response to environmental change, and the cost of phenotype, i.e., the loss of fitness due to expressing a fixed phenotype across environments, have been theoretically predicted, empirically such costs remain poorly documented and little understood. Here, we use a plasticity model system, hermaphroditic nematode *Pristionchus pacificus*, to experimentally measure these costs in wild isolates under controlled laboratory conditions. *P. pacificus* can develop either a bacterial feeding or predatory mouth morph in response to different external stimuli, with natural variation of mouth-morph ratios between strains. We first demonstrated the cost of phenotype by analyzing fecundity and developmental speed in relation to mouth morphs across the *P. pacificus* phylogenetic tree. Then, we exposed *P. pacificus* strains to two distinct microbial diets that induce strain-specific mouth-form ratios. Our results indicate that the plastic strain does shoulder a cost of plasticity, i.e., the diet-induced predatory mouth morph is associated with reduced fecundity and slower developmental speed. In contrast, the non-plastic strain suffers from the cost of phenotype since its phenotype does not change to match the unfavorable bacterial diet but shows increased fitness and higher developmental speed on the favorable diet. Furthermore, using a stage-structured population model based on empirically derived life history parameters, we show how population structure can alleviate the cost of plasticity in *P. pacificus*. The results of the model illustrate the extent to which the costs associated with plasticity and its effect on competition depend on ecological factors. This study provides support for costs of plasticity and phenotype based on empirical and modeling approaches.

Keywords: Adaptive plasticity, cannibalism, cost of plasticity, cost of phenotype, Markov population models.

Layman Summary

A genotype able to express a range of phenotypes in response to environmental conditions, that is to demonstrate developmental plasticity, would be a Darwinian demon, able to infinitely adapt and outcompete those genotypes that require a genetic change to express a phenotype fit to an environment. It has been suggested that the absence of such demons in nature is due to the cost of plasticity, i.e., developmental plasticity results in a reduction of biological fitness compared to a genotype that facultatively expresses a phenotype matching the environment. While conceptually simple, measuring the cost of plasticity in nature has proven a major challenge. We use the nematode *P. pacificus* to measure the cost of plasticity. During its development, *P. pacificus* can assume one of two possible mouth forms: predatory or non-predatory. The likelihood of developing any of these two mouth forms is determined by a gene regulatory network, which itself is affected by a wide range of environmental conditions, including diet. We used two strains of *P. pacificus* and grew them on two different bacterial diets. The plastic strain was capable of switching from non-predatory to predatory mouth form depending on the diet, while the non-plastic strain could only express the predatory mouth form on either of the diets. By measuring the number of eggs laid in both strains on each diet, we show that the plastic response is associated with a reduction in fecundity, thus providing a clear example of the cost of plasticity. We then use a stage-structured model to simulate the population dynamics of the plastic and the non-plastic strains. Our simulation shows that the cost of plasticity is highly context dependent, and its ecological ramifications can be greatly influenced by biotic and abiotic factors.

Changing and fluctuating environments are a hallmark of all ecosystems, affecting the life and evolution of all organisms (Pfennig, 2021; Sæther & Engen, 2015). The ability of an organism to respond to changing environments by expressing alternative phenotypes, i.e., phenotypic plasticity, can, in theory, facilitate adaptation, as it makes various trait optima across time and space in a given environment accessible to a genotype without the need for genetic change (Pfennig, 2021; Pigliucci, 2001; West-Eberhard, 2003). Indeed, many case studies in plants, insects, vertebrates, and nematodes have indicated the importance of phenotypic plasticity for promoting adaptations across environments and for the evolution of novelty (Moczek et al., 2011; Sommer, 2020; West-Eberhard, 2003). We refer to this type of phenotypic plasticity that facilitates adaptation as "adaptive plasticity." However,

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not all examples of phenotypic plasticity are adaptive (Murren et al., 2015). Additionally, plastic genotypes can vary in their degree of plasticity across different conditions (Scheiner, 1993; Schlichting & Pigliucci, 1998). Such assumptions imply the existence of constraints on the evolution of adaptive plasticity, and a huge and growing body of research has been dedicated to identify such hypothetical constraints (Auld et al., 2010; Callahan et al., 2008; DeWitt et al., 1998; Murren et al., 2015; Pfennig, 2021; Snell-Rood et al., 2010). Theoretically, several factors can hinder the evolution of adaptive plasticity: limited genetic variation, weak selection, and the unreliability of environmental signals (Pfennig, 2021; Schlichting & Pigliucci, 1998; Snell-Rood et al., 2010). Most importantly, however, it has been argued that fitness costs will limit the evolution of plastic phenotypes (Murren et al., 2015). Such arguments are largely theoretical, and detecting the cost of adaptive plasticity remains a formidable challenge. For example, a meta-analysis of 27 studies on the cost of plasticity in animals and plants concluded that most of the studies did not have adequate statistical power to detect the cost associated with plasticity (Van Buskirk & Steiner, 2009). Here, we use the plasticity model system, the nematode *Pristionchus pacificus* (Supplementary Figure S1), to obtain experimental evidence for plasticity-associated costs.

To understand the costs associated with adaptive plasticity, one must consider the potential trade-offs between plastic versus non-plastic genotypes in a comparative framework (Figure 1A). Hypothetically, a non-plastic (fixed) genotype might express a mismatching phenotype in a non-inducing environment, i.e., a low-quality diet. In contrast, a plastic genotype may express another phenotype more suitable for the same environment, which results in higher fitness (Figure 1A). This fitness difference between the two genotypes has been referred to as *the cost of phenotype* (Callahan et al., 2008; Murren et al., 2015). In the relevant inducing environment (i.e., high-quality diet), the plastic genotype is induced to form a new phenotype. This ability to express plasticity can incur lower fitness relative to the non-plastic genotype (Figure 1A). This hypothetical fitness trade-off associated with the plastic genotype has been referred to as *the cost of plasticity* (Callahan et al., 2008; DeWitt et al., 1998; Murren et al., 2015). The *cost of phenotype* can be described as the organismal fitness reduction due to devoting resources to the continuous production of resource-demanding phenotypes. In contrast, *the cost of plasticity* can be defined as the price paid in fitness by a highly plastic genotype compared to a less plastic one (Callahan et al., 2008; Murren et al., 2015). Understandably, the terms "cost of phenotype" and "costs of plasticity" are, by virtue of their definitions, ripe for confusion (Auld et al., 2010; Pfennig, 2021). A comprehensive analysis of these constraints would adequately improve our understanding of the role of plasticity in adaptive evolution. However, empirical studies on the costs of phenotype and plasticity remain scarce, especially in metazoans. This has been largely due to two reasons: First, in the wild, conditions often cannot be properly controlled, nor can the effects of various factors be delineated. Second, laboratory experiments are time-consuming, and large organisms cannot be easily investigated. To study the constraints of plasticity, we make use of natural isolates of nematodes that, given their small size and rapid reproduction, can be examined under laboratory conditions.

The nematode *P. pacificus* is an established model system for studying phenotypic plasticity (Sommer & McGaughran, 2013; Sommer et al., 2017). The developmentally plastic mouth of *P. pacificus* can exhibit two distinct forms; the eurystomatous (predatory) morph with a wide stoma and hooked-like teeth or the stenostomatous (non-predatory) morph with a narrow stoma and a single tooth (Supplementary Figure S1a; Bento et al., 2010). *P. pacificus* is a hermaphroditic nematode, and the use of isogenic cultures has facilitated the elucidation of genetic and epigenetic mechanisms underlying this irreversible switch. Specifically, the sulfatase-encoding *eud-1* gene was identified as the key developmental switch that is regulated by various environmental factors and epigenetic mechanisms, and directs a downstream gene regulatory network consisting of more than 20 identified proteins including structural components of mouth formation (Bui et al., 2018; Kieninger et al., 2016; Namdeo et al., 2018; Ragsdale et al., 2013; Sieriebriennikov et al., 2018; Sieriebriennikov et al., 2020; Sun et al., 2022). Importantly, worms respond to surrounding environmental cues to adopt their mouth form in a strain-specific manner, and various environmental stimuli, including temperature, culturing condition, crowding, and diet have been shown to influence mouth-morph ratios (Lenuzzi et al., 2021; Werner et al., 2017,2018). Principally, three major features assist in studying *P. pacificus* mouth-form plasticity. First, the vast collection of naturally occurring wild isolates with hundreds of *P. pacificus* strains being sequenced, accordingly resulted in a highly resolved phylogeny of diverse populations (Supplementary Figure S1b; Rödelsperger et al., 2017). Interestingly, culturing these isolates on the laboratory bacterium *Escherichia coli* displays a range of mouth-morph ratios (Ragsdale et al., 2013). While the parallel formation of both mouth morphs under the same environmental condition represents an unusual type of plasticity that is not seen in the majority of plastic traits, it has allowed unprecedented insight into associated molecular mechanisms and the identification of a large gene regulatory network (Bui et al., 2018; Kieninger et al., 2016; Namdeo et al., 2018; Ragsdale et al., 2013; Sieriebriennikov et al., 2018,2020; Sun et al., 2022). Second, morphological mouth-form plasticity is coupled to behavioral plasticity. Specifically, the predatory form enables predation and cannibalism on other nematodes, while such animals can still feed on bacteria. In contrast, the non-predatory form obligates worms to feed on bacteria (Supplementary Figure S1c; Wilecki et al., 2015). This extension of morphological plasticity to behavior is thought to eliminate resource competitors via predation and the expansion of nutrition (Quach & Chalasani, 2020). Finally, *P. pacificus* is a thoroughly studied soil nematode that is reliably found in association with scarab beetles with recent studies describing the dynamics and succession of nematodes on the beetle carcass after the insect's death (Renahan et al., 2021; Renahan & Sommer, 2021).

Switching between the predatory and non-predatory mouth forms is a specific example of phenotypic plasticity, in which an irreversible decision that occurs during the development of *P. pacificus* via a bi-stable developmental genetic switch (Sieriebriennikov et al., 2018). The bias of the developmental switch determines the ratio of mouth morphs with substantial natural variation between populations of *P. pacificus* (Ragsdale et al., 2013). In this respect, mouth-form plasticity in *P. pacificus* is more akin to the switch between lytic and lysogenic cycles in bacteriophage λ (Ptashne, 1986) than wing pattern polyphenism in butterflies that is seasonally controlled (Nijhout, 1994). Importantly, the relative simplicity of mouth-morph plasticity in *P. pacificus*, as well as its isogenic husbandry of genetically diverse strains, makes it ideal to study different facets of phenotypic plasticity. Here, we took advantage of these features to perform a systematic analysis of mouth morphs and their associated costs and extended our empirical findings by simulating ecologically relevant scenarios in spatially homogeneous and spatially structured populations using empirically derived life history parameters.

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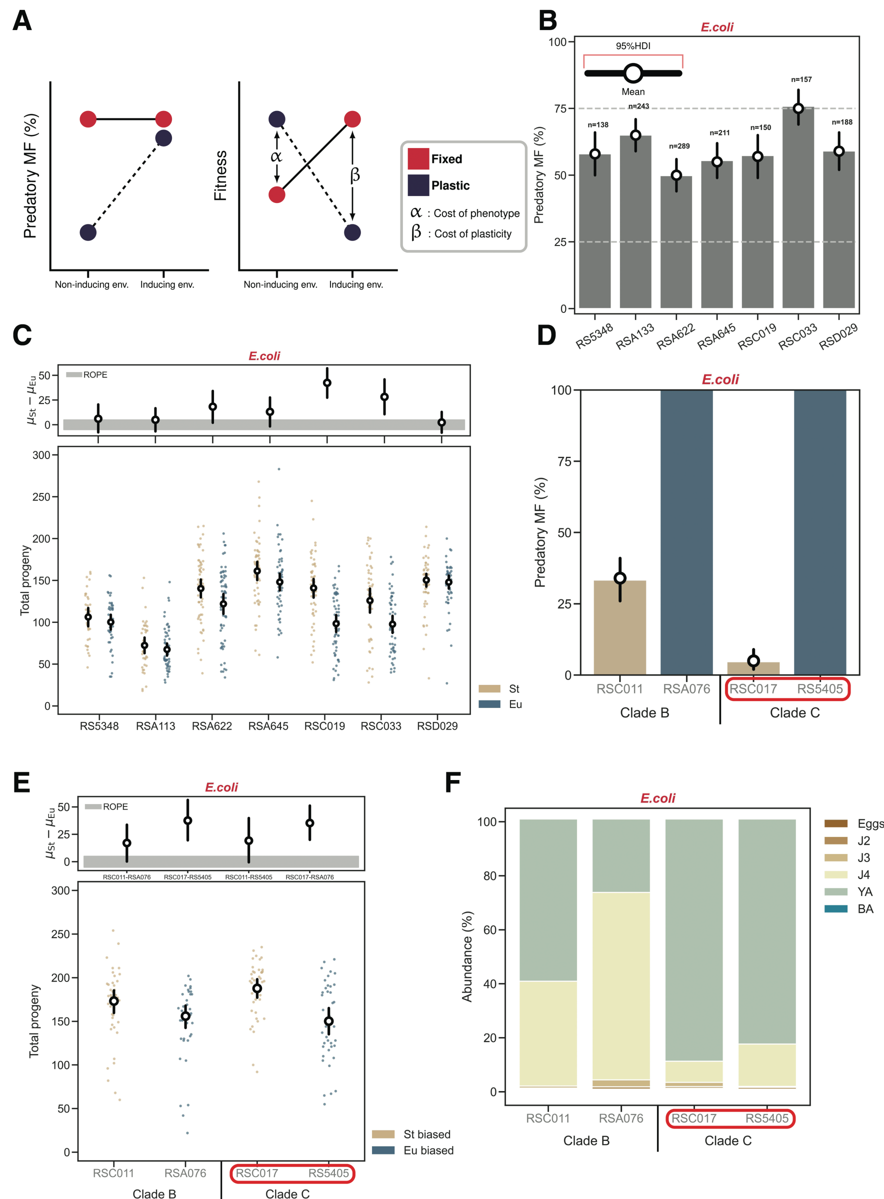

Figure 1. Within and between strains costs of phenotype in P. pacificus: (A) A hypothetical scenario illustrating the cost of plasticity and the cost of phenotype: a plastic genotype switches from the non-predatory to the predatory mouth form when grown in the inducing environment. In contrast, a non-plastic (fixed) genotype constantly expresses the predatory mouth form regardless of environmental changes. Note that the non-plastic (fixed) genotype is constantly displaying the induced phenotype, following the scenario suggested in (Murren et al., 2015). See text for more details. (B) Percentage of predatory mouth form of seven intermediate *P. pacificus* genotypes used in this study. The number of worms assayed for each strain (*n*) is indicated above each of the 95% highest density interval (HDI) for the means. (C) Overall fecundity of the same seven intermediate strains. On average between 51 and 47 predatory and non-predatory mothers per strain were scored, respectively. Two comparisons, RSC019 and RSC033, are outside the region of practical equivalence (ROPE), i.e., means in theses comparisons are different; two other comparisons, RSA622 and RSA645, partially overlap with the ROPE, and the rest of the comparisons include the ROPE, implying no difference. (D) Percentage of predatory mouth form of four biased *P. pacificus* wild isolates representing clades B and C, respectively. For each strain, three biological replicates with a total number of 150 worms per strain were scored. (E) Overall fecundity of the same biased strains. Both non-predatory-biased strains showed credibly higher overall fecundity than the predatory-biased strains. (F) Developmental speed for the biased strains from the two clades. For RSC011, 263; RSA076, 232; RSC017, 320; and RS5405, 306 individuals were staged. Worms were staged according to the following developmental stages: E = eggs; J2, J3, J4 = juvenile stages; YA = young adults with no eggs inside the uterus; BA = breeding adults with eggs inside the uterus. In (B) and (D), the 95%HDI for each strain was estimated using a Bayesian approach to estimate the probability of expressing the predatory mouth morph based on the observations; the 95% HDI for the means and the difference in means in (C) and (E) was calculated using Kruschke's BEST method. We used [−5,5] interval as our ROPE, i.e., differences of means within this interval are practically equal to no difference; the same ROPE and was used for all the analyses in this manuscript (see Supplementary Methods). The pair of strains that were used to measure the costs of plasticity are grouped in red squares in (D–F).

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Methods

For the detailed explanation of experimental assays, including the measurement of fecundity, developmental speed, predation rates, and mouth morph ratios, please see the Supplementary Material.

Stage-structured population model

The population dynamics of *P. pacificus* in different environments based on our laboratory data were modeled as a stage-structured model (Figure 3A), reflecting the life cycle of this nematode in nature (Supplementary Figure S1d). Assume $\mathbf{n}(t)$ to be a 12 × 1 array, where each entry represents the count of each developmental stage of strain *a* in a population at time *t*. The expected composition of strain *a* at time *t* + 1 would be:

$$n(t+1) = A_t n(t) - K(t)e_i - M(t)e_j, \tag{1}$$

where $K(t)$ is the number of J2 individuals of strain $\mathbf{i}$ that were killed at time *t*, $M(t)$ is the number of dauer larvae that emigrated from the population at time *t*, and $\mathbf{e}_i$ and $\mathbf{e}_j$ are unit vectors. For a given *P. pacificus* strain, the transition probabilities and fecundities in the projection matrix depend on the experimentally informed estimates. A simple resource consumption model is included in the model, where individuals in each developmental stage consume food at each step according to their stage-specific consumption rate. Upon depletion of food in a population, J2 larva transition to dauer larva.

To simulate a dispersion-colonization-competition scenario, we constructed a one-dimensional structured population that consisted of *n* localities arranged in a line. At each step, a proportion ω of the dauer larvae from a locality emigrates to its neighboring locality if the neighboring locality has more available food, resulting in a one-way dispersal pattern from a source to a sink. Throughout the model, $n = 12$ and $\omega = 0.1$.

Statistical analyses

For two-group comparisons, we used Kruschke's BEST approach (Kruschke, 2013). The mouth-morph data were modeled as a Bernoulli process. The statistical analyses were carried out with PyMC3 (Salvatier et al., 2016) in Python 3.10, using the No-U-Turn Sampler.

Results

Within and between strains comparisons reveal a cost of phenotype

To measure the cost of phenotype, we selected overall individual fecundity as the primary fitness parameter to capture the reproductive capacity of *P. pacificus* hermaphrodites via selfing (Haldane, 1937; Orr, 2009). For that, we took advantage of the extensive collection of *P. pacificus* natural isolates and selected seven strains with intermediate mouth-morph ratios from across the *P. pacificus* phylogeny (Figure 1B, Supplementary Figure S1b, Supplementary Table S4; Lightfoot et al., 2021; Rödelsperger et al., 2017). Testing for daily fecundity showed that the majority of progeny were laid within a window of 62 hr after maturation (nearly 91%) in an overall window of approximately 158 hr of total egg laying (Supplementary Figure S2a, Supplementary Table S2). These numbers provide a reasonable estimate of lifetime fecundity. In these intra-genotype comparisons, we found a tendency in non-predatory animals to have more progeny than predatory worms. Specifically, the estimated differences in the mean value of fecundity between non-predatory and predatory animals based on the data showed strong and/or partial support in four out of seven comparisons (Figure 1C, Supplementary Table S1), suggesting that the production of the predatory mouth morph can incur a fitness cost. This observation is in concert with a previously report on the slower rate of development in nematodes exhibiting the predatory morph in comparison to non-predatory worms (Serobyan et al., 2013).

Next, we measured fecundity and developmental speed in *P. pacificus* natural isolates that show a biased mouth-morph ratio, i.e., strains that would produce an abundance of non-predatory or predatory mouth morphs on standard laboratory *E. coli* food (Figure 1D, Supplementary Table S4). We selected two pairs of closely related strains from the diverged clades B and C of *P. pacificus* from La Réunion island (Rödelsperger et al., 2014). We found that in both pairs, the non-predatory-biased strains produce more overall progeny than the predatory-biased strains (Figure 1E, Supplementary Table S1). Specifically, given the same time window of the first 62 hr, the St-biased strains had a 21% and 17% higher fecundity in clades B and C, respectively (Supplementary Figure S3b, Supplementary Table S2). Similarly, the non-predatory-biased strains showed a higher developmental speed (Figure 1F, Supplementary Table S3). For example, 75 hr after egg laying, nearly 60% of the non-predatory-biased strain RSC011 reached adulthood, whereas only 27% of the predatory-biased strain RSA076 reached the same stage. Note that inter-clade comparisons show considerable differences in these isolates' developmental speed, which is due to the genetic background. Together, both comparisons illustrate the cost of producing the predatory phenotype.

Across-conditions testing indicates a cost of plasticity

Next, we wanted to determine if a cost of mouth-form plasticity exists in *P. pacificus*. Such a cost of plasticity would be evident when testing a non-plastic genotype relative to a plastic genotype under different conditions (Callahan et al., 2008; DeWitt et al., 1998; Murren et al., 2015; Pigliucci, 2001). Therefore, we performed a cross-condition test by conducting experiments on two distinct food sources, the standard *E. coli* condition used in the previous section, and a *Novosphingobium* diet. The bacterial species *Novosphingobium* was found to be naturally associated with *P. pacificus* and was proven to increase intraguild predation in the *P. pacificus* reference strain PS312 (Akduman et al., 2018, 2020). However, this association was never studied in non-domesticated wild isolates of *P. pacificus*. Therefore, we grew two of the biased strains with different mouth-morph ratios on *Novosphingobium;* the highly non-predatory-biased strain RSC017 and the highly predatory-biased strain RS5405. Indeed, RSC017 showed a substantial increase of the predatory morph of 84% on *Novosphingobium*, indicating strong plasticity. In contrast, the predatory-biased strain RS5405 remained highly predatory in the new condition (Figure 2A, Supplementary Table S4). Thus, we established two distinct food conditions that differentially affect plasticity levels of the two isolates. We refer to RSC017 and RS5405 as plastic and non-plastic strains, respectively.

Theoretically, the cost of plasticity would be displayed in the strain that exhibits a change in mouth-morph ratio upon altering food conditions. Indeed, we found that the plastic strain has lower fecundity and slower developmental speed on *Novosphingobium* when compared to the non-plastic strain (Figure 2B,C, Supplementary Tables S1 and S3). Thus, a strain that plastically responds to a dietary change with the formation of the predatory mouth morph exhibits reduced fitness under these novel conditions indicating a cost of plasticity. In contrast, the non-plastic strain exhibits higher levels of fecundity and

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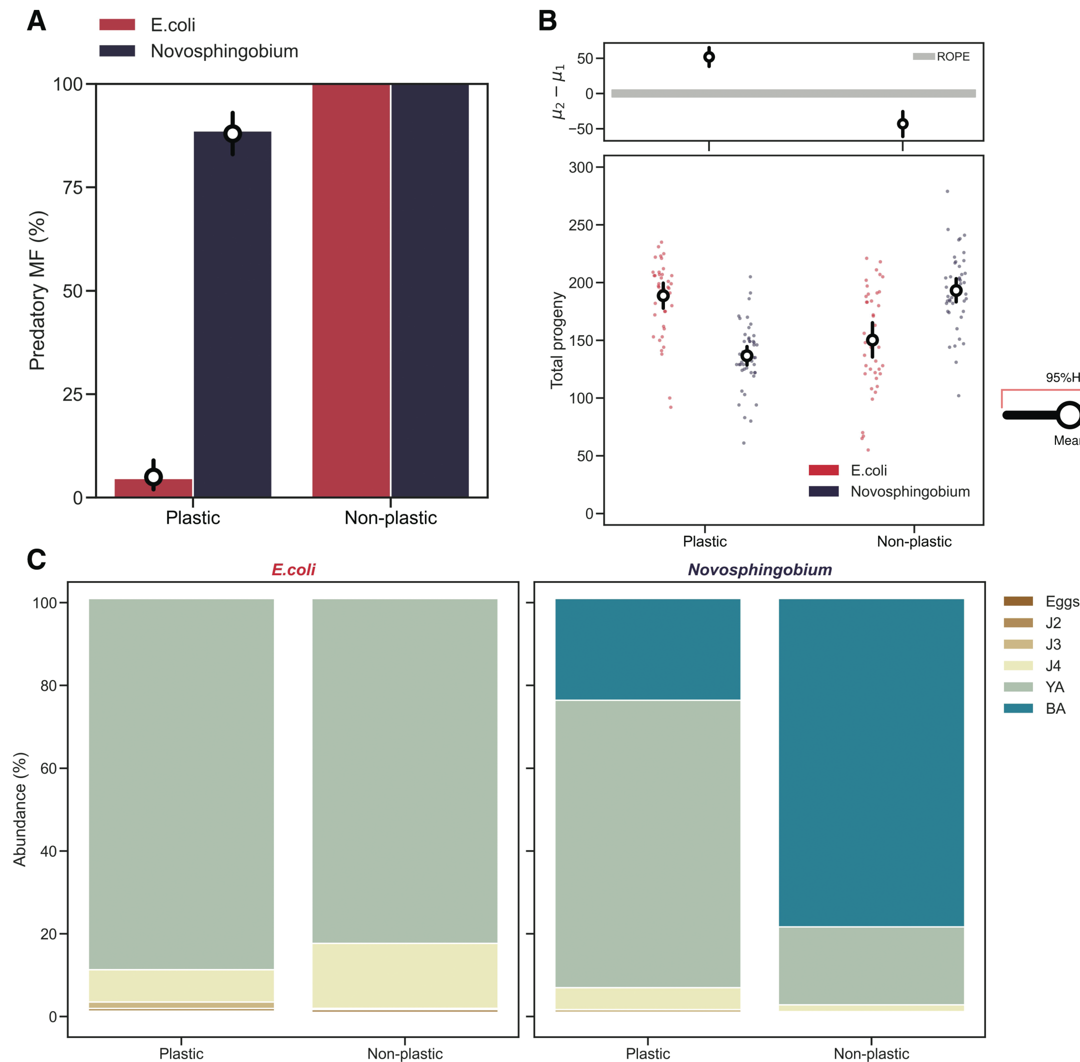

Figure 2. Cost of plasticity across conditions: (A) Percentage of predatory mouth form on E. coli and *Novosphingobium* for *P. pacificus* strains, representing the plastic and non-plastic strains. A total of 150 animals were used to score the mouth-morph ratio for each strain per condition, indicating three biological replicates. (B) Overall fecundity of the *P. pacificus* strains on both food conditions. The top panel indicates the 95% HDI for the estimated difference in means for each pairwise comparison calculated using Kruschke's BEST method (see Supplementary Methods). Comparable numbers of mothers were used: the plastic strain (*E. coli* = 40, *Novosphingobium* = 47); the non-plastic strain (*E. coli* = 40, *Novosphingobium* = 43). (C) Developmental speed for the two genotypes on both conditions. Individual worms were staged 75 hr after mothers were killed; for the plastic strain (*E. coli* = 320, *Novosphingobium* = 301); the non-plastic strain (*E. coli* = 306, *Novosphingobium* = 489) individuals were staged. Worms were staged according to the following developmental stages: E = eggs; J2, J3, J4 = juvenile stages; YA = young adults with no eggs inside the uterus; BA = breeding adults with eggs inside the uterus.

developmental speed on *Novosphingobium* (Figure 2B,C, Supplementary Tables S1 and S3). Thus, a strain that is preferentially predatory under both food conditions exhibits increased fitness when exposed to this new diet. These findings indicate a cost of mouth-morph plasticity in response to dietary induction, raising a fascinating question: which cost plays a larger role in shaping the population dynamics and, consequently, the evolution of mouth-morph ratios?

