Title

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

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Abstract

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 associated costs of both plasticity and phenotype 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 three *P. pacificus* strains to two distinct microbial diets that induce strain-specific mouth-form ratios. Our results indicate that a highly-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, a non-plastic strain suffers from the cost of phenotype in unfavorable conditions, but shows increased fitness and higher developmental speed under favorable conditions. Furthermore, we computationally illustrate the consequences of the costs of plasticity and phenotype on the population dynamics in spatially-homogeneous and spatially-structured populations using empirically-derived life history parameters for modeling. This study provides comprehensive support for the costs of plasticity and phenotype based on empirical and modeling approaches.

Keywords: Cost of plasticity- Cost of phenotype- cannibalism- adaptive plasticity- Markov population models.

Introduction

Changing and fluctuating environments are a hallmark of all ecosystems, affecting the life and evolution of all organisms [1,2]. The ability of an organism to respond to changing environments by expressing alternative phenotypes 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 [2–4]. 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 [3,5,6]. We refer to this type of phenotypic plasticity that facilitates adaptation “adaptive plasticity”. However, it is important to emphasize that not all examples of phenotypic plasticity are adaptive and conceptually, an organism cannot be infinitely plastic to cope with any environment [7]. Additionally, plastic genotypes can vary in their degree of plasticity across different conditions [8,9]. 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 [2,7,10–13]. Theoretically, several factors can hinder the evolution of adaptive plasticity; namely, limited genetic variation, weak selection, and the unreliability of environmental signals [2,9,11]. Most importantly, however, it has been argued that fitness costs will limit the evolution of plastic phenotypes [7]. Such arguments are largely theoretically based, and detecting the cost of adaptive plasticity remains a formidable challenge. Here, we use the plasticity model system, the nematode *Pristionchus pacificus* (Fig. 1), to obtain experimental evidence for plasticity-associated costs.

In general, to understand the costs associated with adaptive plasticity, one first has to consider the potential trade-off in a non-plastic genotype: assuming that the non-plastic genotype expresses a phenotype adapted to environment II, the same phenotype could be less suited in environment I, resulting in a fitness reduction (Fig. 2a). This fitness trade-off has been referred to as *cost of phenotype* by several authors [7,13]. In contrast, a genotype expressing a plastic phenotype induced only by environment II can have lower fitness than a less-plastic or non-plastic genotype in that environment (Fig. 2a). This hypothetical fitness trade-off associated with a plastic genotyped was referred to as *cost of plasticity* [7,13]. Understandably, the terms ‘cost of phenotype’ and ‘costs of plasticity’ are, by virtue of their definitions, ripe for confusion [2,10]. 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. This has been largely due to two reasons: Firstly, in the wild, conditions can often not be properly controlled and the effect of various factors not be delineated. Secondly, laboratory experiments are time consuming and, in particular, 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 [14,15]. The developmentally plastic mouth of *P. pacificus* can exhibit two distinct forms; the eury stomatous (Eu) morph with a wide stoma and hooked-like teeth, or the stenostomatous (St) morph with a narrow stoma and a single tooth (Fig.1a)[16]. *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 [17–23]. 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 [24–26]. 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, resulting in a highly resolved phylogeny of diverse populations (Fig.1b) [27]. Interestingly, culturing these isolates on the laboratory bacterium *E. coli* displays a range of mouth-morph ratios, *i.e.*, some strains are Eu-biased, others are St-biased, while some express intermediate mouth-form ratios (see below) [17]. Second, morphological mouth-form plasticity is coupled to behavioral plasticity. Specifically, the Eu form enables predation and cannibalism on other nematodes, while such animals can still feed on bacteria. In contrast, the St form obligates worms to feed on bacteria (Fig.1c) [28]. This extension of morphological plasticity to behavior is thought to be involved in intraguild predation, *i.e.*, the elimination of resource competitors via rival killing and the expansion of nutrition [29]. Finally, the natural habitat of *P. pacificus* and its relatives has been intensively studied. *P. pacificus* is a 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 (Fig.1d)[30]. These experiments revealed a vital response of mouth-form acquisition in response to the presence of competing nematodes on the insect carcass indicating the ecological significance of adaptive plasticity [31].