The cost of phenotype maximizes the benefits of plasticity

To investigate how the cost of plasticity and the cost of phenotype would manifest in the wild, we constructed a stage-structured model to simulate population dynamics of the plastic and the non-plastic strains on both tested food sources (Figure 3A). For modeling, we used the fecundity measurements from the lab and scaled the developmental rates of the model based on the laboratory estimates of the developmental speed of *P. pacificus* (see Supplementary Methods). First, we tested the population dynamics of the selected strains in separation, i.e., without interactions or competition. Surprisingly, the change from *E. coli* to *Novosphingobium* has only a minor effect on the final population size of the plastic strain (Supplementary Figure S4a). The reduction in fecundity on *Novosphingobium* relative to *E. coli* is presumably compensated by the increase in developmental speed on

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Novosphingobium. To test the hypothesis that faster developmental speed was indeed compensating for the cost of plasticity (i.e., lower fecundity), we simulated the dynamics of the plastic strain by assuming no change in developmental speed. The results of this simulation confirmed this expectation (Supplementary Figure S5). In contrast, in the non-plastic strain, the increase in fecundity and developmental speed on *Novosphingobium* results in a higher frequency of all developmental stages compared to its dynamic on *E. coli* (Supplementary Figure S4b). Importantly, the between strains cost of phenotype is clearly displayed when comparing the frequencies of the two strains on *E. coli* (Supplementary Figure S4a,b). Thus, comparing both populations' trajectories without involving interactions reveals that the cost of the phenotype has a larger effect on the population dynamics than the cost of plasticity.

The cost of plasticity manifests in a competition setup

In nature, *P. pacificus* does not occur in isolation, rather, it competes with other nematodes over resources. Additionally, given the coupling between morphological and behavioral plasticity, predatory worms are able to predate while non-predatory worms are not. Testing the costs of plasticity and phenotype in a competition setup might shed light on the evolution of the predatory mouth morph. Therefore, we first tested if the predation rate positively correlates with the proportion of predatory individuals in wild isolates. To avoid the compounding effect of relatedness on predation (Lightfoot et al., 2021), we selected *C. elegans* as prey for *P. pacificus* predators. Indeed, testing nine *P. pacificus* wild isolates with different mouth morph bias shows that morphological and behavioral plasticity positively correlate (Supplementary Figure S6). Second, we measured predation rates of the plastic and the non-plastic strains against one another by testing predation rates over the two food sources *E. coli* and *Novosphingobium* (Figure 3B).

Next, we used the experimentally obtained predation values for each food source to simulate the effect of interactions between strains on their dynamics in a spatially homogeneous population (see Supplementary Methods, Supplementary Figure S7). Specifically, we used these estimates to simulate the interactions between the two isolates in a population with an equal number of young adults (YAs) of the plastic and non-plastic strains at the start of the simulation. Notably, simulated populations were completely dominated by the non-plastic strain for both food conditions. In addition, rapid elimination of the plastic strains prevents the formation of its dauer larvae, as J2 animals of this strain were completely eradicated by the non-plastic strain (Figure 3C,D). Thus, the cost of plasticity greatly affects the dynamics of the plastic strain in a spatially homogenous population.

Spatial structure significantly affects population dynamics

While modeling the interaction of the plastic and the non-plastic strains in a population without any spatial structure is informative, a more realistic scenario would involve dispersal from different populations upon the depletion of food on the beetle carcass and competition over the nutrient-rich carcasses in the vicinity. Exploring such scenarios in the lab would be a tremendous undertaking. Therefore, we extended our model to include a stepping-stone migration scenario to illustrate the effect of costs of plasticity and phenotype on the competitive dynamics between *P. pacificus* strains in a structured population. We constructed a simple structured population by arranging n localities in one dimension. Each simulation starts with 50 YAs of the plastic strain in the first locality and 50 YAs of the non-plastic strain in the nth locality, with the rest of the localities being empty. All the localities contain a fixed amount of resources, and dauer larvae migrate with a fixed rate from a food-poor locality to a neighboring food-rich locality (Figure 3E). The simulation concludes when all the food in every locality has been depleted.

Based on these simulations, on *E. coli*, higher fecundity of the plastic strain allows adults of this isolate to completely dominate the structured population even in the face of predation (Figure 3F, Supplementary Figure S8a). Although the predation rate of the non-plastic strain is higher, even dauer larvae of the plastic strain continue to fully dominate the structured population (Supplementary Figure S9). These results support a considerable cost of phenotype for the non-plastic strain in the spatially structured population. In addition, in a scenario without predatory interactions, the frequency of the plastic strain decreases only marginally (Figure 3F, Supplementary Figure S9). This finding results from the change in the number of migratory dauer larvae of the non-plastic strain (Supplementary Figure S8a,b). Most importantly, the pace in which the plastic population grows results in exceptionally high numbers of predators belonging to the plastic strain, which outcompete the non-plastic strain in the presence of interaction. Thus, the cost of phenotype substantially influences the abundance of the non-plastic strain, in particular in the presence of interactions.

On *Novosphingobium*, higher fecundity and faster developmental speed of the non-plastic strain turn this isolate into a formidable adversary for the plastic strain. Therefore, the frequencies of adults and dauer larvae of the plastic strain are extremely reduced in the structured population (Figure 3F, Supplementary Figure S9). However, when interactions are limited, in contrast to *E. coli*, the frequencies would slightly increase (Figure 3F, Supplementary Figure S9). This is due to the non-plastic strain profiting from a higher growth rate and higher predation on *Novosphingobium*, but only higher growth when interactions are eliminated (Supplementary Figure S8c,d). Thus, the cost of plasticity would greatly affect the abundance of the plastic strain when competing with a predator under this condition.

Initial food source also affects population dynamics

To capture how significantly the costs of plasticity and phenotype would affect the dynamics of structured populations, we simulated two scenarios where each isolate would start with a favorable food source; *E. coli* for the plastic strain, and *Novosphingobium* for the non-plastic strain, or the unfavorable food source; *Novosphingobium* for the plastic strain, and *E. coli* for the non-plastic strain (Figure 3F). A pair of food sources were labeled "favorable" or "unfavorable" for a strain given the relative fecundity of the strain on each source. Interestingly, the results indicate that the initial condition in which each population starts dramatically affects which strain would ultimately dominate the structured population. When the conditions are favorable for both strains, the cost of plasticity of the plastic strain is greater than the cost of the phenotype of the non-plastic strain. In contrast, the relationship between the costs reverses under conditions that are unfavorable to both strains. Thus, the interaction of the cost of phenotype and the cost of plasticity is context dependent. Together, these simulations reveal that spatial structure and initial food sources could affect the population dynamics with different consequences for the costs of plasticity and phenotypes on

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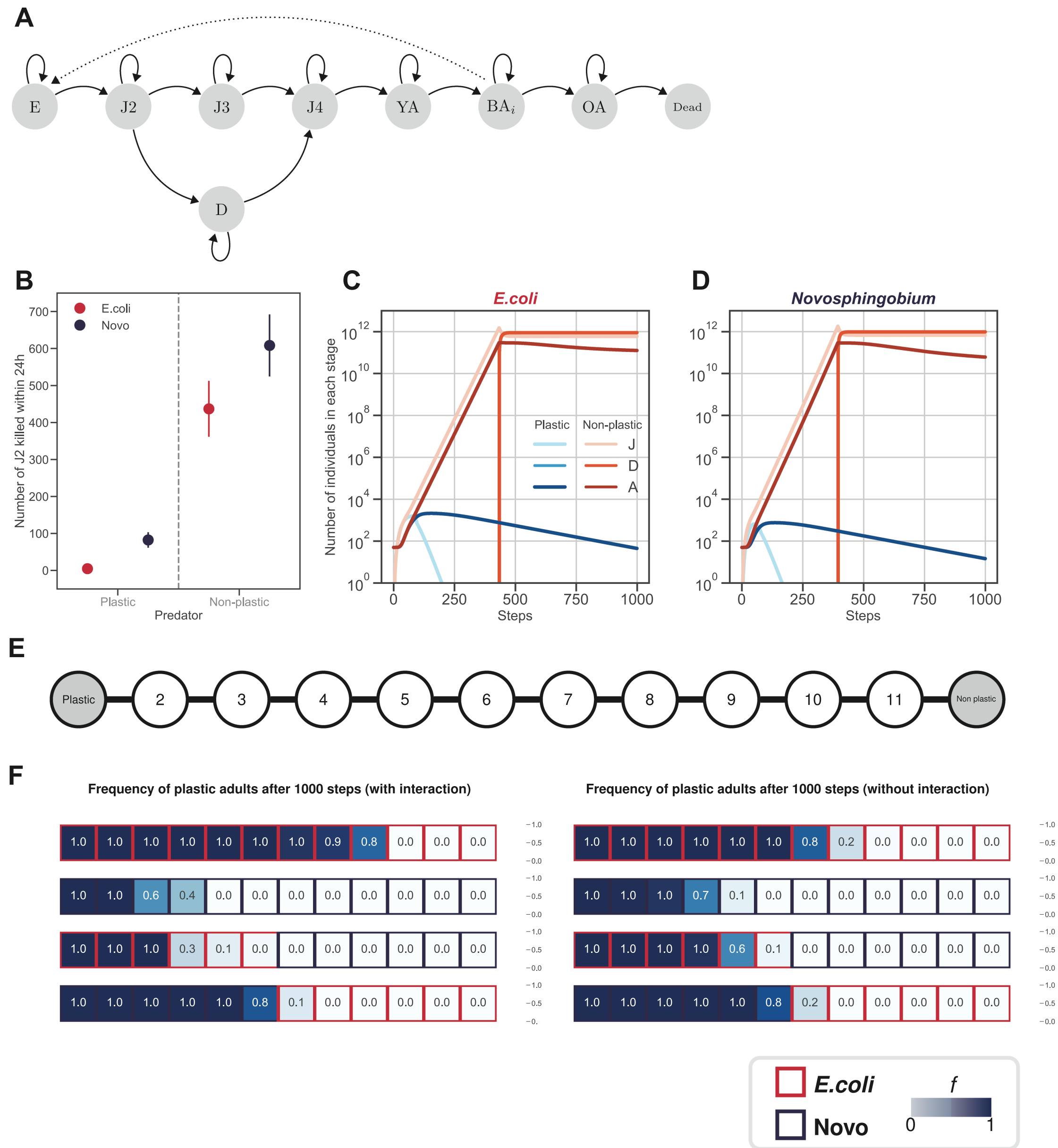

Figure 3. Costs of phenotype and plasticity in spatially homogeneous and structured populations: (A) Life cycle of P. pacificus as a Markov chain (E: egg, J2-4: juvenile stages, D: dauer, YA: young adult, BA_i: breeding adult of the day *i*, OA: old adult). Note that J1 larvae in *P. pacificus* remain in the egg shell and are considered part of E in our model. Solid arrows represent the transition between different developmental stages. Egg laying by BA adults is indicated by a dotted arrow. Five different breeding adults are included in the model (BA_1 to BA_5). (B) Standard inter-strain corpse assay. Each dot represents a mean of five replicates, and error bars represent standard deviation. For each replicate, 20 adult predators were added to ≈ 3000 J2 prey, and corpses were screened after 24 hr. (C,D) Simulation of the effect of with-strain predation on population dynamics in a spatially-homogeneous population. Using predation rate estimates from the corpse assay, we simulated the interaction of the plastic and the non-plastic strain on *E. coli* and *Novosphingobium*. In both conditions, the non-plastic strain drives the plastic strain into extinction. Simulations in (C) and (D) start with 50 YAs of each strain. The initial food supply, $S_0 = 12 \times 10^{12}$. On *E. coli*, for both strains, $\gamma_E = 0.0415$, $\gamma_{J2} = 0.055$, $\gamma_{J3} = 0.085$, $\gamma_{J4} = 0.07$, $\gamma_{YA} = 0.1$, $\gamma_{Bi} = 0.0415$, $\sigma_{OA} = 0.995$ (see Supplementary Methods). On *Novosphingobium*, $\gamma_{YA} = 0.13$ for the plastic strain and $\gamma_{YA} = 0.4$ for the non-plastic strain to account for the change in the developmental speed observed in the experiment. Predation rates: on *E. coli*, $\eta_{RSC017} = 1.7 \times 10^{-4}$ and $\eta_{RS5405} = 3.3 \times 10^{-4}$; on *Novosphingobium*, $\eta_{RSC017} = 6.4 \times 10^{-5}$ and $\eta_{RS5405} = 4.7 \times 10^{-4}$. The same parameters are used in the subsequent simulations. Note that *J* is the sum of all juvenile stages and A the sum of YA, BA_i, and OA stages. (E) A structured population, consisting of 12 localities arranged in a line was used to simulate a dispersion-colonization-competition scenario. Each simulation starts with 50 YAs of the plastic strain on the first locality and 50 YAs of the non-plastic strain on the 12th locality. At each step, ωdauer larvae migrate from population *i* to *j* if *j* has more food than **i**. (F) The frequency of the plastic strain adults (YA, BA_i, OA) 12 localities with or without interaction (i.e., predation) after 1000 steps. As previously noted, 1000 steps represent an arbitrary endpoint, which roughly corresponds to 10 generations. At the start of the simulation, for each strain in localities 1 and 12, $n_E = n_{J2} = n_{J3} = n_{J4} = n_{BA} = n_{OA} = 0$, and $n_{YA} = 50$, while $m = 0.1$. The initial food supply, $S_0 = 10^{12}$ in each locality.

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the two isolates. However, such projections about the population dynamics of these strains of *P. pacificus* should be taken with caution, as many aspects of *P. pacificus* population dynamics and its dispersal patterns in the wild remain poorly understood.

Discussion

Experiential detection of the costs associated with plasticity, especially in metazoans, has proved to be a daunting challenge. For instance, the predator-induced spine of *Daphnia pulex* was reported to show mild support for both the costs of production and maintenance (Scheiner & Berrigan, 1998). Similarly, in the Scandinavian frog, *Rana temporaria*, the costs of metamorphic size were shown to exhibit a plasticity cost in southern populations, whereas northern populations displayed no such costs (Merilä et al., 2004). Van Buskirk and Steiner (2009) in their meta-analysis, concluded that costs of plasticity are mostly low if existing at all. However, the same authors suggested that these costs may influence adaptive evolution under stressful conditions. Additionally, a meta-analysis on aquatic gastropods argued for further empirical investigations to better quantify the energetic costs of plasticity of shell formation (Bourdeau et al., 2015). A more recent study on the cannibalistic cane toads, signifies favoring canalized defenses over plasticity, providing the high cost of plasticity rather than the cost of phenotype (Devore et al., 2021). This diversity of findings indicates the need for establishing a comprehensive empirical framework to address both theoretical and conceptual asserts.

The results obtained in *P. pacificus*, likely benefited from the binary and easily distinguishable state of the polyphenic trait and the isogeneic nature of all tested strains, which facilitate empirical measurements of fitness components, i.e., fecundity and developmental speed, in the laboratory. Additionally, accounting for two fitness components assisted in the transition from abstract measures to simulating a range of ecologically relevant scenarios. Our study complements previous knowledge with a systematic analysis of defined costs in an evolutionary adaptive trait.

It has been argued that resource polyphenism, i.e., the environmental induction of alternative phenotypes to use different resources, such as the development of cannibalistic morphs as a response to environmental stress (Pfennig & McGee, 2010)—is the most relevant of discrete plastic response. Cannibalism provides trophic and survival advantages by either extending energy resources or eliminating competition (Church & Sherratt, 1996; Claessen et al., 2004). It has been suggested that the predatory mouth form in *P. pacificus* boosts survivorship under severe conditions (Serobyan et al., 2014), and reduces competition on the basis of genomic relatedness (Lightfoot et al., 2021). Nevertheless, various *P. pacificus* natural isolates are either predominantly non-predatory or intermediately so. Our results suggest that, in isolation, the fitness payoff incurred by the predatory-biased population makes it inferior to the non-predatory-biased strain (Supplementary Figure S4b,c). Strikingly, our computational model indicates this cost of phenotype to be more detrimental when both isolates are interacting in a spatially homogenous population. The effect of growth rate, developmental speed, and predation are highly context dependent, as shown by our simulations under different starting conditions, resulting in different population dynamics (Figure 3F, Supplementary Figures S8 and S9).

The effect of population structure and the non-homogenous distribution of resources in the environment on the outcome of competition between a plastic and non-plastic strain illustrates the complex nature of the ecological consequences of the cost of plasticity. The role of phenotypic plasticity, and dispersal, in invasions, have long been appreciated (Sharma et al., 2005), based on the assumption that plasticity provides a "Jack-of-all-trades" strategy. This assumption has been challenged (Hulme, 2007), and does not explain the pattern we observe in our model. The models proposed to predict the population-level consequences of plasticity thus far (reviewed in Wennersten and Forsman [2012]) have been almost entirely conceptual. A promising recent attempt by Brass et al. (2021) incorporates plasticity in a continuous-time stage-structured model to predict the ecological effects of plasticity, but their model differs from ours, since they include plasticity as maternally determined phenotypic variation within a species and do not explore the effect of population structure nor the non-homogenous resource distribution on the cost of plasticity. The effects of spatial heterogeneity and dispersal on the evolution of plasticity have been explored before, e.g., Scheiner and Holt (2012) and Edelaar et al. (2017), but our results are not comparable to those, since our model lacks any evolutionary component, e.g., mutation, recombination, etc., and is solely concerned with the short-term ecological consequences of plasticity in *P. pacificus*.

Together, our results suggest a four-pronged explanatory framework, combining the *cost of plasticity, cost of phenotype,* environmental influence, and population structure, each playing a crucial role in adaptive plasticity. However, several questions remain to be answered. For example, measuring predation dynamics and migration rates on beetle carcasses can increase the accuracy of modeling approaches. Also, predator consumption might differ as a functional response to prey density, given search, handling time, foraging efficiency, and predation risks (Holling, 1959a,b; Lima et al., 1985; Sentis et al., 2013; Solomon, 1949). Additionally, in nature, nematode mobility is not restricted to a one-dimensional dispersal. Thus, such parameters merit further empirical and theoretical analyses. Finally, a key question that was hardly identified in other plastic systems is the molecular machinery underlying the production and maintenance of plasticity (Murren et al., 2015; Pigliucci, 2001). In *P. pacificus*, the readily available molecular techniques permit such potential investigations. In conclusion, this study integrates empirical and theoretical approaches to emphasize how different types of costs influence the evolution of adaptive plasticity, while setting the stage for further investigations.

Supplementary material

Supplementary material is available online at *Evolution Letters* (https://academic.oup.com/evlett/qrac001).

Data availability

The software used to run all simulations and conduct all the data analysis was written in Python 3.10.4. For reproducibility, the code and the raw experimental data are available at (https://github.com/Kalirad/cost_of_plasticity).

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Methods

Details of the laboratory assays

Bacterial & nematodes strain culture and maintenance

The two bacteria used as food source under monoxenic conditions were the standard *E. coli* lab strain OP50 and the naturally *Pristionchus*-associated *Novosphingobium* sp. L76 (Akduman *et al.* 2018). OP50 was grown overnight at 37°C in Lysogeny broth medium (LB) without shaking, while *Novosphingobium* sp. L76 was grown overnight at 30°C in Lysogeny broth medium (LB) at 157 rpm. On the following day, 6-cm nematode growth medium (NGM) Petri-dishes were seeded with 300µl of *E. coli* OP50 or *Novosphingobium* and left for overnight incubation (Sieriebriennikov *et al.* 2020). Nematodes were reared on the seeded NGM plates at 20°C. Three adults were passed to new plates every 5 days for *E. coli*, and every 4 days for *Novosphingobium*; giving the difference in developmental speed.

Mouth form phenotyping

Mouth-form scoring was performed on a ZEISS SteREO Discovery.V20 microscope, PlanApo S 1.5x objective with eyepiece PL 10x/23 Br.foc. Mouth-form phenotype was identified according to the mouth width and the shape of the dorsal tooth of young adults as previously reported (Bento *et al.* 2010). For all experiments, three replicates were scored at 20°C on 300µl of the relevant food. The total number of worms scored per strain is as follows: Intra-strain analysis (RS5348= 138, RS113= 243, RSA662= 289, RSA645= 211, RSC019= 150, RSC033= 157, RSD029= 188). In all other analyses, i.e., inter-strain analysis, plasticity cost, and predation assays, we used 150 animals per strain.

Overall and daily self-fecundity measure

Maintenance cultures were first bleached to obtain synchronized eggs before starting an experiment. Bleaching protocol was performed as previously reported in (Stiernagle). Upon synchronization, J4 larvae were individually isolated on separate plates spotted with 20µl of the relevant bacteria. The next day, when worms are young adults, the mouth form was scored to

ensure its consistency with the maintenance culture. For four consecutive days, single worms were transferred to fresh plates every 24 hours. Starting from day 5, worms were kept on the same plate for two more days and then killed. This provides a daily readout for the first four days and a day 5 readout representing the last three days combined. This experimental design was performed given that approximately 90% of the worm's self-progeny is produced within the first three days of adulthood. To obtain fecundity counts, all plates were counted for viable progeny after five days from transferring the mother, thus acquiring both daily and overall self-fecundity. Plates were kept at 20°C across all steps of the experimental design (Fig. S2a).

Developmental speed measure

From maintenance cultures, J4 animals were isolated to avoid any outcrossing of the hermaphrodite worms with spontaneous males in the population. After 24 hours, these worms are developed into breeding adults. Afterwards, 10 breeding adults were placed on a fresh plate with 100µl of the respective food source. Plates were incubated for two hours in order to obtain eggs before the mothers were removed. Note that *P. pacificus* lays its eggs in the 2 or 4-cell stage, resulting in at least 40-50 highly synchronized egg clutches. After 75 hours, worms were observed to determine the developmental stage of the progeny. For each strain, and accordingly for each food condition; 40 − 50 mothers were isolated representing 4-5 biological replicates. Between 232 to 489 progenies were staged for each experiment. Plates were kept at 20°C across all steps of the experimental design (Fig. S2b). The time point of 75hrs was chosen to capture the transition rate from juvenile stages to adulthood (Sommer 2015; Sun *et al.* 2021).

Predation assays: corpse assay

Two types of predation assays were performed in this study; inter-specific and intra-specific predation assays. In the inter-specific predation assays, young adult *P. pacificus* predators, prey on *C. elegans* L1 larvae; while in the intra-specific predation assays, young adult predators of a particular *P. pacificus* strain prey on *P. pacificus* J2 larvae of the other strain.

Corpse assays were performed to quantify both inter as well as intra-specific predation rates. All assays were conducted as previously described in (Lightfoot *et al.* 2019). In short, for the inter-

specific corpse assay, freshly starved *C. elegans* plates were washed with M9 buffer to collect L1 larvae, passed through two Millipore 20µm filters to remove other developmental stages, and followed by centrifugation at 377g/2min to obtain a concentrated larval pellet. One µl of the L1 wash was added onto an empty 6cm (NGM) plate, which represents roughly 3000 larval prey. The *C. elegans* larvae were given at least 1hour window to proportionally spread across the plate. For predators, five *P. pacificus* young adults were blindly picked (independent of mouth-form) from *E. coli* OP50 maintenance cultures. This procedure reflects the predation rates of a population in relevance to mouth-form ratio. Predators were first kept for 10-15 min on an empty plate to reduce body-attached bacteria and were then added to assay plates. The number of corpses was scored after 2 hours with three biological replicates conducted for each assay. For intra-specific predation, we increased the number of predators from 5 to 20 and the assay time from 2 hours to 24 hours as previously reported in (Wilecki *et al.* 2015). In addition, predators were grown on the relevant food source before being blindly picked; i.e., *E. coli* or *Novosphingobium*. For the intra-specific setup, five biological replicates were conducted per assay (Fig. S2c).

Details of the model

To model the dynamics of *P. pacificus* in different environments based on our laboratory data, we envisioned the development of a worm as a finite-state Markov chain. The Markov chain is used to construct a stage-structured population model (for more on this approach to modelling population dynamics, see (Nathan Keyfitz 2005; Caswell 2019)). The projection matrix for this chain is:

$$
\mathbf{A}_{\mathcal{B},\mathcal{S}} =
\begin{array}{c}
\begin{array}{cccccccccc}
\mathrm{E} & \mathrm{J2} & \mathrm{D} & \mathrm{J3} & \mathrm{J4} & \mathrm{YA} & \mathrm{BA}_1 & \cdots & \mathrm{BA}_5 & \mathrm{OA}
\end{array} \\
\begin{bmatrix}
1-\gamma_{\mathrm{E}} & 0 & 0 & 0 & 0 & 0 & F_1 & \cdots & F_5 & 0 \\
\gamma_E & (1-\gamma_{\mathrm{J2}})(1-\gamma^*_{\mathrm{J2}}) & 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 \\
0 & \gamma^*_{\mathrm{J2}} & 1-\gamma_{\mathrm{D}} & 0 & 0 & 0 & 0 & \cdots & 0 & 0 \\
0 & \gamma_{\mathrm{J2}} & 0 & 1-\gamma_{\mathrm{J3}} & 0 & 0 & 0 & \cdots & 0 & 0 \\
0 & 0 & \gamma_{\mathrm{D}} & \gamma_{\mathrm{J3}} & 1-\gamma_{\mathrm{J4}} & 0 & 0 & \cdots & 0 & 0 \\
0 & 0 & 0 & 0 & \gamma_{\mathrm{J4}} & 1-\gamma_{\mathrm{YA}} & 0 & \cdots & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & \gamma_{\mathrm{YA}} & 1-\gamma_{\mathrm{BA}_1} & \cdots & 0 & 0 \\
\vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \cdots & \vdots & \vdots \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 1-\gamma_{\mathrm{BA}_5} & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & \cdots & \gamma_{\mathrm{BA}_5} & \delta_{\mathrm{OA}}
\end{bmatrix}
\end{array},
$$

where γ_i is the probability of transition from developmental stage i into the next developmental stage. In the case of J2, $\gamma_{\mathrm{J2}}^{\square}$ and γ_{J2}^{*} are the probabilities for J2 → J3 and J2 → D, respectively.

Note that as long as food is available ($S_t > 0$), J2 → D transition has a zero probability. We assume that all individuals in each stage survive and develop into the next stage, except for old adults (OA), which have a survival probability, δ_{OA}. In the absence of food, the transition probabilities of all the juvenile stages, as well as the eggs, are zero, while the transition probability for J2 → D is no longer zero (γ^*_{J2} =0.1). The five breeding adult stages (BA_1 to BA_5) each have their own respective per capita fecundity, F_i, for a given bacterial diet (B), based on the daily self-fecundity experiment. For a given *P. pacificus* strain, the transition probabilities and fecundities in the projection matrix depend on the experimentally-informed estimates. The transition probabilities and fecundities for the plastic and the non-plastic are listed in **Table** 1.

Transition probabilities			
	E. coli	*Novosphingobium*	Starvation
E > J2	0.0415	0.0415	0
J2 > dauer	0	0	0.1
J2 > J3	0.055	0.055	0
J3 > J4	0.085	0.085	0
Dauer > J4	0.1	0.1	0
J4 > YA	0.07	0.07	0
YA > BA_i	0.1	**0.13*, 0.4****	0
BA_i > BA_{i+1}	0.0415	0.0415	0.0415
Fecundities			
	E. coli	*Novosphingobium*	
BA_1	22.65*, 19.8**	11.66*, 16.88**	
BA_2	68.45*, 60.3**	62.53*, 80.77**	
BA_3	57.05*, 43.02**	47.13*, 77.7**	
BA_4	33.4*, 19.9**	13.94*, 16.28**	
BA_5	4.97*, 6.6**	0.72*, 1.4**	

Table 1: Parameters used in the model. The fecundity values are based on the average daily number of eggs laid by a given strain on a given food source. The plastic strain specific values is indicated by * and the non-plastic specific values is indicated by **.

The transition probabilities between different stages are set such that the occupancy time for each of the Markov states in our life cycle, *i.e.,* the average time spent over an individuals' life in that state, would correspond to the developmental speed of *P. pacificus* in hours. The mean occupancy time is obtained by calculating the fundamental matrix (**N**) for transition matrix $\mathbf{U}$, where $\mathbf{N} = (\mathbf{I} - \mathbf{U})^{-\mathbf{1}}$. We used a reduced form of our projection matrix that excluded the dauer stage to calculate the fundamental matrix. On *E. coli*, we assume no difference in developmental speed between RSC017 and RS5405. The first column of the fundamental matrix for these strains on OP50 is $[24.1, 18.2, 11.8, 14.3, 10, 24, 24, 24, 24, 24, 200]$, implying that an egg spends on average 24.1 hours in the egg stage, 18.2 in J2, 11.8 in J3, 14.3 in J4, 10 in YA, 24 in each of the five breeding adult stages, and 200 hours (roughly 8.5 days) in the old adult stage before dying. These values are in line with the laboratory measurements of developmental speed (Sommer 2015; Sun *et al.* 2021). We adjusted the probability of YA → BA_1 such that the duration of YA stage on *Novosphigobium* would reduce to ≈ 8 and ≈ 6 hours for RSC017 and RS5405, respectively.

Consumption

Resource consumption is included in the model by assuming fixed consumption rates for each developmental stage. Given food source S_t, if there exist *m* developmental stages in the population at *t* and n_i individuals belong to developmental stage *i*, the amount of available food in the next step will be:

$$\boldsymbol{S_{t+1} = S_t - \sum_{i=1}^{m} \rho_i n_i} \quad , \tag{1}$$

where ρ_i is the per capita consumption rate for developmental stage *i*.

Predation

If a population consists of two strains, *i* and *j*, the number of surviving J2 individuals of strain *i* at time *t* + 1 is:

$$\boldsymbol{V_i(t+1) = V_i(t) - \eta_{ji} P_j(t) V_i(t)} \quad , \tag{2}$$

where η_{ji} is the rate at which adults from strain *j* kill J2s of strain i, $P_j(t)$ is the

number of predatory adults of strain j in the population at time t, and $V_i(t)$ is the number of J2s of strain i. For the plastic strain, the expected number of predatory adults equals the number of adults in the population multiplied by the probability of developing the predatory mouth form on a given bacterial diet.

Population dynamic

Assume $\mathbf{n}(t)$ to be a 12 × 1 array, where each entry represents the count of each developmental stage of strain a in a population at time t. The expected composition of strain a at time $t + 1$ would be:

$$\boldsymbol{n}(\boldsymbol{t}+\mathbf{1}) = \boldsymbol{A_t n}(\boldsymbol{t}) - \boldsymbol{K}(\boldsymbol{t})\boldsymbol{e_i} - \boldsymbol{M}(\boldsymbol{t})\boldsymbol{e_j} \quad , \tag{3}$$

where $K(t)$ is the number of J2 individuals of strain i that were killed at time t, $M(t)$ is the number of dauer larvae that emigrated from the population at time t, and $\boldsymbol{e_i}$ and $\boldsymbol{e_j}$ are unit vectors.

Estimating the predation parameter

To estimate the predation parameter for η_{ji}, we fitted the solution to the difference equation 2,

$$\boldsymbol{V_i}(\boldsymbol{t}) = \boldsymbol{V_i}(\mathbf{0})\left(\mathbf{1} - \boldsymbol{\eta_{ji} P_j}(\boldsymbol{t})\right)^{\boldsymbol{t}} \tag{4}$$

to our empirical data from killing assays. Each killing assay starts with ≈ 3000 J2 worms of strain i ($V_i(0)$= 3000) and 20 adults of strain j. The number of corpses is counted after 24 hours. Assuming a fixed killing rate over the duration of the killing assay, we estimated the η_{ji} that would result in the number of corpses observed in our assay.

Details of the statistical analyses

To analyze the experimental data, instead of taking the Frequentist approach, we opted for Bayesian alternatives. To calculate the probability of developing the predatory mouth morph, we assumed the number of observed predatory worms in a sample of n worms follows the likelihood function y$\sim Bernnoulli(\theta)$, where, as our prior, we assume θ is drawn from a beta distribution with $\alpha = \beta = 1$, which corresponds to a uniform distribution. For our Bayesian estimation for

comparing two groups, equivalent to t-test, for two samples, a and b, we follow Kruschke's BEST approach (Kruschke 2013, 2015): we define likelihood functions, $y_i^a \sim \mathcal{T}(\nu, \mu_a, \sigma_a)$ and $y_i^b \sim \mathcal{T}(\nu, \mu_b, \sigma_b)$. As our prior, we assume that the mean of each sample is from a normal distribution, with the mean and twice the standard deviation of a pooled sample. For the standard deviation, we assume a wide uniform prior, $Unifrom(1{,}300)$. Following Kruschke, we use $\nu = 30$; at higher values of ν, the t-distribution converges to the normal distribution. Such an approach is preferable to the standard t-test, since it compares means and standard deviations between to the two groups. The mean and the 95% highest density interval of our estimates of the parameters of interests, difference in means and difference in standard deviations of two groups, as well as the effects size are reported. This approach lacks the simple and, somewhat deceptive, clarity of Null hypothesis significance testing, but the Bayesian approach is more scientifically appealing, and it enables side-stepping the many issues with p-value (Wasserstein & Lazar 2016). All statistical analyses were conducted with PyMC3 in Python 3.10.4, using the No-U-Turn Sampler. In every analysis, we used effective sample size of $\geq 10{,}000$ for stable estimates of HDIs and ensured that all the 4 MCMC chain had converged, i.e., $\hat{R} = 1$ (Kruschke 2021). The code used to analyze the data with PyMC3 3.11.4 (Salvatier *et al.* 2016) are included in Jupiter notebooks and are accessible on our Github repository associated with this manuscript. The detailed results of the BEST approach can be found in Tables S6-S8.

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Supplementary figures and tables captions

Fig. S1: Mouth-form plasticity in *P. pacificus*: (a) Mouth morph dimorphism in *P. pacificus*. The bacterivorous St morph (non-predatory) possesses a single tooth with a narrow buccal cavity, whereas the predatory Eu morph (predatory) displays two teeth with a broad buccal cavity. **(b)** *P. pacificus* phylogenetic tree representing the genomic relationship between 323 *P. pacificus* wild isolates. The three major clades of *P. pacificus* are all represented in this study. Note that clades A, B, and C have approximately 1% inter-clade genomic divergence. (Adopted from Rödelsperger *et al.* (2017). **(c)** *P. pacificus* adult preying on a *C. elegans* worm. **(d)** *P. pacificus* life cycle including four juvenile stages before reaching adulthood. In stressful conditions; *e.g.,* food depletion, juvenile worms develop into the alternatively dauer stage, which also represents the dispersal stage. Upon suitable conditions, worms exit the dauer stage and resume normal development.

Fig. S2: Schematic representation of the experimental setup: (a) Daily and overall fecundity measurement. *P. pacificus* animals were transferred on a daily basis. The overall self-progeny was counted as the sum of the seven days. **(b)** Developmental speed measurement. *P. pacificus* cultures were initiated by isolating J4 animals from the maintenance culture. Mothers were kept at the same plate for 2 hours, which results in the production of synchronized eggs. After 75 hours, worms were observed to determine the developmental stage of the progeny. **(c)** *P. pacificus* standardized corpse assay. Either *P. pacificus* or *C. elegans* larvae were collected as prey. Prey larvae and predator adults were added to assay plates. Time upon corpse scoring and specifications of the experiment varies according to the interaction type, *i.e.,* intra or inter-specific perdition setup. (Adopted from Lightfoot *et al.* (2021)).

Fig. S3: Daily self-fecundity: (a-c) Daily count for four intermediate *P. pacificus* wild isolates on *E. coli* (a), two pairs of biased *P. pacificus* wild isolates on *E. coli* (b), and two selected *P. pacificus* wild isolates on *Novosphingobium* (c), respectively

Fig S4: Population dynamics of the plastic and the non-plastic strains on *E. coli* and *Novosphingobium* in isolation: Simulations based on empirical data demonstrate the differential

response of the strains on both food conditions. The number of adults and dauer larvae for the plastic strain in *E. coli* relative to their counts on *Novosphingobium* are 1.05 and 1.09, respectively. For the non-plastic strain, the number of adults and dauer larvae in *E. coli* relative to their counts on *Novosphingobium* are 2.28 and 0.82, reflecting the faster YA to BA_1 and higher fecundity of the non-plastic strain on *Novosphingobium*. Note that J is the sum of all juvenile stages. Upon the depletion of food, juvenile stages and eggs stop transitioning into the next developmental stage, except for J2, which develops into dauer larvae. Simulations in (a) and (b) start with 50 YAs of a strain. The initial food supply, $S_0 = 12 \times 10^{12}$. On E. coli, for both strains, $\gamma_{\mathrm{E}} = 0.0415$, $\gamma_{\mathrm{J2}} = 0.055$, $\gamma_{\mathrm{J3}} = 0.085$, $\gamma_{\mathrm{J4}} = 0.07$, $\gamma_{\mathrm{YA}} = 0.1$, $\gamma_{\mathrm{Bi}} = 0.0415$, $\sigma_{\mathrm{OA}} = 0.995$ (see *Supplementary Methods*). On *Novosphingobium*, $\gamma_{\mathrm{YA}} = 0.13$ for the plastic strain and $\gamma_{\mathrm{YA}} = 0.4$ for the non-plastic strain to account for the change in the developmental speed observed in the experiment.

Fig. S5: Interplay between developmental speed and fecundity in the plastic strain: To test our hypothesis that faster developmental speed was indeed compensating for the cost of plasticity, (i.e., lower fecundity), we simulated the dynamics of the plastic strain by assuming no change in developmental speed. The difference in the steady-state counts of adult stages and dauer stage on *E. coli* versus *Novosphingobium* compared to Fig. S4a supports this hypothesis.

Fig. S6: Inter-specific predation assay: A negative-binomial model was fitted to the observed relationship between the mouth-form ratio and the number of corpses counted in the experimental corpse assay. The credible estimate for the negative-binomial model are $\beta 0 = 2.19$ ($1.83 \geq$ 95%HDI ≤ 2.58) and $\beta 1 = 1.41$ ($0.99 \geq$ 95%HDI ≤ 1.82). The 95% HDP region indicates the highest posterior density. The orange dots are the mean estimate sampled from the posterior predictive distribution.

Fig. S7: Estimating predation rates from experimental data: The dotted lines indicated the number of corpses observed after 24 hours of the experimental corpse assay. The solid lines indicate $V_i(t) = V_i(0)\left(1 - \eta_{ji} P_j(t)\right)^t$ with a given killing rate, η_{ji}, that generates the same number of corpses as the killing assay for a given strain on a bacterial diet. η_{ji} was obtained by solving the equation for $t = 24$.

Fig. S8: The effect of interaction on the pattern of dauer larvae dispersal: We measured the total number of the plastic and the non-plastic strains dauer larvae that migrated from each of the 12 localities to a neighboring locality in the one-dimensional stepping stone model during the simulation shown in Fig 3f. The first locality (1) was the starting locality for the plastic strain, and the last locality (12) was the starting locality for the non-plastic strain. The number of migrating dauer larvae for source locality (i) is the total number of dauer larvae dispersed from (i) to locality ($i+1$), provided that ($i+1$) contained food. (a-b) are the dynamics on *E. coli*, while (c-d) are the dynamics on *Novosphingobium*.

Fig S9: Final frequency of dauer larvae in a dispersion-colonization-competition scenario: The frequency of the plastic strain dauer larvae across 12 localities with (a) and without (b) interaction (i.e., predation) after 1000 steps. The results based on the simulations used in Fig. 3f.

Supplementary tables (1-5) represent raw data for overall fecundity, daily fecundity, developmental speed, mouth-form ratio, corpse assay; respectively.

Citations:

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Rödelsperger, C., Meyer, J.M., Prabh, N., Lanz, C., Bemm, F. & Sommer, R.J. (2017). Single-Molecule Sequencing Reveals the Chromosome-Scale Genomic Architecture of the Nematode Model Organism Pristionchus pacificus. *Cell Rep.*, 21, 834-844.
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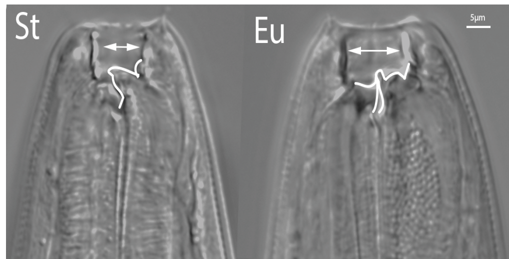
Supplementary Figure S1

a



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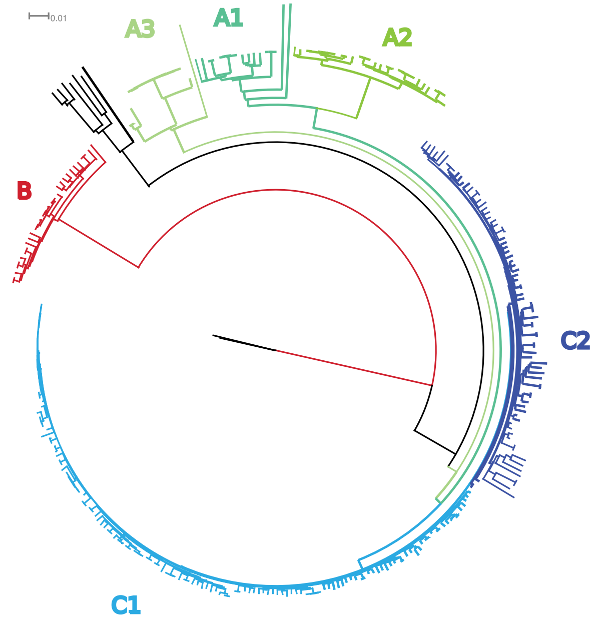

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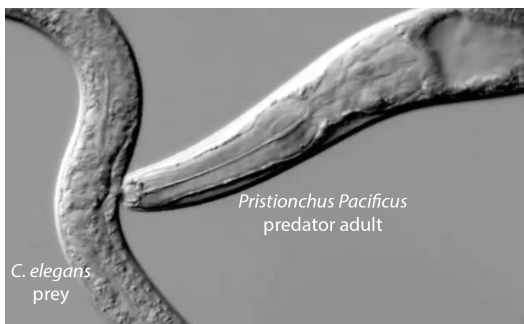

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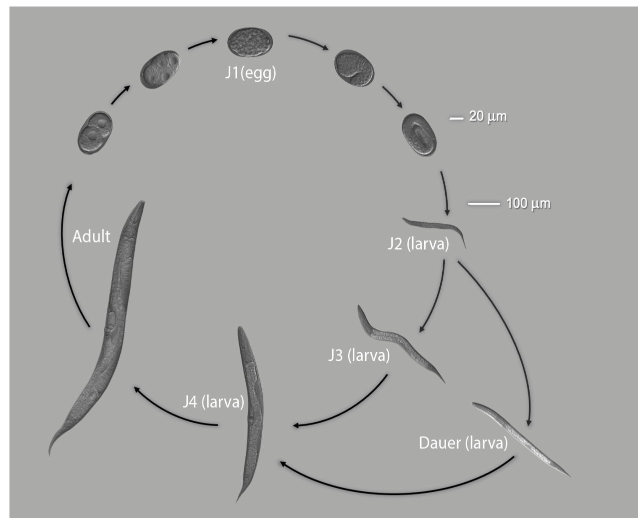

Supplementary Figure S2

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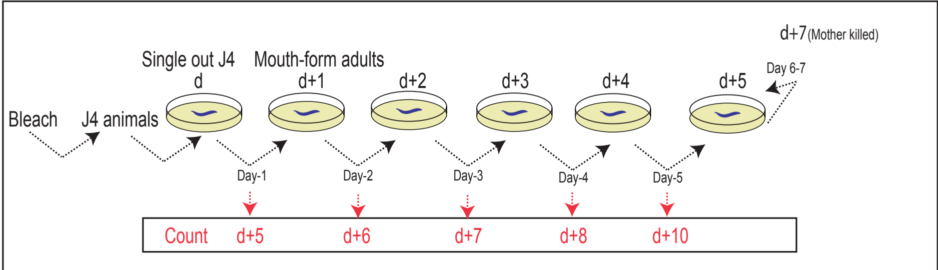

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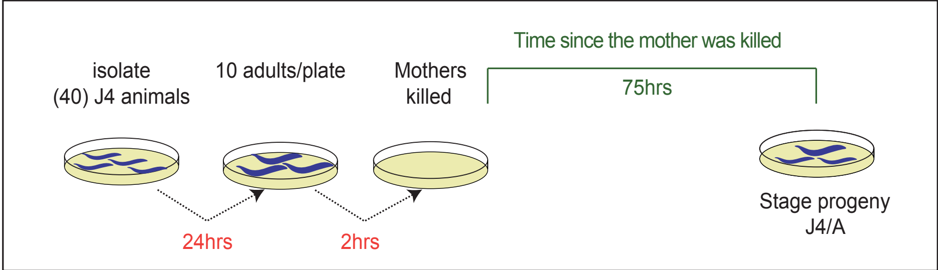

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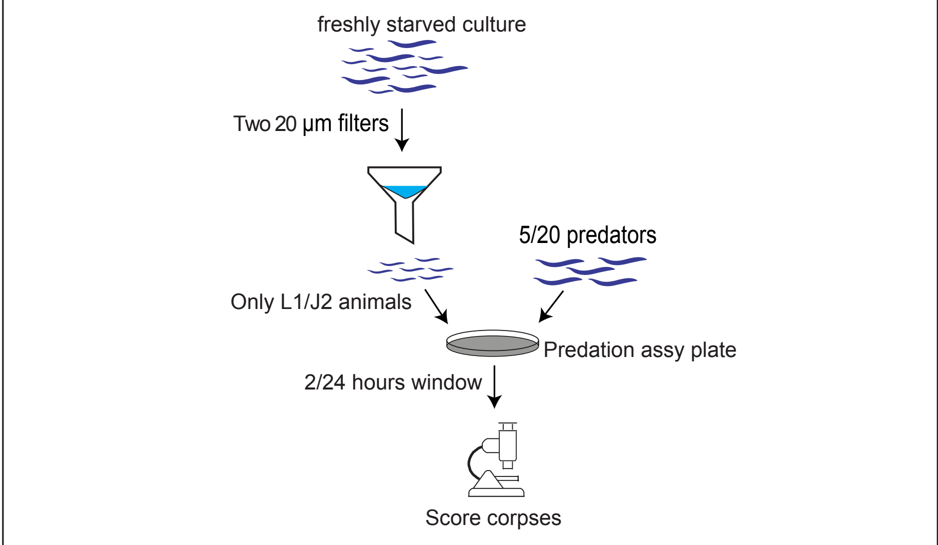

Supplementary Figure S3

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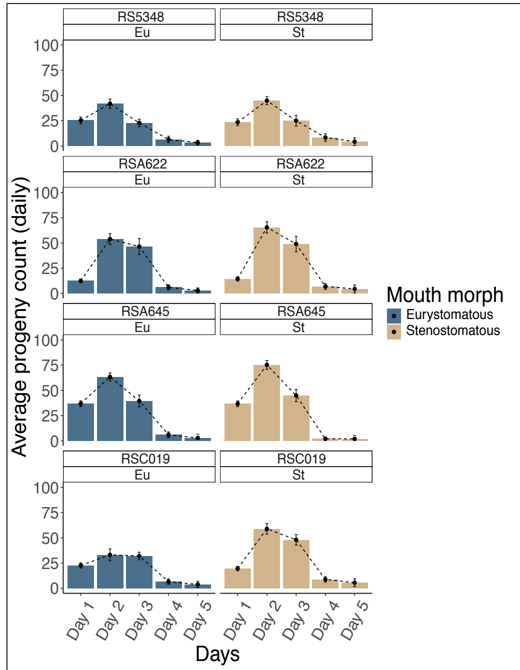

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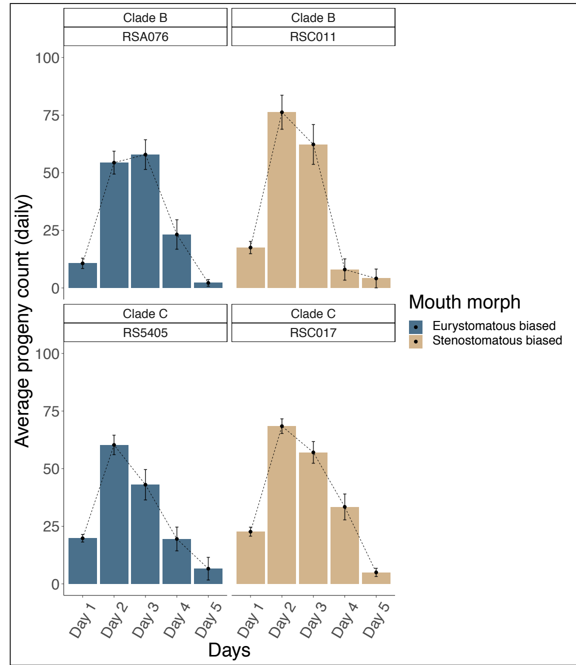

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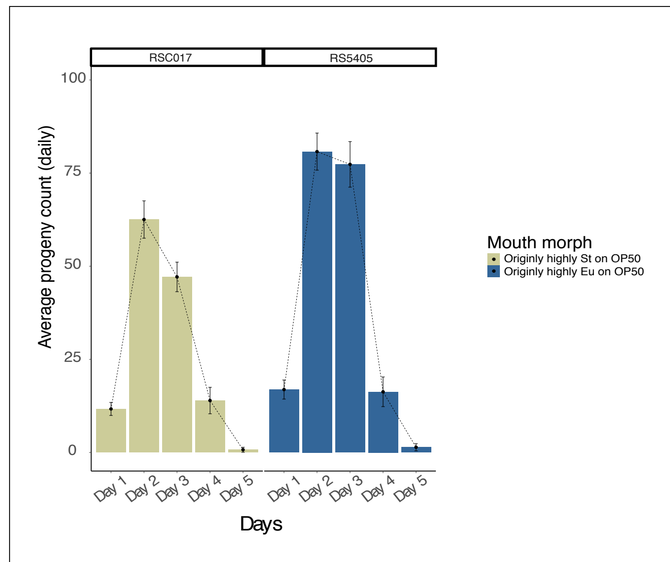