It should be noted that, switching between the Eu and St mouth forms is a specific example of phenotypic plasticity, where the plastic phenotype of an individual can assume one of two alternative mouth morphs, an irreversible decision that occurs during the development of *P. pacificus* via a bi-stable developmental genetic switch [19]. The bias of the developmental switch determines the ratio of mouth morphs with substantial natural variation between populations of *P. pacificus* [17]. In this respect, mouth-form plasticity in *P. pacificus* is more akin to the switch between lytic and lysogenic cycles in bacteriophage λ [32] than wing pattern polyphenism in butterflies that is seasonally controlled [33]. Importantly, the relative simplicity of mouth-morph plasticity in *P. pacificus* - binary readout, isogenic husbandry of genetically diverse strains and strain-specific bi-stability - makes it ideal to study different facets of phenotypic plasticity beyond the identification of associated genetic and epigenetic mechanisms.

Here, we took advantage of these features to perform a systematic analysis of mouth morphs and their associated costs. The cost of plasticity and cost of phenotype can, theoretically, be measured by comparing the fitness of individuals of the same strain with an identical genetic background but opposite phenotypes, or a pair of *P. pacificus* strains, one plastic and one non-plastic, in two different environments (Fig. 2a). Such theoretical expectation relies on a set of assumptions, chief amongst them is the notion that a trait is adapted to a given environment. While establishing an adaptive value for a given trait is a non-trivial issue, we attempt to solve this and related issues by experimentally measuring the costs of phenotype and plasticity under laboratory conditions, using fecundity and developmental speed as fitness parameters in strains sampled across the *P. pacificus* phylogenetic tree. Subsequently, we focused on the response of three *P. pacificus* strains, with varying levels of plasticity, to two different bacterial diets. We extended our empirical findings via simulating ecologically relevant scenarios in spatially-homogeneous and spatially-structured populations using empirically-derived life history parameters. Our study provides comprehensive support for the costs of plasticity and phenotype based on empirical and computational approaches.

Results

Within and between strain comparisons reveal a cost of phenotype

First, we measured the cost of phenotype by studying whether the formation of the predatory mouth morph negatively affects fecundity. 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 (Fig.1b)[27,34]. The ratios of these strains were considered intermediate since they were neither predominantly expressing the predatory (Eu) nor predominantly expressing the non-predatory (St) morph on the standard laboratory food source *E. coli* (Fig. 2b, *SI Appendix*; Table. S4). It should be noted that “intermediate” in this context does not imply an exact 50-50 chance of expressing either of the mouth morphs. Instead, it merely indicates that both alternative morphs can be easily found in a lab grown culture. Therefore, these strains allow testing whether there is a cost of phenotype within the same genetic background and by obtaining animals of both morphs on the same petri dish. To measure the cost of phenotype, we selected overall individual fecundity as primary fitness parameter to capture reproductive capacity of *P. pacificus* hermaphrodites via selfing [35–37]. Testing for daily fecundity showed that the majority of progeny were laid within a window of 62 hours after maturation (nearly 91%) in an overall window of appr. 158 hours of total egg-laying (*SI Appendix*; Fig. S1a& Table. S2). Consequently, the number of eggs laid in this window provide a reasonable estimate of the life-time fecundity. In these intra-genotype comparisons, we found a tendency of St animals to have more progeny than Eu worms. Specifically, the estimated differences in the mean value of fecundity between St and Eu animals based on the data showed strong and/or partial support in four out of seven comparisons (Fig. 2c, *SI Appendix*; Table S1; Table S6). These findings suggest that the production of the predatory mouth morph can incur a cost in the form of fewer progeny. This observation is in concert with a previously reported slower rate of development conceived by nematodes exhibiting the predatory morph in comparison to non-predatory worms [38].

Second, we measured fecundity and developmental speed in *P. pacificus* natural isolates that show a biased mouth-morph choice, *i.e.*, strains that would produce an abundance of St or Eu mouth morphs on the standard laboratory food source *E. coli* (Fig. 2d, *SI Appendix*; Table S4). We selected two pairs of closely-related strains from the diverged clades B and C of *P. pacificus* from La Réunion island [39]. We found that in both pairs the St-biased strains produce more overall