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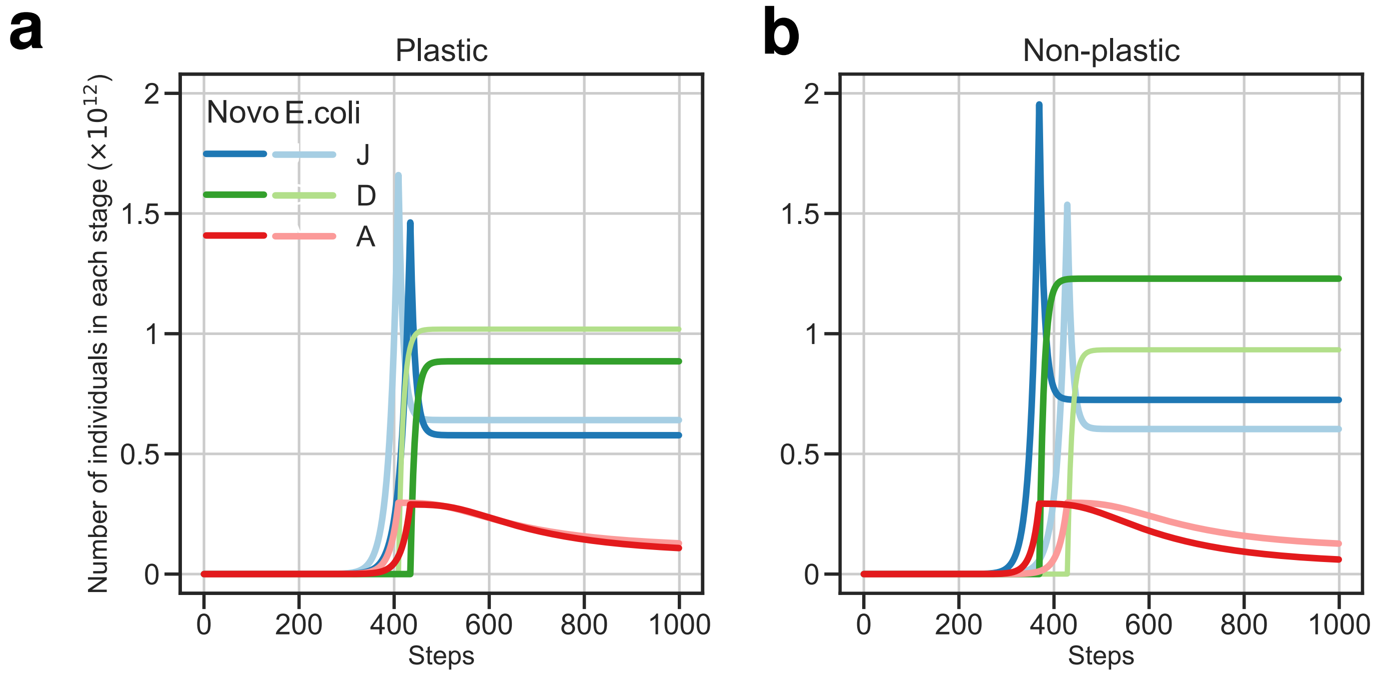
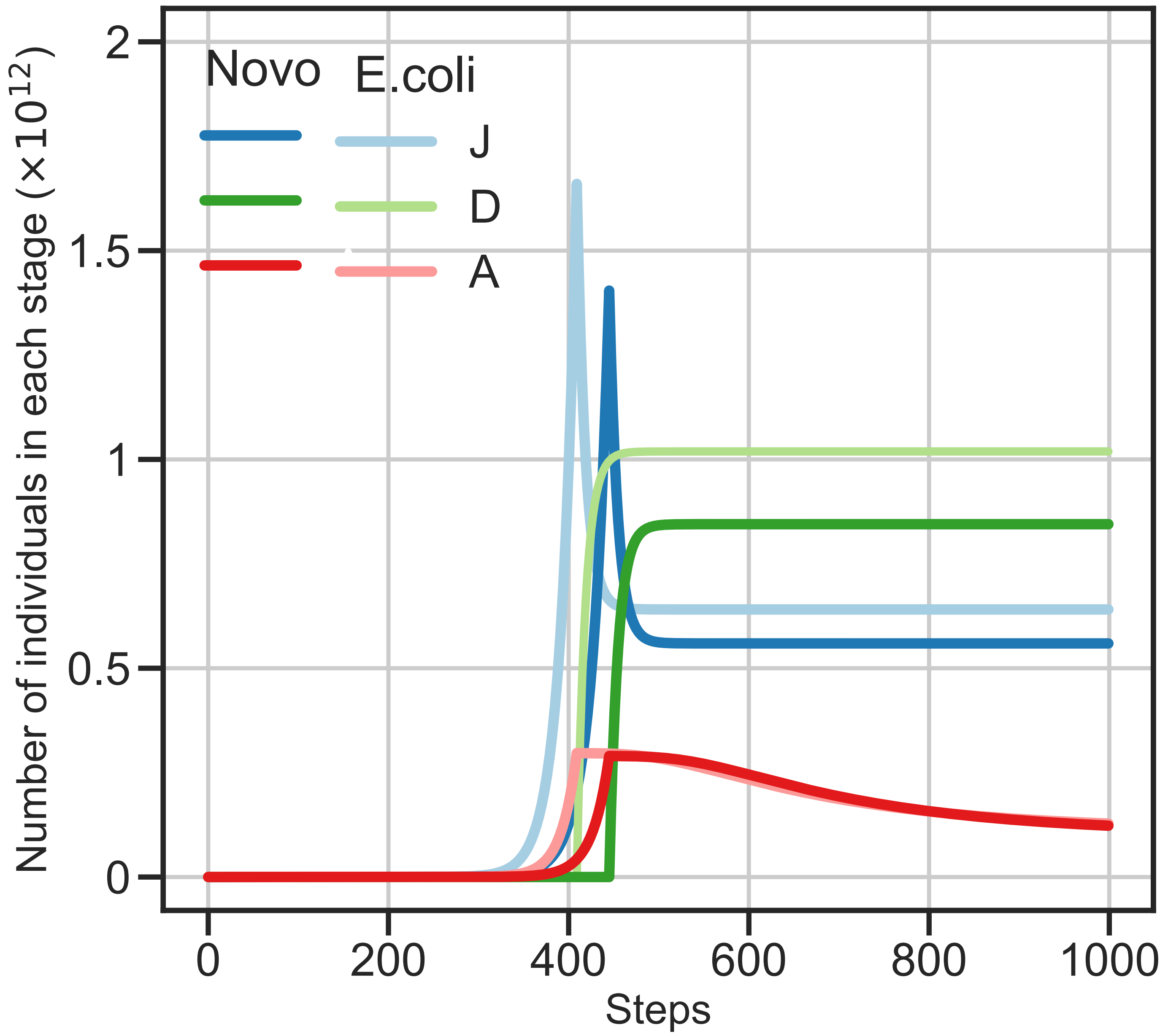
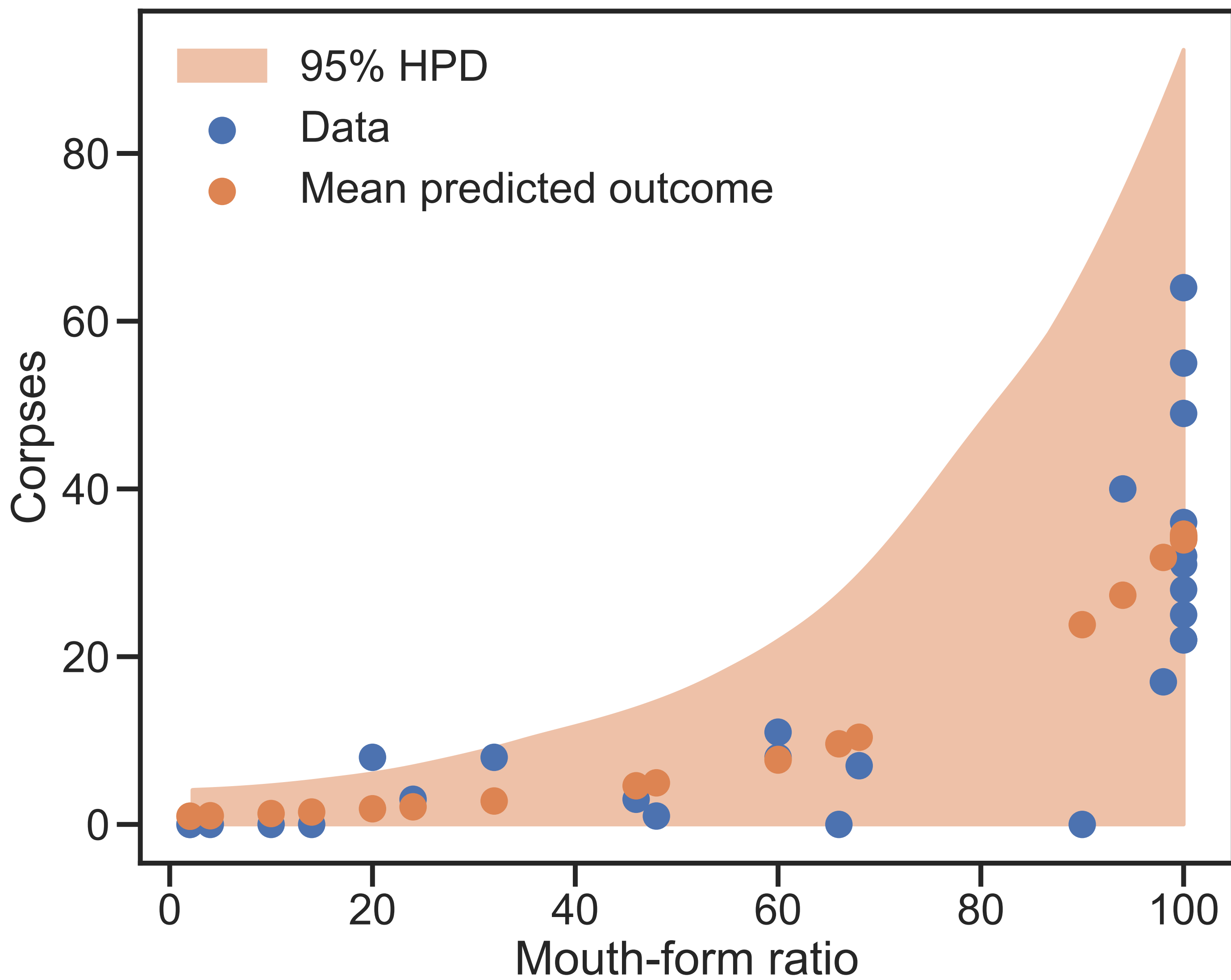


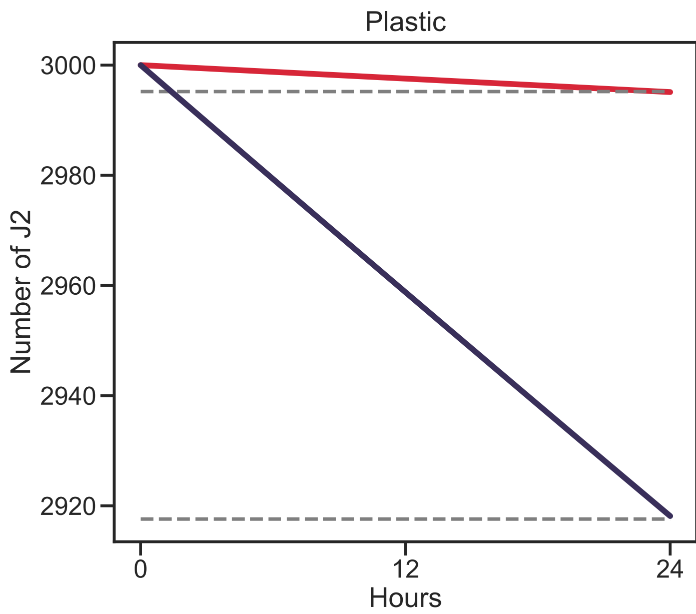
Supplementary Figure S5

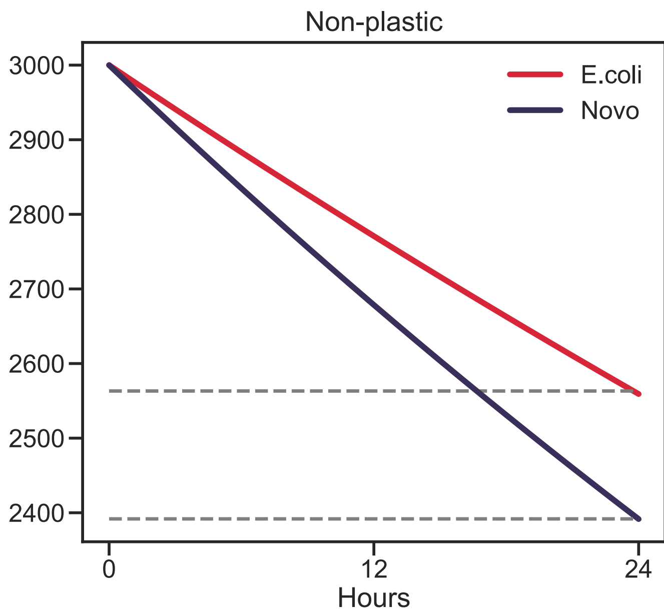


Supplementary Figure S6

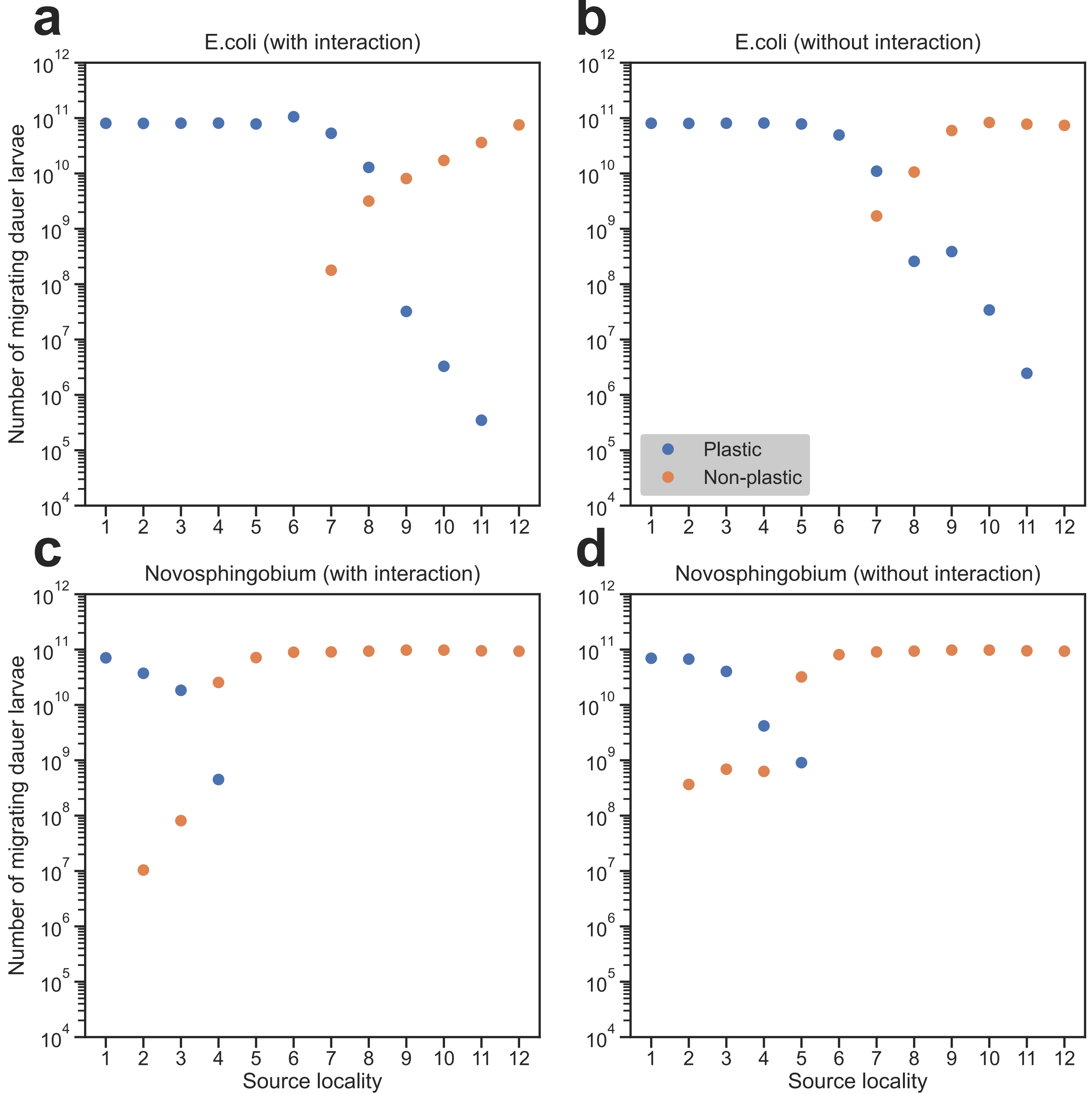


Supplementary Figure S7

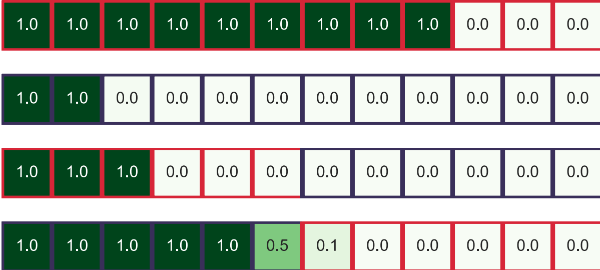


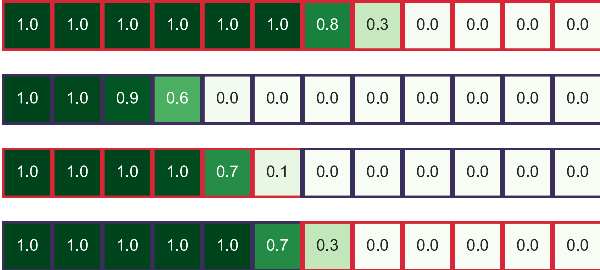


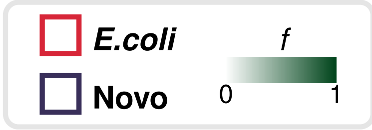
Supplementary Figure S8



Supplementary Figure S9







Supplementary Table S1

Strain	Location/clade	Number of Eu individuals	Number of St individuals	Overall fecundity mean Eu	Overall fecundity mean St	Standard deviation Eu	Standard deviation St	Expirmental setup	Condition
RS5348	La Reunion, Trois Bassins (TB)/C	44	33	99,75	105,9393939	29,03576383	30,79259184	intra-genotype cost of phenotype	*E. coli*
RSA113	La Reunion, Trois Bassins (TB)/C	54	41	68,18518519	72,92682927	25,92738341	29,5375949	intra-genotype cost of phenotype	*E. coli*
RSA622	Mauritius, Sugarcane Institute (MU)/C	50	49	121,5833333	140,0169492	45,12216625	40,81180168	intra-genotype cost of phenotype	*E. coli*
RSA645	Mauritius, Lakaz Chamarel Med.pla (MU)/A	55	54	148,6363636	160,8333333	38,48056924	40,74344489	intra-genotype cost of phenotype	*E. coli*
RSC019	La Reunion, Colorado (CO)/C	54	53	98,2037037	140,8490566	36,15030448	39,93330797	intra-genotype cost of phenotype	*E. coli*
RSC033	La Reunion, Grand Etang Lake-3 (GE)/C	54	47	97,92592593	125,5744681	36,31302685	48,02433472	intra-genotype cost of phenotype	*E. coli*
RSD029	La Reunion, Nez de Boeuf (NB)/B	46	54	143,3478261	146,7407407	31,13784221	32,59365906	intra-genotype cost of phenotype	*E. coli*

Strain	Location/clade	Number of Eu individuals	Number of St individuals	Overall fecundity mean Eu	Overall fecundity mean St	Overall fecundity Eu+St	Standard deviation Eu	Standard deviation St	Standard deviation Eu+St	Expirmental setup	Condition
RSC011	La Reunion, Coteau Kerveguen (CK)/ B	9	32	173,2222222	166,75	168,1707317	36,04087186	45,10131963	42,93710659	inter-genotype cost of phenotype	*E. coli*
RSA076	La Reunion, Nez de Boeuf (NB)/ B	40	0	148,3	0	148,3	42,70002702	0	42,70002702	inter-genotype cost of phenotype	*E. coli*
RS5405	La Reunion, Trois Bassins (TB)/ C	40	0	149,625	0	149,625	45,37348571	0	45,37348571	inter-genotype cost of phenotype	*E. coli*
RSC017	La Reunion, Colorado (CO)/ C	0	40	0	186,525	186,525	0	33,05394775	33,05394775	inter-genotype cost of phenotype	*E. coli*

Strain	Location/clade	Number of Eu individuals	Number of St individuals	Overall fecundity mean Eu	Overall fecundity mean St	Overall fecundity Eu+St	Standard deviation Eu	Standard deviation St	Standard deviation Eu+St	Expirmental setup	Condition
RS54054	La Reunion, Trois Bassins (TB)/ C	43	0	193,0465116	0	193,0465116	33,0273721	0	33,0273721	Cost of plasticity	*Novosphingobium*
RSC017	La Reunion, Colorado (CO)/ C	47	0	136,2553191	0	136,2553191	27,9887152	0	27,9887152	Cost of plasticity	*Novosphingobium*

Supplementary Table S2

Strain	Mean progeny count	Days	Standard deviation	Number of individuals	Condition	Percentage	Animals mouth form	Expirmental setup
RS5348	25,31818182	Day 1	11,19961	44	*E. coli*	25,3816371	Eu	intra-genotype cost of phenotype
RS5348	41,90909091	Day 2	15,71522	44	*E. coli*	42,0141262	Eu	intra-genotype cost of phenotype
RS5348	22,79545455	Day 3	12,53011	44	*E. coli*	22,852586	Eu	intra-genotype cost of phenotype
RS5348	6,431818182	Day 4	10,78414	44	*E. coli*	6,44793803	Eu	intra-genotype cost of phenotype
RS5348	3,29545	Day 5	7,79558	44	*E. coli*	3,30370927	Eu	intra-genotype cost of phenotype
RS5348	23,42424242	Day 1	9,87747	33	*E. coli*	22,1110036	St	intra-genotype cost of phenotype
RS5348	44,9393	Day 2	11,41528	33	*E. coli*	42,4198574	St	intra-genotype cost of phenotype
RS5348	24,93939394	Day 3	15,51801	33	*E. coli*	23,5412108	St	intra-genotype cost of phenotype
RS5348	8,363636364	Day 4	11,01368	33	*E. coli*	7,89474384	St	intra-genotype cost of phenotype
RS5348	4,272727273	Day 5	11,39976	33	*E. coli*	4,03318435	St	intra-genotype cost of phenotype
RSA645	36,83636364	Day 1	11,11349	55	*E. coli*	24,7828746	Eu	intra-genotype cost of phenotype
RSA645	63,34545455	Day 2	14,95081	55	*E. coli*	42,617737	Eu	intra-genotype cost of phenotype
RSA645	39,54545455	Day 3	22,02593	55	*E. coli*	26,6055046	Eu	intra-genotype cost of phenotype
RSA645	6,036363636	Day 4	10,45619	55	*E. coli*	4,06116208	Eu	intra-genotype cost of phenotype
RSA645	2,872727273	Day 5	14,02650	55	*E. coli*	1,93272171	Eu	intra-genotype cost of phenotype
RSA645	36,74074074	Day 1	10,71621	54	*E. coli*	22,8439839	St	intra-genotype cost of phenotype
RSA645	75,22222222	Day 2	16,58104	54	*E. coli*	46,7702936	St	intra-genotype cost of phenotype
RSA645	44,92592593	Day 3	22,62979	54	*E. coli*	27,9332182	St	intra-genotype cost of phenotype
RSA645	2,018518519	Day 4	5,06371	54	*E. coli*	1,25503742	St	intra-genotype cost of phenotype
RSA645	1,925925926	Day 5	12,64143	54	*E. coli*	1,1974669	St	intra-genotype cost of phenotype
RSA622	12,4	Day 1	6,99927	50	*E. coli*	10,1987663	Eu	intra-genotype cost of phenotype
RSA622	53,86666667	Day 2	18,98391	50	*E. coli*	44,304318	Eu	intra-genotype cost of phenotype
RSA622	46,46666667	Day 3	28,50600	50	*E. coli*	38,2179575	Eu	intra-genotype cost of phenotype
RSA622	6	Day 4	9,42949	50	*E. coli*	4,93488691	Eu	intra-genotype cost of phenotype
RSA622	2,85	Day 5	9,37329	50	*E. coli*	2,34407128	Eu	intra-genotype cost of phenotype
RSA622	14,37288136	Day 1	7,74351	49	*E. coli*	10,2651011	St	intra-genotype cost of phenotype
RSA622	65,42372881	Day 2	20,17271	49	*E. coli*	46,725578	St	intra-genotype cost of phenotype
RSA622	48,96610169	Day 3	27,42071	49	*E. coli*	34,9715531	St	intra-genotype cost of phenotype
RSA622	6,898305085	Day 4	10,12626	49	*E. coli*	4,92676431	St	intra-genotype cost of phenotype
RSA622	4,355932203	Day 5	14,28860	49	*E. coli*	3,11100351	St	intra-genotype cost of phenotype
RSC019	22,61111111	Day 1	9,51546	54	*E. coli*	23,024703	Eu	intra-genotype cost of phenotype
RSC019	33,18518519	Day 2	22,06515	54	*E. coli*	33,7921931	Eu	intra-genotype cost of phenotype
RSC019	32,01851852	Day 3	13,87340	54	*E. coli*	32,6041863	Eu	intra-genotype cost of phenotype
RSC019	6,611111111	Day 4	9,97434	54	*E. coli*	6,73203847	Eu	intra-genotype cost of phenotype
RSC019	3,777777778	Day 5	11,58344	54	*E. coli*	3,84687913	Eu	intra-genotype cost of phenotype
RSC019	19,52830189	Day 1	7,97015	53	*E. coli*	13,8647019	St	intra-genotype cost of phenotype
RSC019	58,88679245	Day 2	19,53071	53	*E. coli*	41,8084394	St	intra-genotype cost of phenotype
RSC019	48,05660377	Day 3	19,27475	53	*E. coli*	34,119223	St	intra-genotype cost of phenotype
RSC019	8,773584906	Day 4	9,97434	53	*E. coli*	6,22906899	St	intra-genotype cost of phenotype
RSC019	5,603773585	Day 5	14,41999	53	*E. coli*	3,97856664	St	intra-genotype cost of phenotype
RSC011	17,53658537	Day 1	8,553062495	41	*E. coli*	10,4278463	highly st	inter-genotype cost of phenotype
RSC011	76,24390244	Day 2	23,9629923	41	*E. coli*	45,3372009	highly st	inter-genotype cost of phenotype
RSC011	62,26829268	Day 3	27,51064081	41	*E. coli*	37,026831	highly st	inter-genotype cost of phenotype
RSC011	8	Day 4	14,91978552	41	*E. coli*	4,75707034	highly st	inter-genotype cost of phenotype
RSC011	4,12195122	Day 5	13,14000594	41	*E. coli*	2,45105149	highly st	inter-genotype cost of phenotype
RSA076	10,675	Day 1	7,230233886	40	*E. coli*	7,1982468	highly Eu	inter-genotype cost of phenotype
RSA076	54,375	Day 2	16,02031963	40	*E. coli*	36,6655428	highly Eu	inter-genotype cost of phenotype
RSA076	57,875	Day 3	21,06624728	40	*E. coli*	39,0256237	highly Eu	inter-genotype cost of phenotype
RSA076	23,2	Day 4	20,65491808	40	*E. coli*	15,6439649	highly Eu	inter-genotype cost of phenotype
RSA076	2,175	Day 5	4,684330125	40	*E. coli*	1,46662171	highly Eu	inter-genotype cost of phenotype
RSC017	22,65	Day 1	6,290204024	40	*E. coli*	12,1431444	highly st	inter-genotype cost of phenotype
RSC017	68,45	Day 2	10,19684797	40	*E. coli*	36,6974936	highly st	inter-genotype cost of phenotype
RSC017	57,05	Day 3	15,19606899	40	*E. coli*	30,5857124	highly st	inter-genotype cost of phenotype
RSC017	33,4	Day 4	18,09348941	40	*E. coli*	17,9064469	highly st	inter-genotype cost of phenotype
RSC017	4,975	Day 5	5,757882	40	*E. coli*	2,66720279	highly st	inter-genotype cost of phenotype
RS5405	19,8	Day 1	5,302152441	40	*E. coli*	13,2696386	highly Eu	inter-genotype cost of phenotype
RS5405	60,3	Day 2	13,61597855	40	*E. coli*	40,4120813	highly Eu	inter-genotype cost of phenotype
RS5405	43,025	Day 3	18,94457165	40	*E. coli*	28,8346567	highly Eu	inter-genotype cost of phenotype
RS5405	19,4878049	Day 4	16,20667795	40	*E. coli*	13,0604105	highly Eu	inter-genotype cost of phenotype
RS5405	6,6	Day 5	4,684330125	40	*E. coli*	4,42321288	highly Eu	inter-genotype cost of phenotype
RSC017	11,67391304	Day 1	6,09208721	47	*Novosphingobium*	8,58419751	on *E. coli* highly St	cost of plasticity
RSC017	62,53191489	Day 2	17,5261929	47	*Novosphingobium*	45,9816949	on *E. coli* highly St	cost of plasticity
RSC017	47,12765957	Day 3	13,86835155	47	*Novosphingobium*	34,6544588	on *E. coli* highly St	cost of plasticity
RSC017	13,93617021	Day 4	12,39460657	47	*Novosphingobium*	10,2477068	on *E. coli* highly St	cost of plasticity
RSC017	0,723404255	Day 5	2,337800226	47	*Novosphingobium*	0,53194203	on *E. coli* highly St	cost of plasticity
RS5405	16,88372093	Day 1	8,555725742	43	*Novosphingobium*	8,76315975	on *E. coli* highly Eu	cost of plasticity
RS5405	80,76744186	Day 2	16,65595448	43	*Novosphingobium*	41,9207353	on *E. coli* highly Eu	cost of plasticity
RS5405	77,34146341	Day 3	20,45382126	43	*Novosphingobium*	40,1425493	on *E. coli* highly Eu	cost of plasticity
RS5405	16,27906977	Day 4	13,30471735	43	*Novosphingobium*	8,44932759	on *E. coli* highly Eu	cost of plasticity
RS5405	1,395348837	Day 5	3,193293082	43	*Novosphingobium*	0,72422808	on *E. coli* highly Eu	cost of plasticity

Supplementary Table S3

Strain	Total count	J1(eggs)	J2	J3	J4	Young Adult (YA)	Adult with eggs(BA)	Number of Mothers	%J1(eggs)	%J2	%J3	% J4	%YA	%BA	Condition
RSC017	70	0	1	0	8	61	0	9	0	1,428571	0	11,42857	87,14286	0	*E. coli*
RSC017	72	0	0	3	6	63	0	9	0	0	4,166667	8,333333	87,5	0	*E. coli*
RSC017	66	1	1	1	8	55	0	10	1,515152	1,515152	1,515152	12,12121	83,33333	0	*E. coli*
RSC017	62	0	0	1	0	61	0	9	0	0	1,612903	0	98,3871	0	*E. coli*
RSC017	50	0	0	0	3	47	0	10	0	0	0	6	94	0	*E. coli*
RS5405	69	0	0	0	13	56	0	10	0	0	0	18,84058	81,15942	0	*E. coli*
RS5405	51	0	0	0	10	41	0	10	0	0	0	19,60784	80,39216	0	*E. coli*
RS5405	71	0	1	0	12	58	0	10	0	1,408451	0	16,90141	81,69014	0	*E. coli*
RS5405	56	0	1	0	7	48	0	10	0	1,785714	0	12,5	85,71429	0	*E. coli*
RS5405	59	0	0	1	6	52	0	10	0	0	1,694915	10,16949	88,13559	0	*E. coli*
RSC011	68	0	0	1	31	36	0	10	0	0	1,470588	45,58824	52,94118	0	*E. coli*
RSC011	98	0	1	1	33	63	0	9	0	1,020408	1,020408	33,67347	64,28571	0	*E. coli*
RSC011	41	0	0	0	21	20	0	10	0	0	0	51,21951	48,78049	0	*E. coli*
RSC011	56	0	0	0	17	39	0	9	0	0	0	30,35714	69,64286	0	*E. coli*
RSA076	50	0	1	1	38	10	0	10	0	2	2	76	20	0	*E. coli*
RSA076	59	0	1	4	44	10	0	9	0	1,694915	6,779661	74,57627	16,94915	0	*E. coli*
RSA076	70	0	0	1	53	16	0	9	0	0	1,428571	75,71429	22,85714	0	*E. coli*
RSA076	53	0	0	0	26	27	0	9	0	0	0	49,0566	50,9434	0	*E. coli*
RSC017	72	0	0	0	5	55	12	10	0	0	0	6,944444	76,38889	16,66667	*Novosphingobium*
RSC017	87	0	0	0	2	67	18	10	0	0	0	2,298851	77,01149	20,68966	*Novosphingobium*
RSC017	65	0	0	0	6	40	19	9	0	0	0	9,230769	61,53846	29,23077	*Novosphingobium*
RSC017	77	0	0	2	3	47	25	10	0	0	2,597403	3,896104	61,03896	32,46753	*Novosphingobium*
RS5405	124	0	0	0	3	46	75	10	0	0	0	2,419355	37,09677	60,48387	*Novosphingobium*
RS5405	131	0	0	1	1	21	108	10	0	0	0,763359	0,763359	16,03053	82,44275	*Novosphingobium*
RS5405	126	0	0	0	0	13	113	10	0	0	0	0	10,31746	89,68254	*Novosphingobium*
RS5405	108	0	0	0	4	12	92	10	0	0	0	3,703704	11,11111	85,18519	*Novosphingobium*

Supplementary Table S4

Strain	Condition	Number of Eu animals	Total number of animals counted	%Eu	Expirmental setup
RSA133	*E. coli*	28	47	59,57447	intra-genotype cost of phenotype
RSA133	*E. coli*	63	93	67,74194	intra-genotype cost of phenotype
RSA133	*E. coli*	67	103	65,04854	intra-genotype cost of phenotype
RSD029	*E. coli*	33	47	70,21277	intra-genotype cost of phenotype
RSD029	*E. coli*	38	71	53,52113	intra-genotype cost of phenotype
RSD029	*E. coli*	40	70	57,14286	intra-genotype cost of phenotype
RSC033	*E. coli*	32	45	71,11111	intra-genotype cost of phenotype
RSC033	*E. coli*	47	58	81,03448	intra-genotype cost of phenotype
RSC033	*E. coli*	40	54	74,07407	intra-genotype cost of phenotype
RSC019	*E. coli*	33	50	66	intra-genotype cost of phenotype
RSC019	*E. coli*	30	50	60	intra-genotype cost of phenotype
RSC019	*E. coli*	23	50	46	intra-genotype cost of phenotype
RS5348	*E. coli*	30	53	56,60377	intra-genotype cost of phenotype
RS5348	*E. coli*	20	35	57,14286	intra-genotype cost of phenotype
RS5348	*E. coli*	30	50	60	intra-genotype cost of phenotype
RSA645	*E. coli*	57	111	51,35135	intra-genotype cost of phenotype
RSA645	*E. coli*	34	50	68	intra-genotype cost of phenotype
RSA645	*E. coli*	26	50	52	intra-genotype cost of phenotype
RSA622	*E. coli*	32	116	27,58621	intra-genotype cost of phenotype
RSA622	*E. coli*	96	143	67,13287	intra-genotype cost of phenotype
RSA622	*E. coli*	16	30	53,33333	intra-genotype cost of phenotype
RSC017	*E. coli*	1	50	2	inter-genotype cost of phenotype
RSC017	*E. coli*	5	50	10	inter-genotype cost of phenotype
RSC017	*E. coli*	1	50	2	inter-genotype cost of phenotype
RS5405	*E. coli*	50	50	100	inter-genotype cost of phenotype
RS5405	*E. coli*	50	50	100	inter-genotype cost of phenotype
RS5405	*E. coli*	50	50	100	inter-genotype cost of phenotype
RSC011	*E. coli*	10	50	20	inter-genotype cost of phenotype
RSC011	*E. coli*	16	50	32	inter-genotype cost of phenotype
RSC011	*E. coli*	24	50	48	inter-genotype cost of phenotype
RSA076	*E. coli*	50	50	100	inter-genotype cost of phenotype
RSA076	*E. coli*	50	50	100	inter-genotype cost of phenotype
RSA076	*E. coli*	50	50	100	inter-genotype cost of phenotype
RSA619	*E. coli*	49	50	98	Predation assay
RSA619	*E. coli*	50	50	100	Predation assay
RSA619	*E. coli*	47	50	94	Predation assay
RSA639	*E. coli*	50	50	100	Predation assay
RSA639	*E. coli*	50	50	100	Predation assay
RSA639	*E. coli*	50	50	100	Predation assay
RSA635	*E. coli*	45	50	90	Predation assay
RSA635	*E. coli*	34	50	68	Predation assay
RSA635	*E. coli*	30	50	60	Predation assay
RS5200	*E. coli*	7	50	14	Predation assay
RS5200	*E. coli*	12	50	24	Predation assay
RS5200	*E. coli*	2	50	4	Predation assay
RSC017	*Novosphingobium*	44	50	88	cost of plasticity
RSC017	*Novosphingobium*	47	50	94	cost of plasticity
RSC017	*Novosphingobium*	42	50	84	cost of plasticity
RS5405	*Novosphingobium*	50	50	100	cost of plasticity
RS5405	*Novosphingobium*	50	50	100	cost of plasticity
RS5405	*Novosphingobium*	50	50	100	cost of plasticity

Supplementary Table S5

Prey	Predator	Corpses	Condition	Experimental setup
C. elegans (N2)	*P. Pacificus* (RSA076)	28	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSA076)	31	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSA076)	49	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSC011)	8	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSC011)	8	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSC011)	1	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RS5405)	32	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RS5405)	36	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RS5405)	25	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSC017)	0	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSC017)	0	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSC017)	0	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSC019)	0	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSC019)	11	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSC019)	3	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSA639)	55	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSA639)	64	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSA639)	22	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RS5200)	0	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RS5200)	3	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RS5200)	0	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSA635)	0	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSA635)	7	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSA635)	8	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSA619)	17	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSA619)	22	*E. coli*	inter-specific assay
C. elegans (N2)	*P. Pacificus* (RSA619)	40	*E. coli*	inter-specific assay
RSC017	RSC017	0	*E. coli*	intra-specific assay
RSC017	RSC017	0	*E. coli*	intra-specific assay
RSC017	RSC017	0	*E. coli*	intra-specific assay
RSC017	RSC017	0	*E. coli*	intra-specific assay
RSC017	RSC017	0	*E. coli*	intra-specific assay
RSC017	RS5405	443	*E. coli*	intra-specific assay
RSC017	RS5405	539	*E. coli*	intra-specific assay
RSC017	RS5405	340	*E. coli*	intra-specific assay
RSC017	RS5405	496	*E. coli*	intra-specific assay
RSC017	RS5405	366	*E. coli*	intra-specific assay
RS5405	RSC017	7	*E. coli*	intra-specific assay
RS5405	RSC017	4	*E. coli*	intra-specific assay
RS5405	RSC017	10	*E. coli*	intra-specific assay
RS5405	RSC017	0	*E. coli*	intra-specific assay
RS5405	RSC017	3	*E. coli*	intra-specific assay
RS5405	RS5405	0	*E. coli*	intra-specific assay
RS5405	RS5405	0	*E. coli*	intra-specific assay
RS5405	RS5405	0	*E. coli*	intra-specific assay
RS5405	RS5405	0	*E. coli*	intra-specific assay
RS5405	RS5405	1	*E. coli*	intra-specific assay
RSC017	RSC017	0	*Novosphingobium*	intra-specific assay
RSC017	RSC017	0	*Novosphingobium*	intra-specific assay
RSC017	RSC017	0	*Novosphingobium*	intra-specific assay
RSC017	RSC017	0	*Novosphingobium*	intra-specific assay
RSC017	RSC017	0	*Novosphingobium*	intra-specific assay
RSC017	RS5405	587	*Novosphingobium*	intra-specific assay
RSC017	RS5405	691	*Novosphingobium*	intra-specific assay
RSC017	RS5405	720	*Novosphingobium*	intra-specific assay
RSC017	RS5405	530	*Novosphingobium*	intra-specific assay
RSC017	RS5405	513	*Novosphingobium*	intra-specific assay
RS5405	RSC017	102	*Novosphingobium*	intra-specific assay
RS5405	RSC017	90	*Novosphingobium*	intra-specific assay
RS5405	RSC017	42	*Novosphingobium*	intra-specific assay
RS5405	RSC017	87	*Novosphingobium*	intra-specific assay
RS5405	RSC017	91	*Novosphingobium*	intra-specific assay
RS5405	RS5405	0	*Novosphingobium*	intra-specific assay
RS5405	RS5405	0	*Novosphingobium*	intra-specific assay
RS5405	RS5405	0	*Novosphingobium*	intra-specific assay
RS5405	RS5405	0	*Novosphingobium*	intra-specific assay
RS5405	RS5405	0	*Novosphingobium*	intra-specific assay

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5 # High nutritional conditions influence feeding plasticity in
6 # *Pristionchus pacificus* and render worms non-predatory
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9
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22 **Abstract**

23 Developmental plasticity, the ability of a genotype to produce different phenotypes in response
24 to environmental conditions, has been subject to intense studies in the last four decades. The
25 self-fertilizing nematode *Pristionchus pacificus* has been developed as a genetic model
26 system for studying developmental plasticity due to its mouth-form polyphenism that results in
27 alternative feeding strategies with a facultative predatory and a non-predatory mouth form.
28 Many studies linked molecular aspects of the regulation of mouth-form polyphenism with
29 investigations of its evolutionary and ecological significance. Also, several environmental
30 factors influencing *P. pacificus* feeding structure expression were identified including
31 temperature, culture condition and population density. However, the nutritional plasticity of the
32 mouth form has never been properly investigated although polyphenisms are known to be
33 influenced by changes in nutritional conditions. For instance, studies in eusocial insects and
34 scarab beetles have provided significant mechanistic insights into the nutritional regulation of
35 polyphenisms but also other forms of plasticity. Here, we study the influence of nutrition on
36 mouth-form polyphenism in *P. pacificus* through experiments with monosaccharide and fatty
37 acid supplementation. We show that in particular glucose supplementation renders worms
38 non-predatory. Subsequent transcriptomic and mutant analyses indicate that *de novo* fatty
39 acid synthesis and peroxisomal beta-oxidation pathways play an important role in the
40 mediation of this plastic response. Finally, the analysis of fitness consequences through
41 fecundity counts suggests that non-predatory animals have an advantage over predatory
42 animals grown in the glucose-supplemented condition.
43

44 Keywords: *Pristionchus pacificus*, developmental plasticity, polyphenism, nutrition, glucose,
45 fatty acids
46

47 **Research highlights**

48 This study represents the first systematic attempt to investigate the influence of nutrition on
49 mouth-form polyphenism in the genetic model organism *Pristionchus pacificus*. Through
50 glucose and oleic acid supplementation we show that high nutritional conditions influence
51 feeding plasticity and render worms non-predatory. Mutant analysis indicates a role of *de novo*
52 fatty acid synthesis and peroxisomal beta-oxidation pathways for these responses.
53

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54 **Introduction**

55 Developmental plasticity allows organisms to alter their developmental trajectory to execute
56 distinct phenotypes in response to environmental cues with polyphenisms representing the
57 most remarkable form of plasticity by exhibiting environment-sensitive alternative phenotypes
58 without intermediate forms (West-Eberhard, 2003). In general, developmentally plastic
59 responses can be observed at physiological, morphological, and behavioural levels, including
60 life history traits of organisms. Many case studies have shown developmental plasticity to be
61 ubiquitous in nature. It has been suggested to facilitate adaptation to novel or heterogenous
62 environments and to play an important role for evolutionary diversification and the creation of
63 novelty (Ghalambor et al., 2007; Pigliucci, 2001; West-Eberhard, 2003). Multiple
64 environmental factors have been shown to influence polyphenisms including temperature,
65 seasonal changes, and population density (see reviews; Simpson et al., 2011; Yang &
66 Pospisilik, 2019). Specifically, nutrition is an important regulator of polyphenisms. Several
67 studies in insects have highlighted the importance of nutritional conditions during development
68 for the generation of discrete phenotypes, revealing genetic and epigenetic mechanisms
69 involved (Casasa et al., 2020; Chandra et al., 2018; Gotoh et al., 2014; Kamakura, 2011;
70 Kucharski et al., 2008). For instance, caste polyphenism in honeybee and horn polyphenism
71 in dung beetles represent some of the extreme cases of nutrition-induced developmental
72 plasticity (Emlen, 1997; Haydak, 1970; Moczek, 1998). Nonetheless, our understanding of
73 how nutrition affects the expression of polyphenic traits in other taxa remains limited.

74 Besides insects, nematodes provided substantial mechanistic insights into the
75 regulation of polyphenisms in the last decade. In particular, the free-living hermaphrodite
76 *Pristionchus pacificus* has been a model system for the studies of developmental plasticity.
77 Research in *P. pacificus* has provided insights into the genetic and epigenetic regulation as
78 well as the ecological and evolutionary significance of plasticity (Schroeder, 2021; Sommer,
79 2020). In nature, *P. pacificus* can be found in soil and on scarab beetles (chafers, stag beetles,
80 dung beetles), which, after their death, provide a nutritious environment for the nematode
81 through proliferation of bacteria and fungi on the carcass (Herrmann et al., 2006; Meyer et al.,
82 2017; Ragsdale et al., 2015; Renahan et al., 2021; Renahan & Sommer, 2022). In contrast to
83 *Caenorhabditis elegans* and most other free-living nematodes, *P. pacificus* shows a
84 remarkable mouth-form dimorphism, which influences its dietary niche and behaviour. During
85 postembryonic development, genetically identical *P. pacificus* worms form one of two,
86 alternative morphs, the so-called eurystomatous (Eu) and stenostomatous (St) mouth forms
87 (Figure 1a) (Bento et al., 2010). While the Eu morph enables facultative predatory feeding on
88 other nematodes by exhibiting two “teeth” and a wide mouth, the St morph leads to strict
89 microbial feeding and forms only one tooth and a narrow mouth (Theska et al., 2020). Major

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90 components of the gene regulatory network that govern mouth-form plasticity have been
91 identified in this model (Bui et al., 2018; Casasa et al., 2023; Levis & Ragsdale, 2023; Namdeo
92 et al., 2018; Ragsdale et al., 2013; Serobyan et al., 2016; Sieriebriennikov et al., 2018, 2020;
93 Werner et al., 2023). For instance, a supergene locus with the sulfatase *eud-1* and two alpha-
94 N-acetylglucosaminidase genes (*nag-1* and *nag-2*), as well as the sulfotransferase, *seud-*
95 *1*/*sult-1*, act as part of a main switch in mouth-form determination (Bui et al., 2018; Namdeo
96 et al., 2018; Ragsdale et al., 2013; Sieriebriennikov et al., 2018). They are involved in the
97 regulation of the evolutionarily conserved nuclear hormone receptors *nhr-40* and *nhr-1* that
98 have been co-opted for the regulation of mouth-form plasticity and act downstream of *eud-1*
99 and *seud-1/sult-1* (Sieriebriennikov et al., 2020; Theska & Sommer, 2024). It is important to
100 note that the understanding of the molecular regulation of feeding structure polyphenism is far
101 from completion with many epigenetic aspects still to be identified (Brown et al., 2024; Werner
102 et al., 2023).

103 Mouth-form plasticity in *P. pacificus* is influenced by several environmental factors such
104 as culture condition, population density, and temperature (Lenuzzi et al., 2023; Werner et al.,
105 2017, 2018). Previous studies have also revealed prominent roles for nematode metabolites
106 (e.g. ascarosides) and dietary bacteria in the mouth-form decision and predatory feeding
107 (Akduman et al., 2020; Bose et al., 2012; Dardiry et al., 2023). In contrast, our knowledge of
108 how changes to the nutritional status of worms affect mouth-form plasticity is still scarce. Bento
109 et al. (2010) have shown that starvation promotes the development of the Eu morph. More
110 recent genetic work by Casasa and co-workers identified the Mediator subunit MDT15/MED15
111 to regulate mouth-form polyphenism, which is known to have a conserved role in metabolic
112 responses to nutritional stress (Casasa et al., 2023). These observations suggests that mouth-
113 form plasticity is sensitive to nutritional conditions in particular to low nutrition. However,
114 mouth-form and fitness consequences of a high nutritional condition have never been
115 investigated in *P. pacificus*. One approach of studying the effect of nutrition on nematode
116 plasticity is to introduce supplements, such as monosaccharides and fatty acids, into the
117 standard diet to increase fat storage in nematodes, which is a strong alteration to the nutritional
118 status. This aided research in *C. elegans* to discover novel functions of molecular factors
119 involved in lipid metabolism and associated biological processes (Alcántar-Fernández et al.,
120 2018; Han et al., 2017; Lee et al., 2015; Nomura et al., 2010; Wan et al., 2022). Note that
121 while nutrient supplementation is well established in *C. elegans*, the role of direct and indirect
122 effects of supplementation through the bacterial diet cannot be disentangled. Very likely, the
123 effects observed in such experiments are due to both direct and indirect uptake of nutrients.
124 Moreover, nutrient supplementation studies have prioritised investigating lipid storage as an
125 indicator of nutritional status. Nematodes lack an adipose tissue for lipid storage; however,
126 they generate lipid droplets in many cells including the intestine to serve this function. In

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127 addition, storage lipids can be visualised through staining methods such as Oil Red O (ORO),
128 which is a reliable post-fix technique, providing a proxy for lipid storage when it is quantitatively
129 measured (O'Rourke et al., 2009).

130 Here, we study the influence of nutrition on mouth-form plasticity in *P. pacificus* by
131 establishing experimental setups in which worms are grown in "high nutrition" dietary
132 conditions that promote fat storage. These conditions are obtained through supplementation
133 of monosaccharides and fatty acids (mainly glucose and oleic acid) into the nematode growth
134 medium (NGM) (Figure 1b). We also examine the lipid storage in worms via ORO staining and
135 imaging analysis. We show that fat storage-promoting conditions, in particular glucose
136 supplementation, render worms non-predatory. Similarly, we test whether these dietary effects
137 are mediated transgenerationally but find no such influence. In addition, we carry out
138 transcriptomic and subsequent mutant analyses to explore associated molecular
139 mechanisms. Results indicate that *de novo* fatty acid synthesis and peroxisomal beta-
140 oxidation pathways, which are involved in storage and utilisation of lipids, are essential for
141 nutrition-induced mouth-form plasticity. Finally, we examine worm fitness by using fecundity
142 as proxy to explore whether the mouth-form response in the glucose-supplemented dietary
143 condition benefit *P. pacificus*. Our findings suggest that non-predatory animals have a fitness
144 advantage over predatory animals grown under the same nutritional condition. Overall, this
145 work establishes nutritional status as an additional factor influencing mouth-form polyphenism
146 and highlights the beneficial advantage of this plastic ability in *P. pacificus*.
147

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148 ## Materials and methods

149 ### Nematode stock maintenance

150 All *P. pacificus* strains were cultured on 6cm NGM plates with 300µl *E. coli* OP50 provided as
151 food at 20°C as previously described (Sommer et al., 1996). The wild type strain PS312 was
152 used for experimentation unless otherwise noted. Stocks were maintained by passing three
153 young adult hermaphrodites with eggs every five days. Mutant *P. pacificus* strains were
154 similarly cultured and are available from the SommerLab.

155 ### Monosaccharide supplementation experiments

156 Monosaccharides were purchased from Sigma-Aldrich as follows: glucose (D-(+)-Dextrose,
157 product number: D9434), galactose (D-(+)-Galactose, product number: G0750), fructose (D-
158 (−)-Fructose, product number: F0127). All monosaccharides were dissolved in purified and
159 autoclaved water to obtain 1M stock concentrations. Stock solutions were filtered through
160 0.22µm sterile filters before storage. Plate pouring was carried out under a sterile hood. Each
161 monosaccharide stock was diluted into liquid NGM media to obtain desired final
162 concentrations. Monosaccharide-added NGM was then stirred for approximately 1 minute to
163 obtain a homogenous media. Media was then poured into 6cm plates using a sterile 10ml
164 serological pipette, obtaining a volume of 9ml in each plate. Control plates with the same
165 volume were poured from the main NGM media without monosaccharides. Plates were
166 allowed to dry for 2 days and were then seeded with 300µl overnight grown *E. coli* OP50. After
167 two days, three young hermaphrodites with eggs (from stocks grown under standard
168 conditions) were added onto each plate for each strain, condition, and replicate. After adding
169 worms, plates were incubated at 20°C until F1 adults emerged. Plates were then used for
170 mouth-form phenotyping and ORO staining.

171 ### Fatty acid supplementation experiments

172 Fatty acid supplementation was carried out as described by Deline et al. (2013); however,
173 cholesterol was added into the NGM media after autoclaving. Sodium oleate (Sigma-Aldrich,
174 product number: O7501) and sodium linoleate (Sigma-Aldrich, product number: L8134) were
175 used for oleic acid and linoleic acid supplementation, respectively. For each experiment, a
176 0.1M fatty acid working stock was prepared fresh in purified and autoclaved water. Once
177 completely dissolved, the fatty acid working stocks were purged with nitrogen gas to prevent
178 oxidation by air. Plate pouring was carried out as in monosaccharide supplementation except
179 the volume of the NGM in each plate was 8ml. Both supplemented and control plates
180 contained 0.1% Tergitol (NP-40, Sigma-Aldrich, product number: NP40S) to facilitate fatty acid
181 absorption. Once poured, plates were covered with a box to avoid light oxidation. The same

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182 steps as in monosaccharide supplementation were followed for seeding the plates with *E. coli*
183 OP50, culturing nematodes, mouth-form phenotyping and ORO staining.

184 **Mouth-form phenotyping**

185 Mouth-form phenotypes were scored using Zeiss Discovery V.20 stereomicroscope based on
186 mouth-width of individual worms as previously described (Ragsdale et al., 2013; Theska et al.,
187 2020). Each plate was taken as a biological replicate and 30 worms were scored for their
188 mouth-form, unless otherwise mentioned. The percentage of Eu animals of each plate was
189 then calculated. Mouth-form percentage (Eu%) is graphed as a mean of percentages of all
190 plates for each strain in a particular dietary condition.

191 **Transgenerational experiments**

192 For testing potential transgenerational effects, lines were established by picking three young
193 (day 1) adult worms with eggs from standard condition onto oleic acid, glucose, and control
194 plates, respectively. Ten lines were established for each condition and each line was
195 propagated by transferring three young adult worms for five generations on the same
196 condition. In case of contamination, the line was discontinued and not used for counting mouth
197 form or reversal. At every generation, 6-7 lines (among the already established 10 lines) were
198 selected and reverted back to standard culture conditions. For each condition, mouth-form
199 phenotypes were scored for all plates at every generation and reversal.

200 **Oil Red O staining**

201 Post-fix ORO staining method was modified from Li et al. (2016). Oil Red O stock solution was
202 prepared by dissolving 0.5g ORO (Sigma-Aldrich, product number: O0625) in 100ml
203 isopropanol. This stock solution was shaken on a see-saw rocker for several days. To prepare
204 ORO working solution, required amount was taken from the stock and centrifuged for 5
205 minutes at 4500rpm to pellet all ORO-related precipitates. Working ORO solution was
206 prepared fresh by diluting precipitate-free ORO (supernatant) in purified water to obtain 60%
207 ORO. This solution was also shaken on a see-saw rocker for 10 minutes and centrifuged for
208 5 minutes at 4500rpm before staining worms. For worm fixation, a 1% formaldehyde solution
209 was used by diluting 1ml 16% formaldehyde solution (Thermo Scientific, catalogue number:
210 28906) in 15ml 1X PBS (phosphate buffered saline). For dehydrating worms, 60% isopropanol
211 was prepared fresh by diluting isopropanol in purified water. First, worms were washed off the
212 plates with 1X PBS and collected in a 15ml Falcon tube for each sample. Note that, after
213 mouth-form phenotyping, several biological replicates (plates) were washed into one sample
214 tube for each strain for a particular dietary condition. Once worms formed a sediment, extra
215 volume was removed, leaving only 1ml. This volume containing worms was then transferred

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216 to a new 1.5ml tube. To pellet worms after transfer or wash, samples were briefly centrifuged
217 in a quick-spin centrifuge. Worms were fixed with 1% formaldehyde (1ml) for 30 minutes by
218 shaking on a see-saw rocker. Then, samples were frozen in liquid nitrogen (~8 seconds) and
219 thawed under running tap water, three times in total. Samples were washed with 1X PBS three
220 times and were dehydrated in 60% isopropanol (1ml) for 2 minutes. Finally, samples were
221 stained with 0.5ml 60% ORO working solution for 30 minutes on a see-saw rocker. The
222 staining solution was applied through a 0.22μm sterile filter. After staining, samples were
223 washed three times with 1X PBS. Supernatant of each sample was removed, leaving
224 approximately 0.1ml sample of stained worms. Two drops of Vectashield (Biozol, catalogue
225 number: H-1000) was then added into each sample. Samples were gently mixed by pipetting
226 up and down before mounting onto microscope slides. Samples were then imaged for ORO
227 quantification.