progeny than the Eu-biased strains (Fig. 2e, *SI Appendix*; Table S1; Table S7). Specifically, giving the same time window of the first 62 hours, the St-biased strains had a 21% and 17% higher fecundity in clades B and C, respectively (*SI Appendix*; Fig. S2b& Table. S2), which in turn may affect population dynamics and resource competition in the wild. Similarly, the St-biased strains showed a higher developmental speed than the Eu-biased strains (Fig. 2f, *SI Appendix*; Table. S3). For example, 75 hours after egg-laying, nearly 60% of the St-biased strain RSC011 reached adulthood; whereas only 27% of the Eu-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, our pairwise comparisons of total eggs laid by Eu and St individuals in intermediate strains, and the pairwise comparisons of fecundity and developmental speed in four biased strains from two different *P. pacificus* clades clearly illustrate the cost of producing the Eu 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 eminent when testing a less plastic genotype relative to a more plastic genotype under different conditions [4,7,12,13]. 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 from California [40,41]. However, it was never studied in non-domesticated wild isolates of *P. pacificus*. Therefore, we used three strains with different mouth-morph ratios on *E. coli*, and grew them on *Novosphingobium*; the highly St-biased strain RSC017, the intermediate strain RSC019, and the highly Eu-biased strain RS5405. Indeed, RSC017 and RSC019 showed a substantial increase of the Eu morph of 84% and 40% on *Novosphingobium*, respectively, indicating strong plasticity. In contrast, the Eu-biased strain RS5405 remained highly Eu in the new condition (Fig. 3a, *SI Appendix*; Table. S4). Thus, we established two distinct food conditions that differentially affect plasticity levels of the three isolates.

Theoretically, the cost of plasticity would be displayed in the strain that exhibits the largest change in mouth-morph ratio upon altering food conditions. Accordingly, we would expect to detect the

highest effect on fitness in the most plastic strain, and vice versa. Indeed, we found that RSC017 has the lowest fecundity and the slowest developmental speed on *Novosphingobium* when compared to the less-plastic strains (Fig. 3b-c, *SI Appendix*; Table. S1,3, Table S8). Thus, a strain that plastically responds to a dietary change with the formation of the Eu mouth morph exhibits reduced fitness under these novel conditions indicating a cost of plasticity. In contrast, RS5405 exhibits the highest levels of fecundity and developmental speed on *Novosphingobium* (Fig. 3b-c, *SI Appendix*; Table. S1,3). Thus, a strain that is preferentially Eu under both food conditions exhibits increased fitness when exposed to this new diet. Consistent with these observations, RSC019 exhibits intermediate mouth morph ratios in all measurements compared to other strains on *Novosphingobium*; (Fig. 3b-c, *SI Appendix*; Table. S1,3). Taken together, these findings indicate a cost of mouth-morph plasticity in response to dietary induction. In conclusion, we observe a cost of phenotype, as well as, a cost of plasticity in mouth-form plasticity of *P. pacificus*, which raises the 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-classified model to simulate population dynamics of the St-biased strain RSC017 and the Eu-biased strain RS5405; on both tested food sources (Fig. 4a). For modeling, we used the fecundity measurements from the lab and scaled the developmental rates of the model based on the laboratory estimates of developmental speed of *P. pacificus* (see **Materials and Methods**). First, we tested 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 RSC017 (Fig. 4b). The reduction in fecundity on *Novosphingobium* relative to *E. coli* is presumably compensated by the increase in developmental speed on *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 RSC017 by assuming no change in developmental speed. Indeed, the results of this simulation confirmed this expectation (Fig S6). In contrast, in RS5405 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* (Fig. 4c). Importantly, the between strains cost of

phenotype is clearly displayed when comparing RS5405 and RSC017 frequencies on *E. coli* (Fig. 4b-c). Thus, comparing both populations' trajectories without involving interactions reveals that the cost of 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, giving the coupling between morphological and behavioral plasticity, Eu worms are able to predate while St 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 predation rate positively correlates with the proportion of predatory individuals in wild isolates. To avoid the compounding effect of relatedness on predation [59], 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 (*SI Appendix*; Fig. S3). Second, we measured predation rates of the two oppositely biased strains RSC017 and RS5405 against one another by testing predation rates over the two food sources *E. coli* and *Novosphingobium* (Fig. 4d). On an *E. coli* diet, RS5405 predation rates were far higher than RSC017 predation rates; giving their opposite mouth morph bias.

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 **Materials and Methods**, *SI Appendix*; Fig. S4). Specifically, we used these estimates to simulate the interactions between the two isolates in a population with an equal number of RSC017 and RS5405 young adults at the start of the simulation. Notably, simulated populations were completely dominated by RS5405 for both food conditions. In addition, rapid elimination of RSC017 averts the formation of its dauer larvae, as J2 animals of this strain were completely eradicated by RS5405 predators (Fig. 4e, f). Thus, the cost of plasticity greatly affects the dynamics of RSC017 in a spatially-homogenous population.