228 **ORO quantification and analysis**

229 Oil Red O quantification was performed as described in Feldhaus and Piskobulu (2024
230 preprint). Briefly, we imaged worms on an AxioImager.Z1 (Zeiss, Oberkochen, Germany) with
231 a 10x/0.3 objective and an AxioCam 506 mono. For ORO quantification, images were obtained
232 with filter sets for DAPI and Texas Red in transmission mode. The filter-based imaging with a
233 monochrome camera was chosen to remove any potential bias from white balance settings of
234 the camera. Oil Red O absorbance per worm was semi-automatically calculated by manually
235 outlining the worm area and then obtaining the difference between the Texas Red channel
236 (where ORO appears transparent) and the DAPI channel (where ORO absorbs) after
237 correcting for differences in the spectral response for both channels. In total, 15 adult worms
238 were quantified per strain and condition. For each worm, raw integrated density (RawIntDen),
239 from channel 4 of the processed image, was taken as a measure of ORO absorbance. To
240 compare individuals with different body size (e.g. mutants), ORO absorbance (RawIntDen)
241 was normalised by the body area (μm^2) for each worm (RawIntDen/body area). For calculating
242 body area, images of worms from the blue channel (DAPI) were saved in grayscale. The body
243 area was then calculated for each worm by “Threshold” and “Analyze Particles” functions on
244 Fiji (Schindelin et al., 2012). Therefore, the final ORO absorbance value was determined as
245 RawIntDen divided by body area for each worm.

246 **RNA extraction**

247 For RNA extraction, three young adults with eggs, from standard condition, were placed on
248 oleic acid, glucose, and control conditions, respectively. For each condition, we scanned
249 through 5-9 plates and collected 150 F1 young adults without eggs in two separate tubes for
250 RNA extraction (two technical replicates). RNA extraction was carried out using Direct-zol RNA

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251 Microprep Kit (Zymo Research, R2060). Quality of RNA was assessed using NanoDrop.
252 Concentration of RNA was assessed using Qubit 2.0 Fluorometer (Invitrogen). Samples were
253 then diluted to required concentration and sent to NovoGene Co., Ltd for sequencing.

254 **RNA-seq and KEGG enrichment analysis**

255 Raw reads were aligned to the reference *P. pacificus* genome (El Paco) with STAR (version
256 2.7.1a) and quantified with featureCounts from the Subread R package (version 2.0.1) based
257 on the latest gene annotations (Athanasouli et al., 2020; Dobin et al., 2013; Liao et al., 2014).
258 The count matrix was filtered by removing genes with less than 10 reads, reducing the number
259 of genes to be examined from 28896 to 18174 and 18049 for oleic acid and glucose
260 respectively. Differential gene expression analysis was performed for each of the two
261 conditions with DESeq2 (FDR-corrected $P<0.05$, version 1.42.0) (Love et al., 2014). The
262 differentially expressed genes from each condition were tested for overrepresentation of
263 KEGG pathways using Fisher's exact-test with multiple testing correction (Bonferroni
264 corrected $P<0.05$) in R (version 4.3.1), based on the existing KEGG annotations for the *P.*
265 *pacificus* genes (Athanasouli et al., 2023).

266 **Phylogenetic tree**

267 All *C. elegans* fatty acid desaturase protein sequences were obtained from WormBase
268 (www.wormbase.org). Longer isoforms were picked for genes with different isoforms. Each *C.*
269 *elegans* fatty acid desaturase protein was then blasted against *P. pacificus* on the protein
270 database (www.pristionchus.org, El Paco annotation v3, 2020). From each blast result, all *P.*
271 *pacificus* hits were collected and analysed on InterProScan (www.ebi.ac.uk) for conserved
272 protein domains. We found two genes (PPA03289 and PPA05783) which did not contain
273 desaturase domains, which were discarded. The rest of the protein sequences were aligned
274 by ClustalW on MEGA11 software (Tamura et al., 2021). Based on this alignment, a maximum
275 likelihood phylogenetic tree was constructed with LG model, and 100 bootstrap replications.
276 The phylogenetic tree was visualised and edited on FigTree software (version 1.4.4).

277 **CRISPR/Cas9 mutagenesis and identification of mutants**

278 For the generation of mutants by CRISPR, the protocols of Witte et al. (2015) and Han et al.
279 (2020) were followed with minor modifications. All components of the CRISPR/Cas9 complex
280 were purchased from Integrated DNA Technologies (IDT). Each CRISPR RNA (crRNA) was
281 designed to target 20bp upstream of the protospacer adjacent motif (PAM). To construct
282 CRISPR/Cas9 complex, 5µl CRISPR RNA (crRNA) from 100µM stock was mixed with 5µl
283 trans-activating CRISPR RNA (tracrRNA) obtained from 100µM stock (catalogue number
284 1072534). This mixture was first denatured at 95°C for 5 minutes, and then cooled down at

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285 room temperature for annealing (5 minutes). Hybridized crRNA:tracrRNA product (5μl) was
286 mixed with 1μl Cas9 protein from 62μM stock (catalogue number: 1081058) and incubated at
287 room temperature for 5 minutes. Tris-EDTA buffer was then added into the CRISPR/Cas9 mix
288 to obtain a final concentration of 18.1μM for crRNA:tracrRNA hybrid and 2.5μM for Cas9. Co-
289 injection marker, with *Ppa-eft-3* promoter and TurboRFP sequences, was also integrated into
290 the injection mix (Han et al., 2020). Injected worms were allowed to lay eggs for 24 hours.
291 Fluorescent marker-positive plates were used to isolate emerging F1 progeny. From each
292 positive plate, 8 -10 F1 worms were isolated. Each F1 worm was allowed to lay eggs and then
293 was lysed in single worm lysis buffer (10mM Tris–HCl at pH 8.3, 50mM KCl, 2.5mM MgCl2,
294 0.45% NP-40, 0.45% Tween 20, 120µg/ml Proteinase K) in a thermal cycler program with
295 65°C for 1 hour; 95°C for 10 minutes. Lysates were used to carry out polymerase chain
296 reactions to amplify the target region. Mutants in F1 generation (predominantly heterozygotes)
297 were first identified through Sanger sequencing (GENEWIZ Germany GmbH). Subsequent re-
298 isolation and sequencing of the following generation (F2) allowed capturing homozygous
299 mutants. All crRNAs, and related primers for target genes are provided in Table S1.

300 **Genetic repair of *Ppa-pddl-1(tu2028)***

301 To genetically revert *Ppa-pddl-1(tu2028)* back to wild type through CRISPR editing, a crRNA
302 (5'- TACTATCCTCTATCCTATAG-3') was designed targeting 20bp upstream of the TGG PAM
303 site at exon 4 based on the mutant sequence including 7bp insertion. A 100bp wild type repair
304 template, covering the target region, was also used (synthesised by IDT). The repair template
305 was as follows: 5'-
306 CATGAATAGTTCTAACCACTTCTTCTCCTTCAGACATTACTATCCTCTAGTGGCCCTTCTC
307 TGTTTCCTCATGCCAACTGTCGTTCCTGTCTATTATTGG-3'. The repair template was
308 integrated into the CRISPR injection mix at a concentration of 10μM. Homozygous wildtype
309 worms were isolated in F2 generation post injection, validated via sanger sequencing.

310 **Fecundity experiment**

311 To study fecundity of Eu and St morphs, young adult worms without eggs (F1) were isolated
312 from glucose-supplemented diets (80mM concentration) based on their mouth morph and
313 subsequently transferred to standard dietary conditions with 20μl *E. coli* OP50. Worms were
314 passed onto new plates for 8 consecutive days and were removed at the end of 8th day. After
315 five days from inoculation, viable progeny was counted on each plate.

316 **Data visualisation and statistical analysis**

317 All plots were generated in RStudio (version 1.2.5042) using ggplot2 and ggpubr packages.
318 Illustrations were produced in Affinity Designer (version 1.9.2). Statistical significance testing

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319 was carried out by comparing two independent groups, e.g., control vs. treatment, mutant vs.
320 wildtype, Eu vs. St, for ORO absorbance and total fecundity. Briefly, data was first tested for
321 normal distribution mostly via Shapiro-Wilk normality test with additional visual observations.
322 In case of normal distribution, either two sample t-test (equal variance) or Welch two sample
323 t-test (unequal variance) were used. In case of nonnormal distribution, Wilcoxon rank sum-
324 test was used as the nonparametric equivalent. All statistics were performed in RStudio
325 (version 1.2.5042).
326
327

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328 **Results**

329 **Monosaccharide and fatty acid supplementations render worms non-predatory in a**
330 **concentration dependent manner**

331 For all experiments, we studied hermaphrodites of the PS312 strain, the wild type of *P.*
332 *pacificus* that had its genome being sequenced at chromosome level (Dieterich et al., 2008;
333 Rödelsperger et al., 2017). This strain is highly Eu under standard lab conditions on an *E. coli*
334 OP50 diet with more than 95% of animals expressing the Eu morph. First, we performed pilot
335 experiments with monosaccharides ranging from 20mM to 300mM concentrations. Results
336 revealed a concentration dependent effect on mouth-form plasticity. Specifically, the number
337 of St worms increased with increasing concentrations of monosaccharides (Figure S1a).
338 Among all, we prioritised glucose as the main St-inducing monosaccharide and performed
339 further experiment by using 100mM and 150mM concentrations. Results indicated that
340 100mM glucose is sufficient to render worms highly St without exerting observable adverse
341 effects (Figure 2a). Note that even under glucose-supplementation conditions, there is still an
342 effect of population density on mouth-form expression as pilot experiments starting from 10
343 adult worms (Figure S1a) revealed higher Eu ratios than similar glucose concentration
344 experiments with three adult inoculations (Figure 2a). For fatty acid supplementations, we
345 used oleic acid (monounsaturated fatty acid) and linoleic acid (polyunsaturated fatty acid) to
346 test 0.5mM and 1mM concentrations. Similar to monosaccharide supplementations, oleic acid
347 effect on mouth-form plasticity was mediated in a concentration dependent manner (Figure
348 2a). Observations based on morphology and developmental pace of worms at both
349 concentrations suggested that 0.5mM oleic acid would be more ideal for further experiments.
350 Results also indicated that the same concentrations were detrimental for worms in the linoleic
351 acid condition. While worms did not populate the plates at 1mM, they were developmentally
352 slow and almost completely St at 0.5mM linoleic acid (Figure S1b). Therefore, we performed
353 another experiment for linoleic acid with reduced concentrations ranging from 0.01mM to
354 0.2mM. We found that 0.2mM linoleic acid renders worms predominantly St, alleviating the
355 detrimental effect observed at 0.5mM concentration (Figure S1c). Overall, these results show
356 that monosaccharides and fatty acid supplementations induce changes of the mouth-form
357 ratio towards St in *P. pacificus*. For further experiments, we selected oleic acid and glucose
358 as main supplements at 0.5mM and 100mM concentrations, respectively.

359 **Oleic acid and glucose diets do not induce a transgenerational effect on mouth-form**
360 **plasticity**

361 Some dietary and nutritional influences on nematodes are known to be transmitted
362 transgenerationally (Beltran et al., 2020). Therefore, we propagated cultures on oleic acid and

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363 glucose conditions for five generations to explore potential transgenerational effects of these
364 diets on mouth-form plasticity (Figure S2a,b). We found that worms exhibit similar mouth-form
365 ratios relative to their initial response (F1) through generations (Figure S2c,d). When we
366 reverted worms from each generation back to the regular dietary condition without
367 supplements, we did not observe any transgenerational memory of mouth form (Figure S2e,f).
368 Note that over the course of these experiments, we observed more consistent and less
369 variable mouth-form responses in glucose condition (highly St, on average below 20% Eu)
370 relative to oleic acid across different batches.

371 **Oleic acid and glucose supplementations induce fat storage**

372 Next, we performed ORO staining on worm populations reared on oleic acid and glucose diets
373 to visualise and quantify their lipid storage (Figure 1b). Relative to control groups, oleic acid-
374 and glucose-fed worms showed an increase in ORO absorbance which signifies an increase
375 in fat storage (Figure 2b). Whole-body images of stained animals also demonstrated the
376 distribution of storage lipids. Anterior regions of worms clearly indicated lipid accumulation in
377 both oleic acid and glucose conditions (Figure 2c). In particular the head region of the worm
378 allows easy visualization of the influence of supplementation on fat storage (Figure 2c inlets).
379 Thus, both tested supplements induce fat storage in addition to their effect on mouth-form
380 expression.

381 **Differential gene expression analysis points out pathways involved in lipogenesis and**
382 **lipolysis**

383 To understand which molecular factors are involved in mediating this nutrition-induced mouth-
384 form change, we carried out RNA-seq on worm populations reared in supplemented and
385 control conditions (Figure 3a). First, we performed differential gene expression analysis
386 between each supplement and its control, and then a KEGG enrichment analysis of
387 differentially expressed genes in both oleic acid and glucose conditions. KEGG enrichment
388 analysis revealed pathways involved in lipogenesis (“biosynthesis of unsaturated fatty acids”,
389 “fatty acid elongation”) and lipolysis (“peroxisome”, “fatty acid degradation”) (Figure 3b). This
390 suggested that worms do not only store but also actively utilise lipids in supplemented
391 conditions. Furthermore, we found genes which were upregulated and involved in peroxisomal
392 beta-oxidation and biosynthesis of unsaturated fatty acids. Therefore, we selected genes in
393 these pathways as candidates to investigate whether they mediate the effect of nutrient
394 supplementation on mouth-form expression. We utilised CRISPR gene editing technology to
395 introduce mutations in genetic components involved in both pathways as this method allows
396 easy manipulation in *P. pacificus* (Han et al., 2020; Witte et al., 2015). For these experiments,
397 we only used glucose-supplemented diet to induce fat storage and test responses of mutants

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398 because of the higher reproducibility of results obtained through glucose supplementation and
399 also the impracticality of fatty acid supplementations that require preventive measures from
400 oxidation by light and air (see Materials and methods for more details).

401 **Delta-9 desaturase activity is essential for a complete mouth-form response on**
402 **glucose-supplemented diet**

403 We targeted delta-9 fatty acid desaturases for studying the role of the biosynthesis of
404 unsaturated fatty acids and *dhs-28* and *daf-22* for investigating the peroxisomal beta-oxidation
405 pathway on nutritional plasticity of the *P. pacificus* mouth-form. Delta-9 desaturases facilitate
406 lipid storage by acting early in the *de novo* fatty acid synthesis pathway, converting saturated
407 fatty acids into monounsaturated fatty acids (Figure 4a). In *C. elegans,* for instance,
408 simultaneous inhibition of the function of two delta-9 desaturases (*fat-6* and *fat-7*) results in
409 the loss of endogenous unsaturated fatty acids and a significant reduction in lipid storage
410 (Brock et al., 2007). Delta-9 desaturases are activated by the transcription factor *sbp-1* (sterol
411 regulatory element binding protein). Like *C. elegans*, *P. pacificus* has a single *sbp-1* gene
412 (PPA37968), which is a one-to-one ortholog of *Cel-sbp-1*. First, we introduced frameshift
413 mutations in this gene to block the activity of delta-9 desaturases and explore its contribution
414 to mouth-form plasticity. However, homozygous mutants were sterile; therefore, we could not
415 further our investigation with this gene. Next, we specifically targeted individual delta-9
416 desaturases. Interestingly, this gene family has been amplified in *P. pacificus* relative to *C.*
417 *elegans* (Markov et al., 2015). We constructed a phylogenetic tree based on protein
418 sequences of all *P. pacificus* fatty acid desaturase domain-containing genes and related *C.*
419 *elegans* desaturases. We found a total of 10 *P. pacificus* genes which contain a delta-9 fatty
420 acid desaturase-like protein domain (Figure S3). Note that unlike for *sbp-1*, there is no one-
421 to-one orthology relationship between delta-9 fatty acid desaturase genes between *P.*
422 *pacificus* and *C. elegans*, therefore requiring a new nomenclature. Among these genes,
423 ppa_stranded_DN27845_c0_g3_i3, PPA1053, PPA40514, and
424 ppa_stranded_DN27845_c0_g2_i1 showed highest protein sequence similarity with *C.*
425 *elegans* delta-9 desaturases (*fat-5*, *fat-6*, and *fat-7*). In this order, we named these genes as
426 *Ppa-pddl-1* (*Pristionchus* delta-9 desaturase-like-1), *Ppa-pddl-2*, *Ppa-pddl-*3, and *Ppa-pddl-4*
427 (Figure 4b, Figure S3). Among all, *Ppa-pddl-1*, *Ppa-pddl-3*, and *Ppa-pddl-4* are expressed
428 throughout the development of *P. pacificus* based on previous gene expression analysis
429 (Baskaran et al., 2015). Therefore, we prioritised these three genes and introduced mutations
430 through CRISPR. We obtained three frameshift alleles for both *Ppa-pddl-3* (*tu2033*, *tu2034*,
431 *tu2035*) and *Ppa-pddl-4* (*tu2030*, *tu2031*, *tu2032*), and one frame shift allele (*tu2028*) and a
432 3bp insertion allele (*tu2029*) for *Ppa-pddl-1* (Table S2). Among these mutants, *tu2028* and

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433 *tu2029* were developmentally slow and had significantly reduced fat storage until late
434 adulthood under standard conditions.

435 Next, we assessed mouth-form responses of all mutants on glucose-supplemented
436 diets. Results revealed that the response of delta-9 desaturase mutants to glucose is generally
437 incomplete, that is, they have a higher Eu ratio than wild type animals (above 20% Eu) (Figure
438 4c). Compared to all other mutants, *Ppa-pddl-1* mutants were more consistent in their mouth-
439 form response to the glucose-supplemented diet (greater than 40% Eu on average) (Figure
440 4c). Therefore, we further studied *Ppa-pddl-1* using *tu2028* as reference allele. First, we
441 measured the lipid storage profile of *Ppa-pddl-1*(*tu2028)* on standard diet by ORO staining.
442 We found that *Ppa-pddl-1*(*tu2028)* exhibits significantly lower levels of ORO absorbance
443 relative to wild type (Figure S4a,b). To confirm that the response of *tu2028* to glucose-
444 supplemented diet is due to its mutational background in *Ppa-pddl-1* and not to other
445 mutations as a result of potential off target effects by CRISPR, we genetically reverted *Ppa-*
446 *pddl-1*(*tu2028)* back to wild type using CRISPR editing via a repair template. Growing wild
447 type, the repaired strain, and the original *Ppa-pddl-1*(*tu2028)* mutant on glucose-
448 supplemented diets revealed highly similar levels of mouth-form ratios between wild type and
449 repaired worms (Figure 4d). Specifically, both strains were highly St on glucose-supplemented
450 diets. Repaired and wild type strains also showed a similar trajectory of ORO absorbance
451 between control and glucose conditions (Figure 4e). Among all, *Ppa-pddl-1*(*tu2028)* had the
452 weakest ORO absorbance in both conditions (Figure 4e). In addition, we observed that *Ppa-*
453 *pddl-1*(*tu2028)* mutant animals restored their growth on glucose-supplemented diet and also
454 regained their wild type physiological and morphological characteristics upon genetic repair
455 (Figure 4f). Overall, these results highlight the significance of the function of *Ppa-pddl-1* in
456 both lipid storage and mouth-form plasticity.

457 **Peroxisomal beta-oxidation mutants fail to respond to glucose supplementation**

458 Next, we studied mutants of two peroxisomal beta-oxidation genes, *dhs-28* and *daf-22*. *P.*
459 *pacificus* has two copies of both genes in its genome (Markov et al., 2016) (Figure 5a). For
460 *Ppa-dhs-28.1*, we induced mutation in the copy with the sterol carrier protein domain
461 (PPA20393) and obtained two insertion and three deletion alleles (Table S2). All mutants
462 exhibited the same observable characteristics and were morphologically smaller and
463 developmentally slower relative to wild type animals. For *daf-22*, we used the available double
464 mutant *Ppa-daf-22.1 Ppa-daf-22.2* (Markov et al., 2016). Note that mutants of *Ppa-dhs-28.1*
465 were generated in the PS312 wild type background, whereas the *Ppa-daf-22.1 Ppa-daf-22.2*
466 double mutant was generated in RS2333, which is a highly related derivative of PS312 that
467 was used for several studies on dauer development (Falcke et al., 2018; Markov et al., 2016).
468 Therefore, we included both highly similar wild type strains in our experiments as independent

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469 controls. First, we assessed mouth-form responses of three *Ppa-dhs-28.1* mutant alleles
470 (*tu1855*, *tu1856*, and *tu1858*) and the *Ppa-daf-22.1 Ppa-daf-22.2* double mutant on glucose-
471 supplemented diets. Mutants of both genes had a consistently high Eu mouth-form ratio
472 between control and glucose conditions. Next, we simultaneously assessed mouth-form
473 plasticity and lipid storage of *Ppa-dhs-28.1(tu1855*) and the *Ppa-daf-22.1 Ppa-daf-22.2* double
474 mutant on glucose-supplemented diets. Relative to both wild type strains, mutants remained
475 highly predatory and showed no increase in ORO absorbance between control and glucose
476 conditions (Figure 5b,c). We also observed that glucose-supplemented peroxisomal beta-
477 oxidation mutants exhibit delayed development and reduced body size. Moreover, ORO-
478 stained peroxisomal beta-oxidation mutants show a disruption in lipid storage integrity on
479 glucose-supplemented diets (Figure 5d). They also accumulate larger lipid droplets relative to
480 wild type, indicating reduced lipid oxidation. Taken together, these results indicate that the
481 peroxisomal beta-oxidation pathway is required for the mouth-form response to glucose.

482 **Mouth-form plasticity switch genes are required for phenotypic response in glucose**
483 **diet**

484 Next, we tested whether the influence of glucose-supplemented diets on mouth-form plasticity
485 is mediated through the central gene regulatory network of mouth-form plasticity (Figure 6a).
486 For that, we utilised mutants of plasticity switch genes which are St-form defective.
487 Specifically, we used *sult-1(tu1061)* and the double mutant *nag-1(tu1142) nag-2(tu1143*) and
488 assessed their mouth form and lipid storage responses on glucose-supplemented diets. Both
489 mutants were unable to switch from Eu to St on glucose-supplemented diets, indicating that
490 the nutritional effect acts upstream of the plasticity switch module (Figure 6b). However,
491 plasticity switch mutants were able to increase their lipid storage when fed on glucose-
492 supplemented diets (Figure 6c). This suggests that the plasticity switch genes are essential
493 for mouth-form response but not for lipid storage.

494 **Non-predatory worms exhibit a fitness advantage over predatory ones**

495 Our final aim was to explore whether there is a fitness advantage of favouring the development
496 of one morph over the other in a fat storage-inducing condition. For this, we chose fecundity
497 as proxy for fitness to evaluate the hermaphrodites' reproductive success by counting viable
498 progeny produced by isolated individuals. First, we reduced the concentration of glucose from
499 100mM to 80mM to increase the likelihood of obtaining Eu animals. Then, we isolated animals
500 of both morphs grown in the glucose-supplemented diets and measured their fecundity on the
501 standard diet (Figure 7a). Results revealed that St worms on average exhibit higher daily and
502 total fecundity than Eu worms (Figure 7b,c). These findings suggest that promoting the

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503 development of the St morph under such a dietary influence confers a fitness advantage to *P.*
504 *pacificus*.

505 **Discussion**

506 This study highlights the significance of nutrition in mouth-form plasticity in *P. pacificus*. First,
507 we showed that fat storage-promoting conditions induce the non-predatory morph. This
508 suggests that growing in an “overly satiated” nutritional state increases the likelihood of worms
509 developing as non-predatory. These findings are consistent with previous studies, which
510 indicated that opposite dietary condition, *i.e.*, low nutrition, can lead to the predatory morph in
511 *P. pacificus* (Bento et al., 2010); and even a cannibalistic novel predatory mouth-form in
512 another diplogastrid nematode, *Allodiplogaster sudhausi* (Wighard et al., 2024). In addition,
513 results obtained from transgenerational experiments suggest that worms must constantly be
514 kept at overly satiated state during development to obtain and maintain a mouth-form
515 response, at least in the wild type *P. pacificus* PS312 strain.

516 Supplementing NGM agar plates with glucose and oleic acids has been an effective
517 method for delivering monosaccharides and fatty acids to worms via dietary ingestion
518 (Alcántar-Fernández et al., 2018; Deline et al., 2013; Nomura et al., 2010). However, it is
519 important to note that this method does not address any potential indirect effect caused by the
520 interaction between the supplement and the bacteria (Kingsley et al., 2021), which can be a
521 research interest on its own. In our experiments, we did not monitor fatty acid and glucose
522 uptake or content in worms, instead we utilised ORO staining, to monitor the outcome of
523 changes in lipid storage. Our results are concordant with previous findings. For instance,
524 glucose supplementation to NGM plates does not only result in the accumulation of glucose
525 but also triacylglycerols in *C. elegans* (Alcántar-Fernández et al., 2018). Oil Red O stains
526 neutral lipids, mainly triacylglycerols. Studies in *C. elegans* have shown an increase in ORO
527 levels in worms grown in oleic acid- and glucose-supplemented conditions (Choi et al., 2021;
528 Lee et al., 2015). Therefore, ORO does not only provide a way of measuring the nutritional
529 status of worms but also validate the uptake of supplementations. Moreover, our results
530 showed that glucose supplementation consistently induces the St morph in higher proportions
531 relative to oleic acid. Note that we could not rule out the cause of inconsistency and the high
532 variability of mouth-form ratios in oleic acid supplementations. Regardless, we selected
533 glucose-supplemented diet as the main fat storage-promoting condition to induce the non-
534 predatory morph.

535 Further, mutant analyses revealed that genes involved in lipogenesis and lipolysis play
536 a significant role in glucose supplementation-induced mouth-form plasticity. First, we showed
537 that delta-9 desaturase activity, particularly *Ppa-pddl-1*, is required for a complete mouth-form

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538 and lipid storage response. The spatial transcriptome data, published by Rödelsperger et al.
539 (2021), indicates that *Ppa-pddl-1* is enriched in anatomical regions that suggests expression
540 in the intestine. Inactivation of this gene causes a drastic reduction in lipid storage and
541 developmental rate; such phenotypic effect is obtained through simultaneous inhibition of *fat-*
542 *6* and *fat-7* in *C. elegans* (Brock et al., 2007). However, *Ppa-pddl-1* mutant still exhibits a
543 partial ability to respond to glucose-supplemented diet. This suggests that remaining functional
544 delta-9 desaturases may have compensated for the loss of *Ppa-pddl-1*. Therefore, further
545 functional characterization of delta-9 desaturases is required to completely elucidate potential
546 role of this gene family in mouth-form plasticity. Furthermore, growing peroxisomal beta-
547 oxidation mutants on glucose-supplemented diet revealed that these mutants have disrupted
548 lipid storage integrity, which resulted in a weaker ORO absorbance relative to wild type strains.
549 Additional observations in these mutants, such as developmental delay and reduction in body
550 size, suggest that they are not able to nutritionally benefit from the glucose-supplemented diet.
551 Taken together, these findings suggest that pathways associated with storage and utilisation
552 of lipids carry out essential metabolic processes, mediating mouth-form plasticity in a high
553 nutrition environment.
554 Additionally, our findings suggest that the dietary effect of glucose supplementation
555 acts upstream of the plasticity switch module. However, how this dietary effect is mediated to
556 influence downstream components, affecting mouth-form decision remains subject of future
557 research. Of note, *de novo* fatty acid synthesis and peroxisomal beta-oxidation pathways
558 produce signalling molecules which have diverse functions (Artyukhin et al., 2018; Watts,
559 2016). For example, polyunsaturated fatty acids produced by the *de novo* fatty acid synthesis
560 pathway can be integrated into storage lipids or processed further into signalling molecules
561 such as eicosanoids, which can function as ligands for transcription factors, affecting gene
562 expression. Cytochrome P450 enzymes are involved in the production of eicosanoids from
563 polyunsaturated fatty acids (Kulas et al., 2008) and their activity have been associated with
564 several biological functions such as development, dauer formation, and lipid metabolism in *C.*
565 *elegans* (for review, see Larigot et al., 2022). Cytochrome P450-related pathways are enriched
566 (KEGG) in our differential gene expression data for both oleic acid and glucose, suggesting a
567 potential role. Moreover, peroxisomal beta-oxidation pathway produces ascarosides,
568 extracellular signalling molecules which can influence dauer formation and mouth-form
569 plasticity (Butcher, 2017; Butcher et al., 2007; Markov et al., 2016; Werner et al., 2018). Taken
570 together, lipid-mediated signalling may have potential functions in nutrition-induced mouth-
571 form plasticity and therefore further research is required to elucidate associated mechanisms.
572 Finally, we sought to explore whether there is an adaptive value of facilitating the
573 development of the St morph in a high nutrition environment. In *P. pacificus*, while the Eu
574 morph exhibits a fitness advantage associated with its predatory ability (Serobyan et al., 2014),

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575 the St morph claims this advantage through a faster development and a higher fecundity
576 (Dardiry et al., 2023; Serobyan et al., 2013). In addition, highly non-predatory natural isolates
577 of *P. pacificus* have higher fecundity and exhibit faster development on standard dietary
578 condition relative to highly predatory strains, which were isolated from similar localities in
579 nature (Dardiry et al., 2023). Growing worms on glucose-supplemented diet allowed
580 separation of morphs; then assessing their fecundity revealed a fitness disadvantage for the
581 predatory mouth-form. Intriguingly, our findings suggest that glucose supplementation does
582 not offset the fitness cost of producing the Eu phenotype or promote its development to begin
583 with. Hence, the fitness advantage associated with the non-predatory morph suggests that
584 favouring its development, under such dietary effect, can be beneficial for the population.
585 In summary, this study signifies the nutritional sensitivity of mouth-form polyphenism
586 in *P. pacificus* and adds nutritional status as important environmental factor influencing mouth
587 form. We introduced glucose-supplementation as a novel environmental condition to induce
588 non-predatory morph. Our findings suggest that nutritional status of the worm can potentially
589 dictate its mouth-form fate. We also found a strong association between lipid metabolism and
590 mouth-form plasticity. Lastly, glucose-supplementation helped understand fitness
591 consequences of the mouth-form determination, indicating an advantage for the non-predatory
592 morph.
593

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599 **Competing interests**

600 No competing interests declared.

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604 **Data availability**

605 Raw Reads obtained from the RNA-seq were deposited in the European Nucleotide Archive
606 (ENA) under the accession number PRJEB76787.

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864 ## Figure Legends

865 **Figure 1. Mouth-form polyphenism of *P. pacificus*, and the experimental design for**
866 **supplementation studies.** (a) Differential interference contrast (DIC) images of the
867 eurystomatous (Eu) and the stenostomatous (St) mouth morphs. The Eu morph has a wide
868 buccal cavity, a hook-like dorsal tooth (on the left, outlined), and a sub-ventral tooth. The
869 narrow-mouthed stenostomatous (St) morph has a flint-shaped dorsal tooth (on the left,
870 outlined). Scale bar is 5μm. (b) Illustration of the general experimental design using
871 supplementation with monosaccharides and fatty acids.
872
873 **Figure 2. Effect of oleic acid and glucose supplementations on mouth-form plasticity**
874 **and lipid storage in *P. pacificus*.** (a) Concentration-dependent effect of oleic acid and
875 glucose on mouth-form plasticity. N ≥ 3 biological replicates per condition for each
876 concentration. Each faint data point represents a replicate (plate), with 30 animals per replicate
877 being scored for mouth-form percentage (Eu%). Error bars represent s.e.m. (b) Oil Red O
878 absorbance (RawIntDen/body area) obtained from worms grown in oleic acid, glucose, and
879 respective control conditions. N = 15 per condition. Each faint data point represents an
880 individual worm. P values are obtained from Welch two sample t-test and two sample t-test for
881 oleic acid and glucose comparisons, respectively. Bars represent mean values of all samples
882 in each condition. Error bars represent s.d. (c) Representative images of ORO-quantified
883 worms, indicating lipid storage profile. Images are acquired from the blue channel in grayscale.
884 Lipid droplets appear dark. Scale bar is 50μm.
885
886 **Figure 3. Transcriptome analysis.** (a) An illustration of RNA extraction. (b) Overrepresented
887 KEGG pathways of differentially expressed genes for oleic acid and glucose conditions.
888 Statistical analysis by Fisher's exact-test with multiple testing correction (Bonferroni corrected
889 $P<0.05$).
890
891 **Figure 4. Significance of delta-9 desaturase activity in glucose supplementation-**
892 **induced mouth-form plasticity.** (a) Schematic representation of the essential activity of
893 delta-9 desaturases, facilitating fat storage by converting saturated fatty acids into
894 monounsaturated fatty acids. Sterol regulatory element binding protein 1 (SBP-1) activates
895 delta-9 desaturases. (b) A simplified phylogenetic tree of *Pristionchus* delta-9 desaturase-like
896 genes (*Ppa-pddl-1*, *Ppa-pddl-2*, *Ppa-pddl-3*, *Ppa-pddl-4*), and *C. elegans* delta-9 desaturases
897 (*fat-5*, *fat-6*, and *fat-7*). Triangle denotes *P. pacificus* genes which are constitutively expressed
898 during development (Baskaran et al., 2015). The phylogenetic tree is drawn based on the tree
899 in Figure S3. (c) Eu percentages of CRISPR mutants of *Ppa-pddl-1*, *Ppa-pddl-3*, *Ppa-pddl-4*,

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900 and wild type animals in control and glucose conditions. N ≥ 2 biological replicates per strain
901 for each condition. Bars represent mean values of all replicates. Error bars represent s.d. (d)
902 Eu percentages of wild type, *tu2028*(*Ppa-pddl-1*), and CRISPR repaired-*tu2028* strains in
903 control and glucose conditions. N ≥ 3 biological replicates per strain for each condition. (c,d)
904 Each faint data point represents a replicate (plate), with 30 animals per plate being scored for
905 mouth-form percentage (Eu%). (e) ORO absorbance (RawIntDen/body area) obtained from
906 wild type, *tu2028*(*Ppa-pddl-1*), and CRISPR repaired-*tu2028* strains grown on control and
907 glucose conditions. N = 15 per strain for each condition. Each faint datapoint represents an
908 individual worm. (d,e) Error bars represent s.e.m. (f) Representative images of ORO-
909 quantified strains from control (-glucose) and glucose (+glucose) conditions. Images are
910 acquired from the blue channel in grayscale. Lipid droplets appear dark. Scale bar is 100μm.
911
912 **Figure 5. Response of peroxisomal beta-oxidation mutants to glucose-supplemented**
913 **diet.** (a) Schematic of peroxisomal beta-oxidation pathway, indicating duplicate copies in *P.*
914 *pacificus* for *dhs-28* and *daf-22* genes relative to *C. elegans*. (b) Eu percentages of
915 peroxisomal beta-oxidation mutants and wild type strains in control and glucose conditions. N
916 ≥ 2 biological replicate per strain for each condition. Each faint data point represents a
917 replicate (plate), with 30 animals per plate being scored for mouth-form percentage (Eu%). (c)
918 ORO absorbance (RawIntDen/body area) obtained from peroxisomal beta-oxidation mutants
919 and wild type strains grown on control and glucose conditions. N = 15 per strain for each
920 condition. Each faint data point represents an individual worm. (b,c) Error bars represent
921 s.e.m. (d) Representative images of ORO-quantified strains from control (-glucose) and
922 glucose (+glucose) conditions. Images are acquired from the blue channel in grayscale. Lipid
923 droplets appear dark. Scale bar is 100μm.
924
925 **Figure 6. Role of plasticity switch genes in glucose supplementation-induced mouth-**
926 **form plasticity.** (a) Schematic of the mouth-form gene regulatory network, indicating different
927 modules with genetic and epigenetic components. (b) Eu percentages of plasticity switch
928 mutants and wild type strains in glucose and control conditions. N = 3 biological replicates per
929 strain for each condition. Each faint data point represents a replicate (plate), with 30 animals
930 per plate being scored for mouth-form percentage (Eu%). (c) ORO absorbance
931 (RawIntDen/body area) measured in plasticity switch mutants and wild type strains grown on
932 control and glucose conditions. N = 15 per strain for each condition. Each faint data point
933 represents a worm. (b,c) Error bars represent s.e.m.
934

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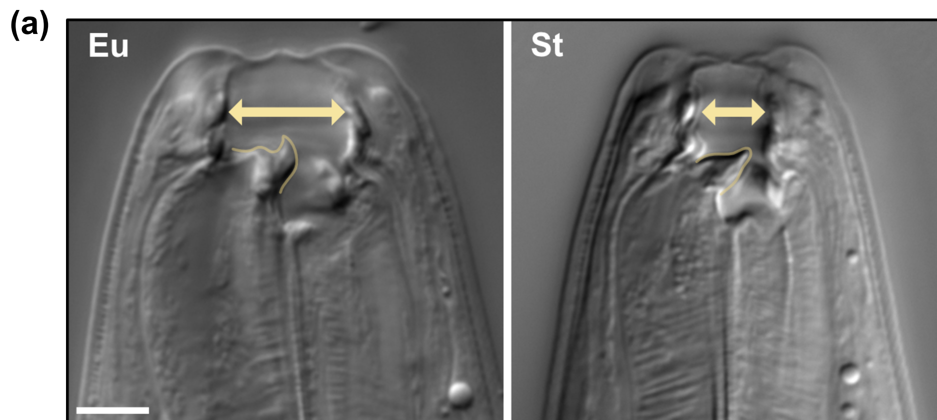
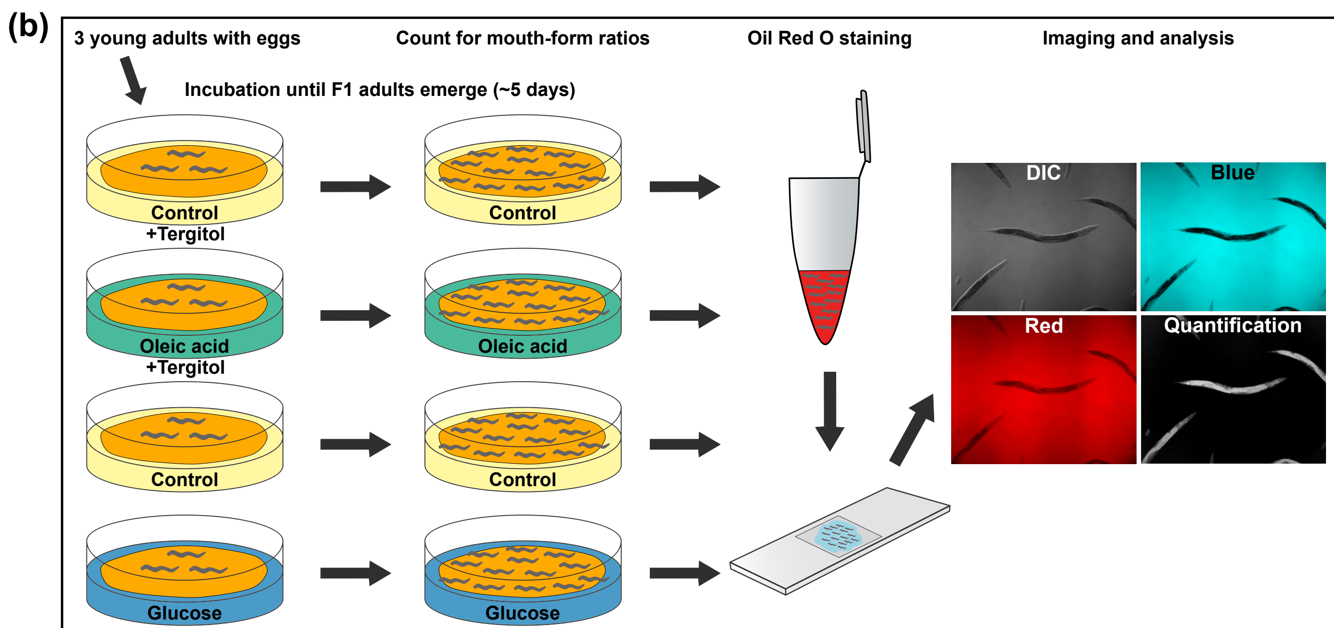
935 **Figure 7. Fecundity of different morphs after glucose supplementation.** (a) Illustration for
936 the experimental design to study fecundity of Eu and St animals obtained from glucose-
937 supplemented condition. (b) Daily fecundity of Eu and St animals. Error bars represent s.e.m.
938 (c) Overall fecundity of Eu and St animals. Bars represent mean values of all individuals for
939 each morph. Error bars represent s.d. P value is obtained from Wilcoxon rank sum test. (b,c)
940 N = 34 per morph. Each faint data point represents a worm.

Figure 1

(a)



(b)



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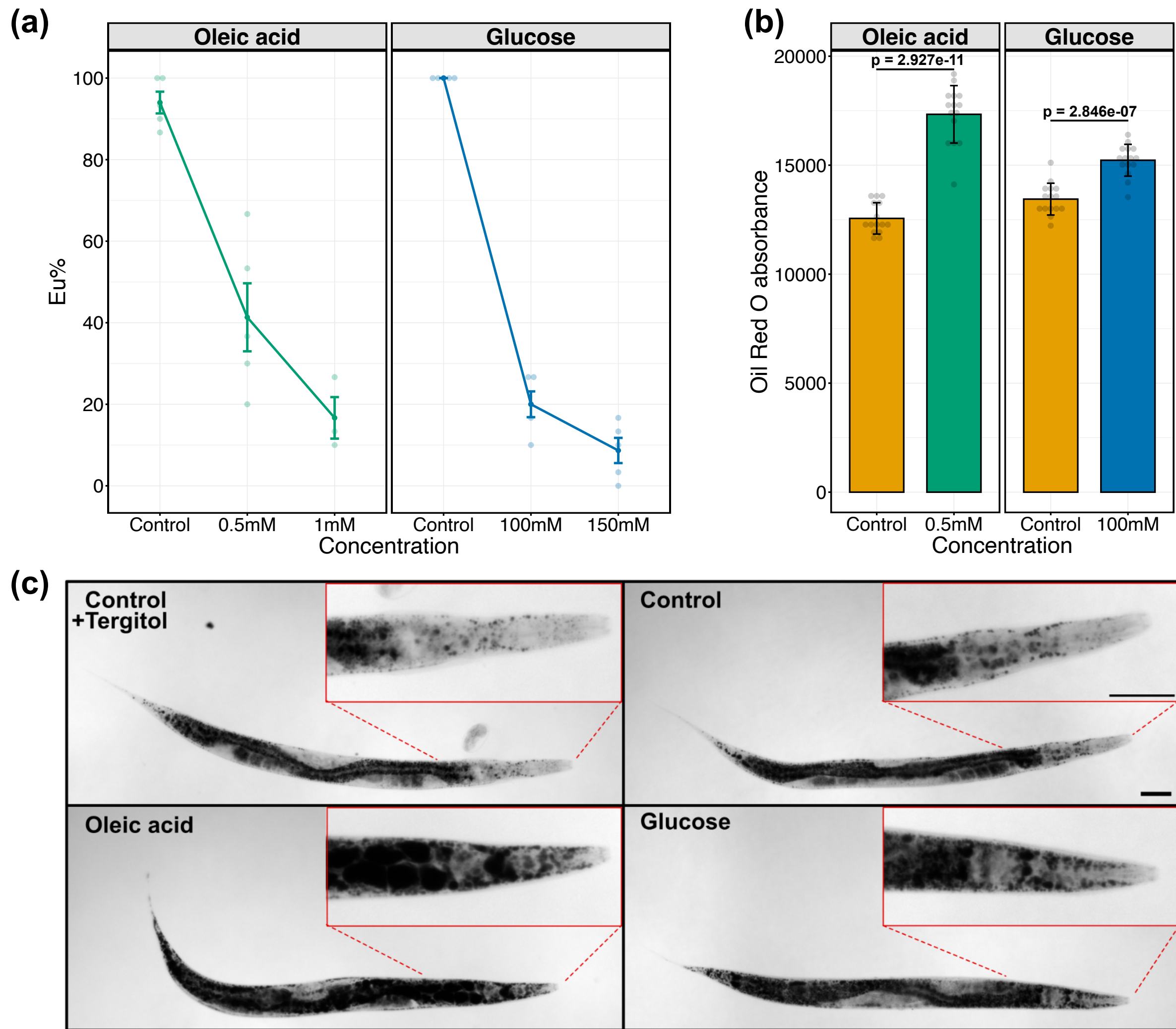

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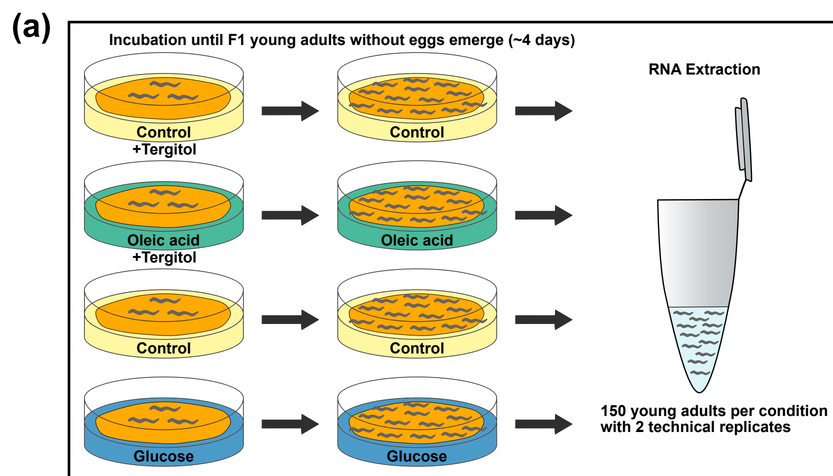

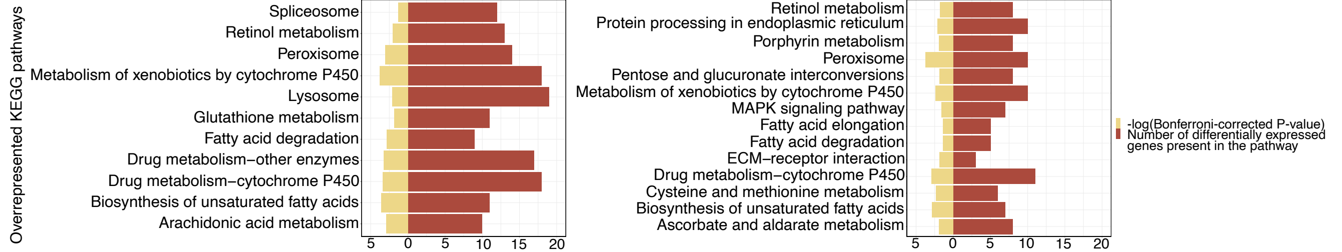

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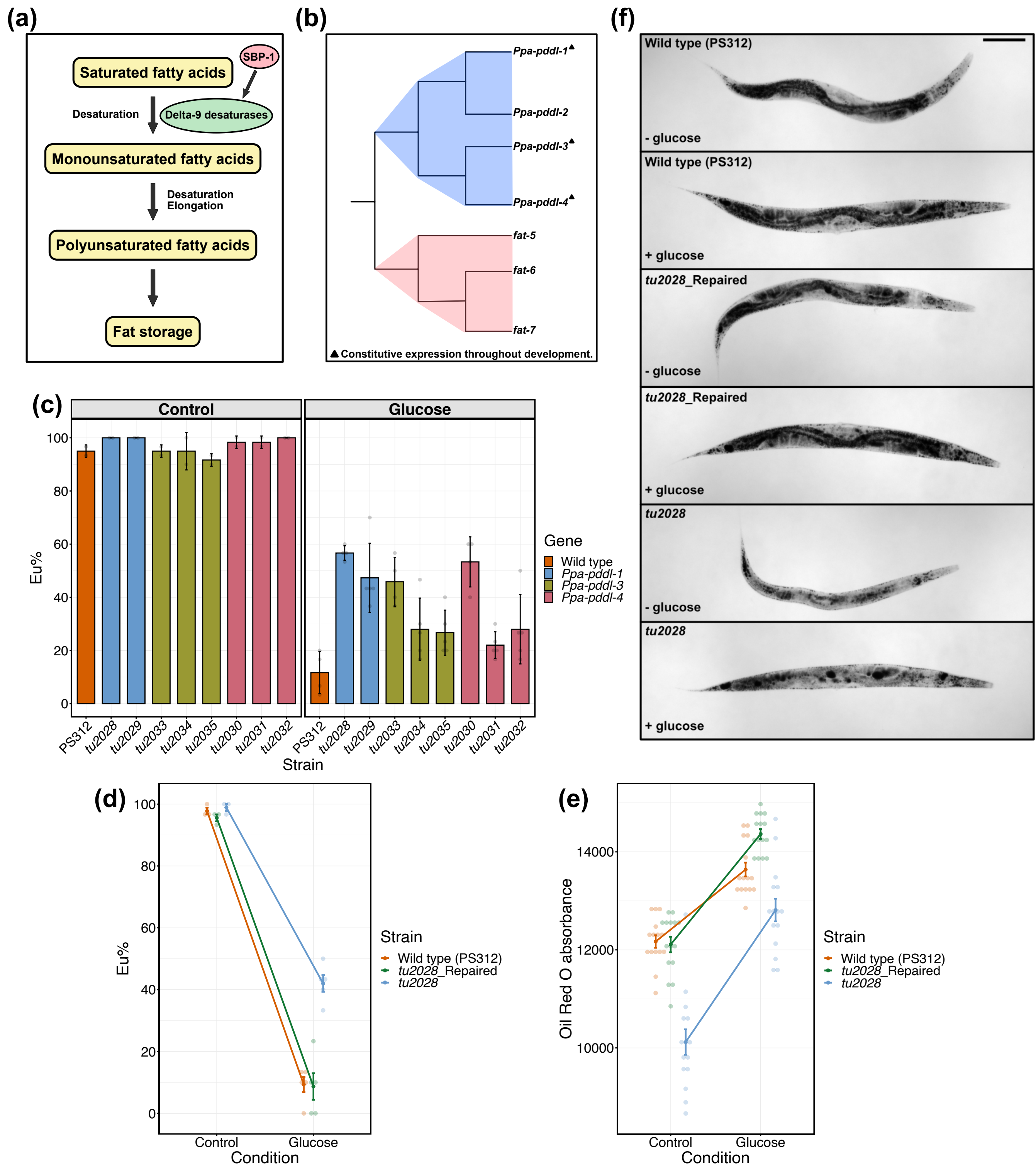
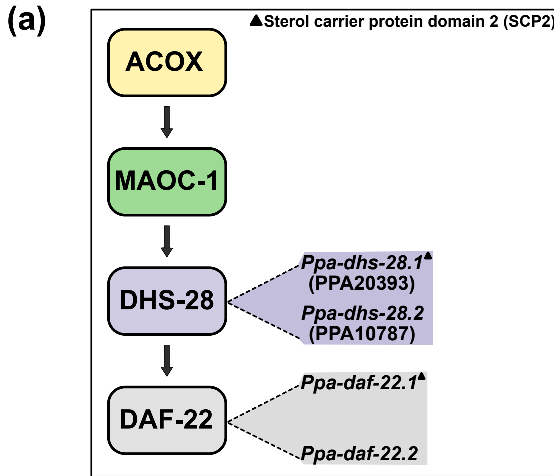
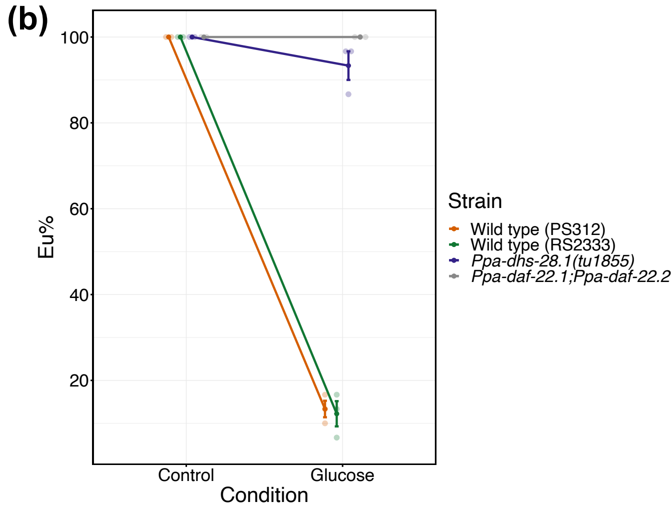
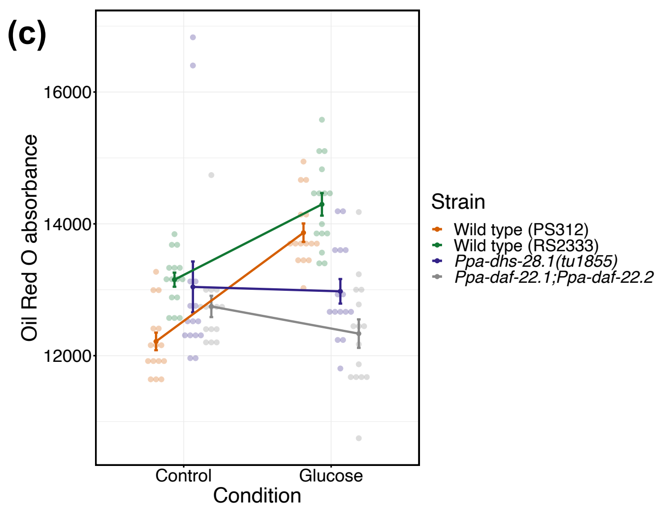