Spatial structure significantly affects population dynamics

While modeling the interaction of RSC017 and RS5405 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 construct a source and sink version of the stepping-stone migration scenario to illustrate the costs of plasticity and phenotype on the dynamics of in a structured population (Fig. 5a). We constructed a simple structured population by arranging n localities in one dimension. Each simulation starts with 50 young adults (YAs) of RSC017 in the first locality and 50 YAs of RS5405 in the n^{th} locality, with reset of localities being empty. All the localities contain a fixed amount of resource and dauer larvae migrate with a fixed rate from a food-poor locality to a neighboring food-rich locality (Fig. 5a). The simulation concludes when all the food in every locality has been depleted.

This modeling approach results in three major findings. First, on *E. coli*, higher fecundity of RSC017 allows adults of this isolate to completely dominate the structured population even in the face of predation (Fig. 5b, *SI Appendix*; Fig. S5a). Although the predation rate of RS5405 is higher, even dauer larvae of RSC017 continue to fully dominate the structured population (Fig. 5c). These results support a considerable cost of phenotype for RS5405 in the spatially-structured population. In addition, in a scenario without predatory interactions, the frequency of RSC017 decreases only marginally (Fig 5b vs. d, c vs. e). This finding results from the change in the number of migratory dauer larvae of the predatory strain RS5405 (*SI Appendix*; Fig. S5a,b). Most importantly, the pace in which the RSC017 population grows, results in exceptionally high numbers of RSC017 predators that outcompete RS5405 in the presence of interaction. Thus, the cost of phenotype substantially influences RS5405 abundance, in particular in the presence of interactions.

Second, on *Novosphingobium*, higher fecundity and faster developmental speed of RS5405 turns this isolate into a formidable adversary for RSC017. Therefore, the frequencies of RSC017 adults and dauer larvae are extremely reduced in the structured population (Fig. 5b, c). However, when interactions are limited, in contrast to *E. coli*, the frequencies would slightly increase (Fig 5b vs. d, c vs. e). This is due to RS5405 profiting from a higher growth rate and higher predation on *Novosphingobium*, but only higher growth when interactions are eliminated (*SI Appendix*; Fig. S5c,d). Thus, the cost of plasticity would greatly affect RSC017 abundance when competing with a predator under this condition.

Initial food source also affects population dynamics

Finally, 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 RSC017, and *Novosphingobium* for RS5405; or the unfavorable food source; *Novosphingobium* for RSC017, and *E. coli* for RS5405 (Fig. 5b-e). 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 RSC017 is greater than the cost of phenotype of PS5405. 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 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 remains poorly understood.

Discussion

Resource polyphenism is one example of developmental plasticity where alternative phenotypes facilitate utilizing different food resources, including developing cannibalistic morphs as a response to environmental stress [42]. Cannibalism provides trophic and survival advantages by either extending energy resources or eliminating competition [43,44]. Evidently, the predatory mouth form in *P. pacificus* has proven to boost survivorship under severe conditions [45], and reduce competition on the basis of genomic relatedness [34]. Nevertheless, various *P. pacificus* natural isolates are either predominantly non-predatory, *i.e.*, St-biased, or intermediate. Here, we could demonstrate the cost sustained by the production of the predatory phenotype (the Eu moth morph). In isolation, the fitness payoff incurred by the Eu-biased population makes it inferior to the St-biased strain (Fig. 4b,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 the different of population dynamics and a different dominance pattern due to the costs of plasticity and phenotype (*SI Appendix*; Fig. S5). Together, our results suggest a four-pronged explanatory framework, combining cost of plasticity, cost of phenotype, environmental influence, and population structure, each playing a crucial role in adaptive plasticity.