Figure 5







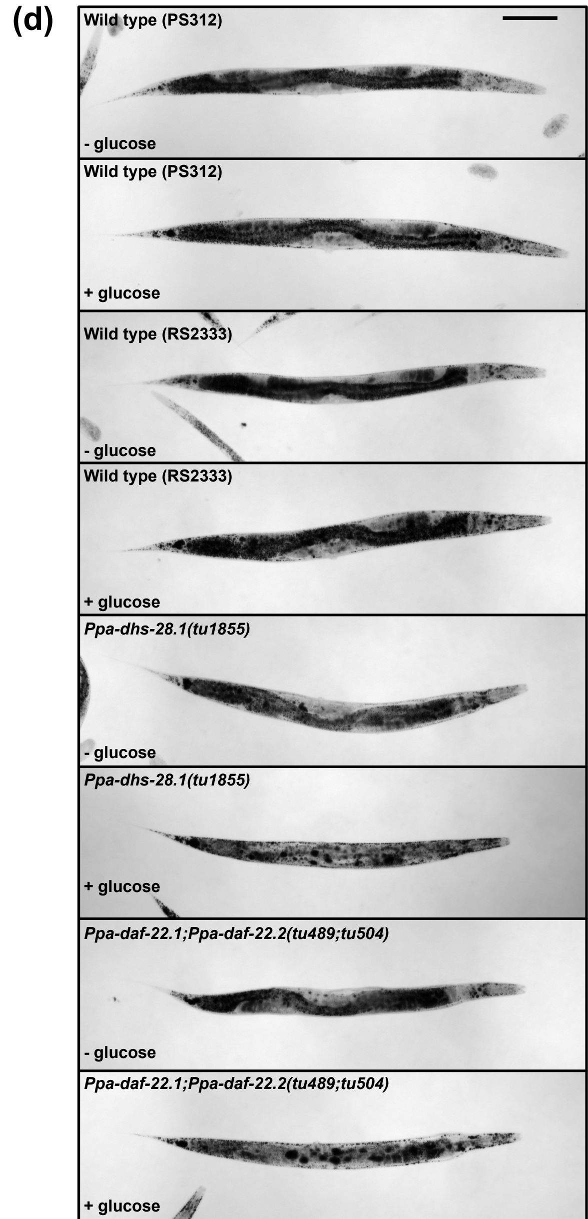


Figure 6

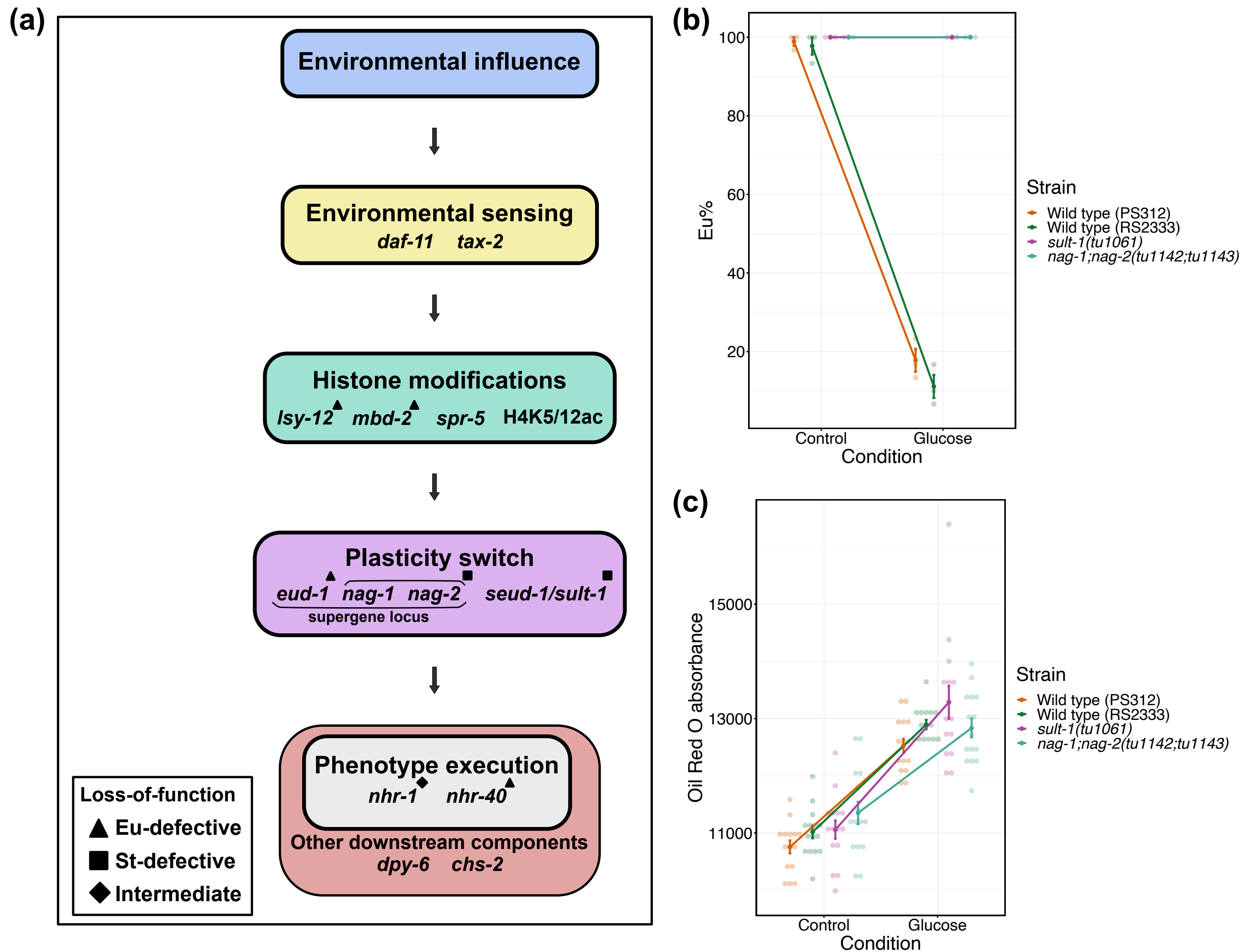
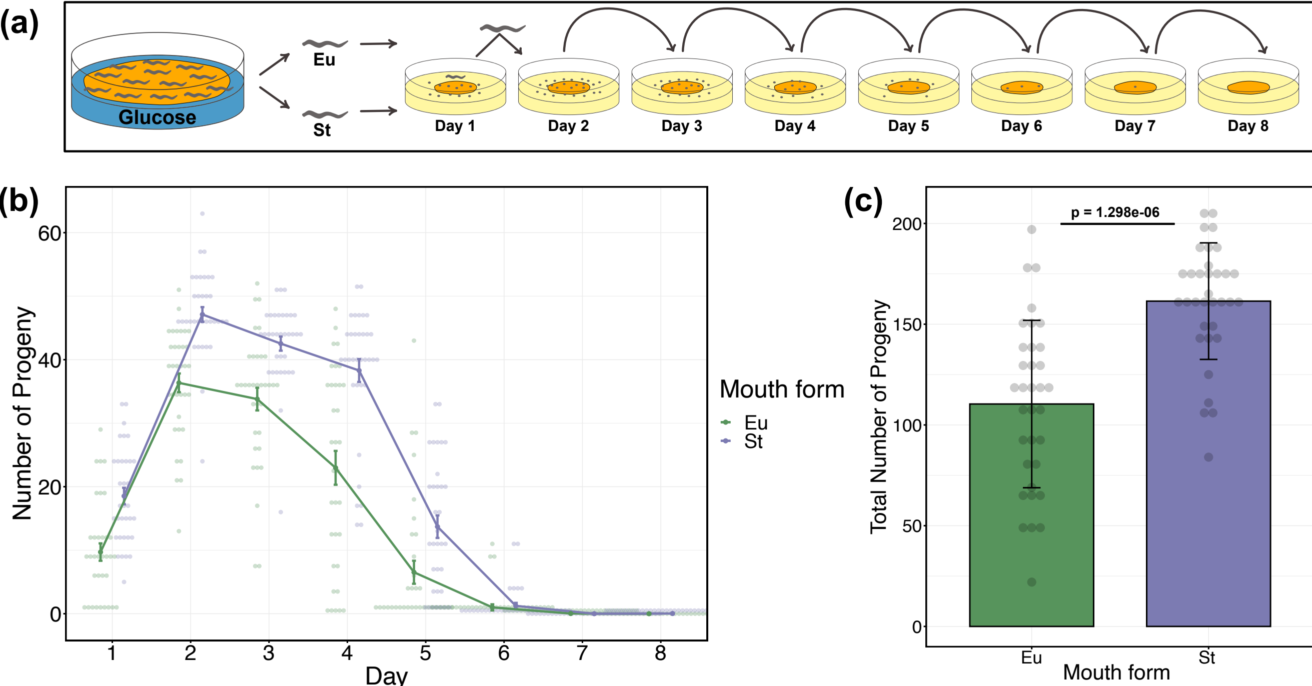


Figure 7



High nutritional conditions influence feeding plasticity in *Pristionchus pacificus* and render worms non-predatory

Supplementary Figures and Tables

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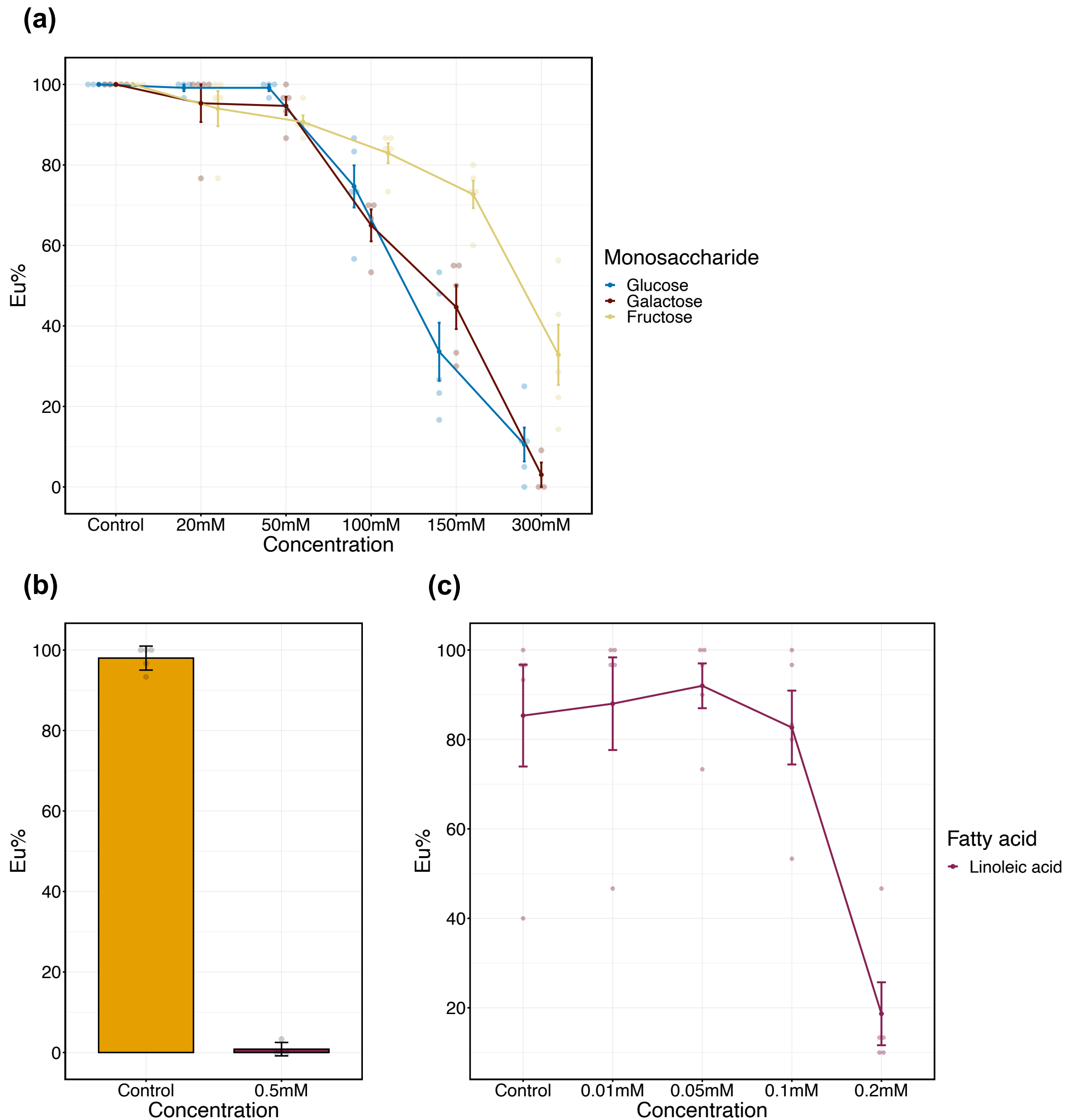
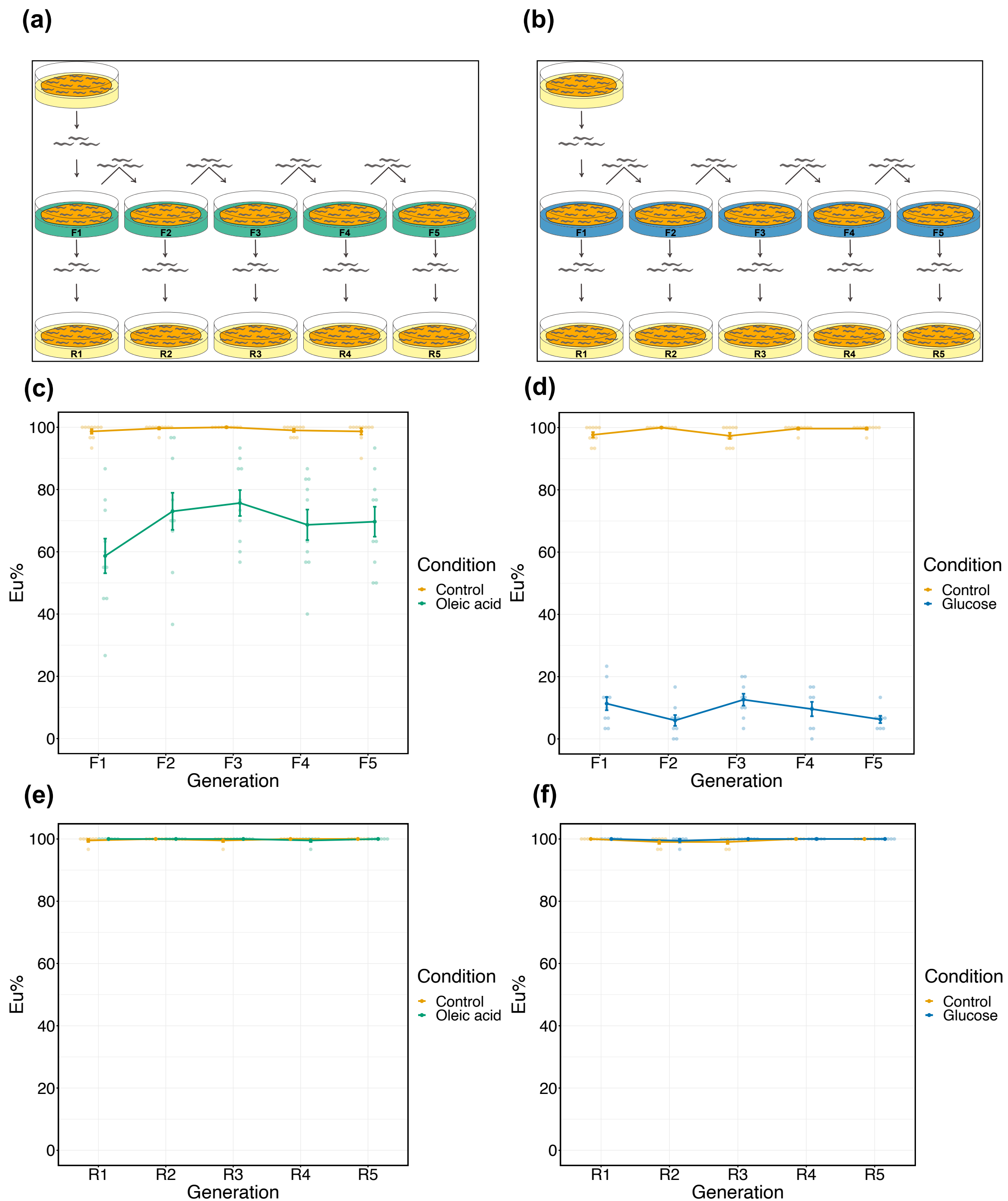


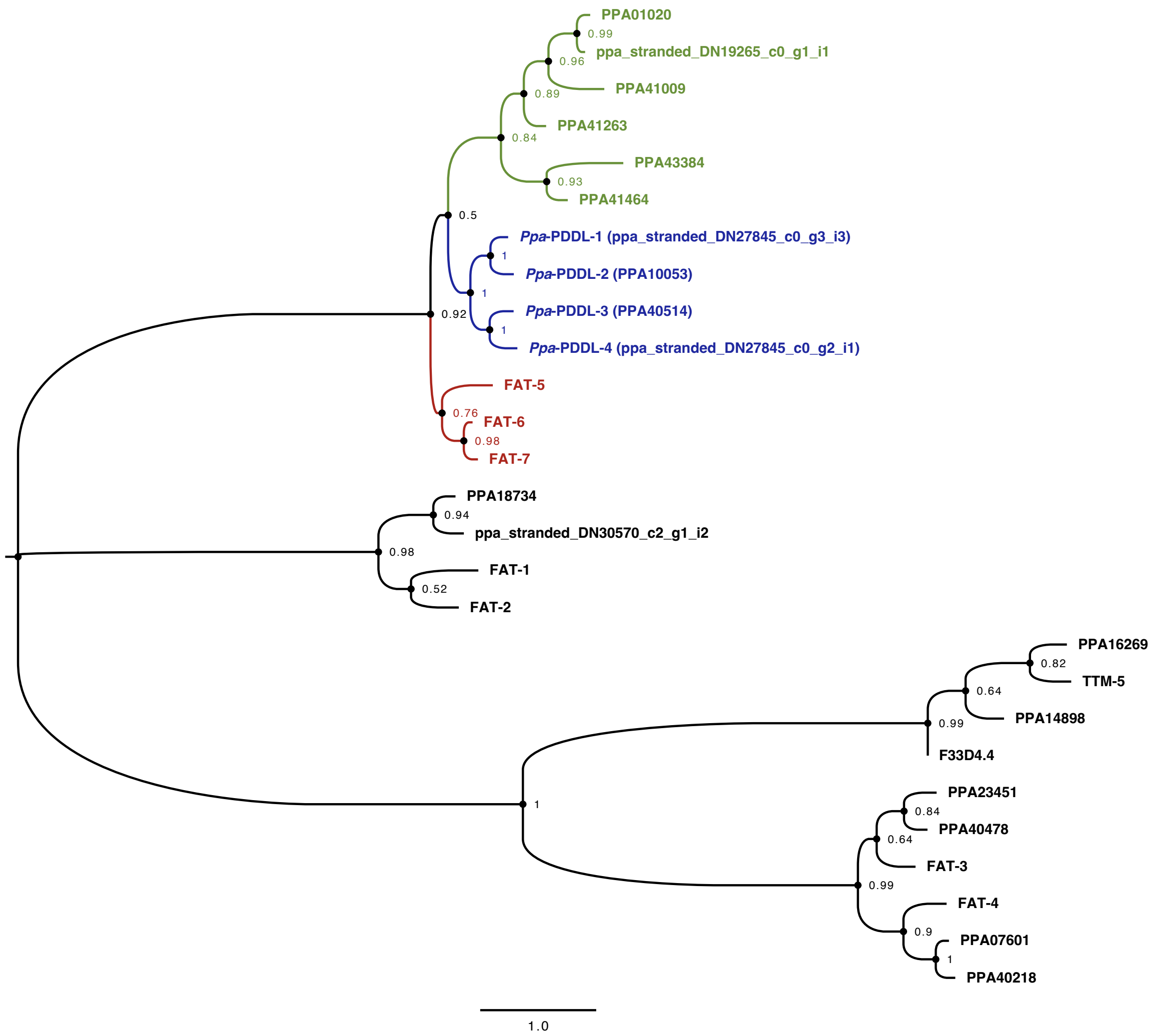
Supplementary Figure S1

Concentration dependent effect of supplements on mouth-form plasticity. (a) Eu percentages of worms grown on different concentrations of monosaccharides. In this pilot experiment, cultures were initiated by inoculating with 10 adult worms, allowing them to lay eggs for 2 hours. This method results in higher Eu percentages than inoculating with 3 worms. N ≥ 3 biological replicates per condition in each concentration. From each replicate (plate), 25-30 worms were scored, except for 300mM concentrations from which 4-28 worms were scored. Error bars represent s.e.m. (b) Effect of 0.5mM linoleic acid on mouth-form plasticity. N ≥ 4 biological replicates per concentration. From each replicate (plate), 28-30 worms were scored. Bars represent mean values of all replicates. Error bars represent s.d. (c) Linoleic acid concentration effect on mouth-form plasticity. N = 5 biological replicates per concentration. From each replicate (plate), 30 worms were scored. Error bars represent s.e.m. (a-c) Each faint data point represents a biological replicate (plate) scored for mouth-form ratio (Eu%).



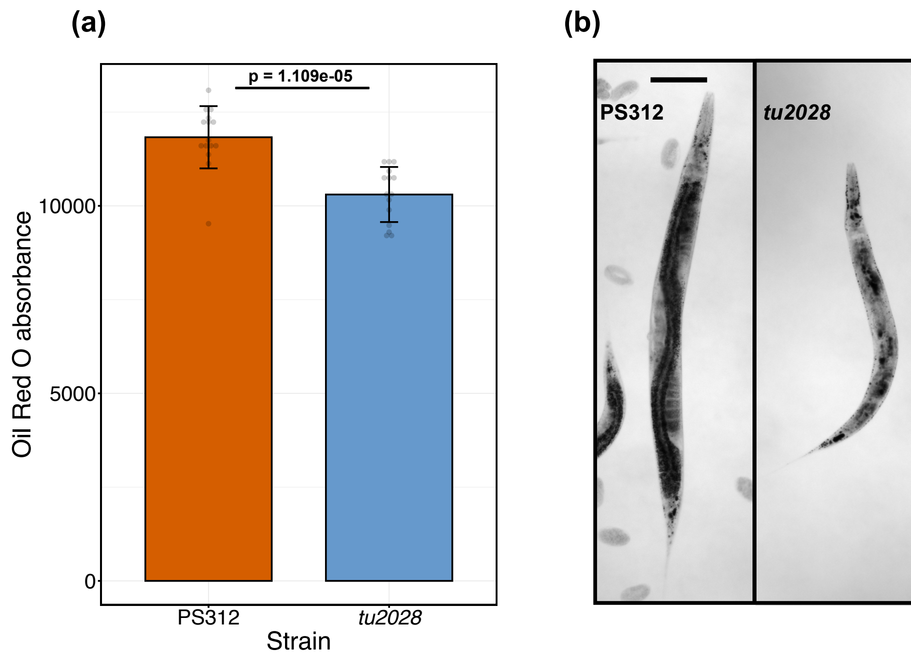
Supplementary Figure S2

Transgenerational effect of oleic acid and glucose supplementations on mouth-form plasticity. (a,b) Illustrations show the experimental design for studying the transgenerational effect of oleic acid (a) and glucose (b) supplementation on mouth-form plasticity. (a,b) The same experimental method was applied for respective control conditions. (c,d) Eu percentages of worms through generations (F1-F5) for oleic acid and control conditions (c); and for glucose and control conditions (d). (c) N = 10 biological replicates per condition for each generation. (d) N ≥ 8 biological replicates per condition for each generation. (e,f) Eu percentage of worms after reversal (F1-F5) for oleic acid and control conditions (e); and for glucose and control conditions (f). (e) N = 7 biological replicates per condition for each generation. (f) N ≥ 6 biological replicates per condition for each generation. (c-f) Each faint data point represents a replicate (plate), with 30 animals per plate being scored for mouth-form percentage (Eu%). Error bars represent s.e.m.



Supplementary Figure S3

Phylogenetic tree of all *P. pacificus* fatty acid desaturase domain-containing proteins with related *C. elegans* desaturases. A maximum likelihood phylogenetic tree, constructed with LG model, and 100 bootstrap replications. Bootstrap values are indicated next to branch nodes. Green and Blue colours denote *Pristionchus* delta-9 desaturase domain-containing proteins. Red colour denotes *C. elegans* delta-9 desaturases.



Supplementary Figure S4

Delta-9 desaturase mutant, *Ppa-pddl-1(tu2028)*, exhibits reduced lipid storage relative to wild type strain (PS312) on standard dietary condition. (a) ORO absorbance (RawIntDen/body area) obtained from wildtype (PS312) and *Ppa-pddl-1(tu2028)* strains. N = 15 per strain. P value is obtained from a two sample t-test. Each faint data point represents a worm. Bars represent mean values of all samples for each strain. Error bars represent s.d. (b) Representative images of ORO-quantified worms, indicating lipid storage profile. Images are acquired from the blue channel in grayscale. Lipid droplets appear dark. Scale bar is 100μm.

Supplementary Table S1. List of crRNA and primer sequences for genes that were studied for mutant analyses.

Gene name	Accession	crRNA	Forward Primer	Reverse Primer
Ppa-pddl-1	ppa_stranded_DN27845_c0_g3_i3	5′-CAGACATTACTATCCTCTAG-3′	5′-CCGTTAGAGTCTACTTCATGCTATGGAA-3′	5′-ATCAACCTGACCATATTTCAGTCTGACC-3′
Ppa-pddl-3	PPA40514	5′-CTATCTCCCCCTTGCGACTC-3′	5′-TTCTGATCTGTGGAACGACCCG-3′	5′-CACGGATTCGACGGGAGTGATG-3′
Ppa-pddl-4	ppa_stranded_DN27845_c0_g2_i1	5′-GTAATTCCCGTCTATTTCTG-3′	5′-TAACGATGTTTTCCTTCAGGTAATGGGC-3′	5′-CTGCTGCTTGTAGATTAGTCCATACAGA-3′
Ppa-dhs-28.1	PPA20393	5′-GGGGAGATCAAGGCAGCCGG-3′	5′-CGATATTGTTGCAGTGAACGAC-3′	5′-CTTCTAGTTACATCAGCTGTCTCG-3′

Supplementary Table S2. List of CRISPR mutants utilised in this study.

Gene Name	Gene Accession	Strain	Allele	Mutation Type	Mutation Location	Source
Ppa-pddl-1	ppa_stranded_DN27845_c0_g3_i3	RS4411	*tu2028*	7bp insertion	exon 4	This paper
Ppa-pddl-1	ppa_stranded_DN27845_c0_g3_i3	RS4412	*tu2029*	3bp insertion	exon 4	This paper
Ppa-pddl-3	PPA40514	RS4401	*tu2033*	5bp deletion	exon 5	This paper
Ppa-pddl-3	PPA40514	RS4402	*tu2034*	7bp deletion	exon 5	This paper
Ppa-pddl-3	PPA40514	RS4403	*tu2035*	7bp deletion	exon 5	This paper
Ppa-pddl-4	ppa_stranded_DN27845_c0_g2_i1	RS4398	*tu2030*	4bp deletion	exon 7	This paper
Ppa-pddl-4	ppa_stranded_DN27845_c0_g2_i1	RS4399	*tu2031*	10bp deletion	exon 7	This paper
Ppa-pddl-4	ppa_stranded_DN27845_c0_g2_i1	RS4400	*tu2032*	28bp insertion	exon 7	This paper
Ppa-dhs-28.1	PPA20393	RS4147	*tu1855*	4bp deletion	exon 3	This paper
Ppa-dhs-28.1	PPA20393	RS4138	*tu1856*	7bp deletion	exon 3	This paper
Ppa-dhs-28.1	PPA20393	RS4141	*tu1857*	8bp insertion	exon 3	This paper
Ppa-dhs-28.1	PPA20393	RS4140	*tu1858*	21bp deletion	exon 3	This paper
Ppa-dhs-28.1	PPA20393	RS4139	*tu1859*	19bp insertion	exon 3	This paper
Ppa-daf-22.1;Ppa-daf-22.2	ppa_stranded_DN16812_c0_g1_i1;PPA41516	RS2770	*tu489;tu504*	7bp deletion;7bp insertion	refer to Markov et al., 2016	Markov et al., 2016
sult-1	PPA12547	RS2974	*tu1061*	10bp deletion	exon 9	Namdeo et al., 2018
nag-1;nag-2	PPA06134;PPA34489	RS3195	*tu1142;tu1143*	1 SNP + 17bp insertion;2 SNPs + 9bp deletion	exon 5;exon 5	Sieriebriennikov et al., 2018