Conceptually, the cost of plasticity has been suggested as a major factor hindering the evolution of adaptive plasticity. However, it has been challenging to experimentally detect these costs, especially in metazoans. For instance, the predator-induced spine of *Daphnia pulex* was reported to show mild support for both the costs of production and maintenance [46]. 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 [47]. Moreover, a meta-analysis of 27 studies on the cost of plasticity, of which only seven were on animals, has concluded that costs of plasticity are mostly low if existing at all [48]. However, the same authors suggested that these costs may influence adaptive evolution under stressful conditions. Additionally, meta-analysis on aquatic gastropods argued for further empirical investigations to better quantify the energetic costs of plasticity of shell formation [49]. 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 [50]. Together, this diversity of findings indicates the need for establishing a comprehensive empirical framework to address both theoretical and conceptual asserts. Our results in *P. pacificus*, likely benefited from the binary and easily distinguishable state of the polyphenic trait, Eu vs. St mouth morph, and the isogeneic nature of all tested strains, which together facilitate empirical measurements of fitness components, *i.e.*, fecundity and developmental speed, of each morph in the laboratory. Additionally, accounting for two fitness components assisted in the transition from abstract measures to simulating a range of ecologically relevant scenarios. Thus, our study complements previous knowledge with a systematic analysis of defined costs in an evolutionary adaptive trait.

Limits of this study

While this study provides comprehensive insight into adaptive plasticity, 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, giving search and handling time, besides foraging efficiency and predation risks [51–55]. Additionally, in nature, nematode mobility is not restricted to a one-dimensional dispersal. Thus, such parameters merits 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 [4,7]. 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.

Figures captions

Fig. 1: Mouth-form plasticity in *P. pacificus*: (a) Mouth-morph dimorphism in *P. pacificus*. The bacterivorous St morph possesses a single tooth with a narrow buccal cavity, whereas the predatory Eu morph 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 Rödelberger 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. 2: Within and between strain costs of phenotype in *P. pacificus*: (a) The cost of plasticity and the cost of phenotype in our model can be illustrated by a hypothetical scenario: The plastic strain A switches from St mouth form to Eu when grown in environment II, but this plastic

response to environment II is accompanied by a reduction in fitness, α , which is the cost of plasticity. Strain B is non-plastic and facultatively expresses the Eu morph in environments I and II, but its phenotype is an adaptation to environment II, while in environment I the same phenotype is accompanied by a reduction in fitness, β , which is the cost of phenotype. It is evident that costs of phenotype and plasticity are, by definition, exclusively meaningful in comparative studies in environments, which can be described as adaptive and non-adaptive with regards to a given trait.

(b) Mouth-morph ratio 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) bar the mean percentage of Eu mouth morph for each strain. (c) Overall fecundity of the same seven intermediate strains. On average 51 and 47, Eu and St mothers per strain were scored, respectively. Bars indicate the 95%HDI for the mean estimated using Kruschke's BEST method (see **Materials and Methods**). The top panel indicates the 95% HDI for the estimated difference in means for each pairwise comparison. We used [-5,5] interval as our region of practical equivalence (ROPE), *i.e.*, differences of means within this interval are practically equal to no difference. Two comparisons, RSC019 and RSC033, are outside the ROPE, *i.e.*, means in these 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. The same ROPE is used for all the subsequent analyses. (d) Mouth-morph ratio of four biased *P. pacificus* wild isolates representing clades B and C, respectively. For each strain, mouth-morph ratios were scored for three biological replicates with a total number of 150 worms per strain. (e) Overall fecundity of the same biased strains. Both St-biased strains showed credibly higher overall fecundity than the Eu-biased strains. The top panel indicates the 95% HDI for the estimated difference in means for each pairwise comparison using Kruschke's BEST method. The 95% HDI for the estimated difference in means in two comparisons does not overlap with the ROPE, indicating difference, while the rest of comparisons only partially overlap with the ROPE. (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 (a) and (d), the 95%HDI for each strain was estimated using a Bayesian approach to estimate the probability of expressing the Eu mouth morph based on the observations (see **Material and Methods**).

Fig. 3: Cost of plasticity across conditions: (a) Mouth-morph ratios on *E. coli* and *Novosphingobium* for three *P. pacificus* strains, representing biased and intermediate mouth-morph ratios. A total of 150 animals were used to score the mouth-morph ratio for each strain per condition, indicating three biological replicates. The 95%HDI of means for each strain was estimated using the Bayesian approach, described in details in **Material and Methods**. **(b)** Overall fecundity of the three strains on both food conditions. The top panel indicates the 95% HDI for the estimated difference in means for each pairwise comparison. In all three pairwise comparisons, the 95% HDI for the estimated difference in means does not overlap with the ROPE, indicating credible differences between each pair. We used [-5,5] interval as our region of practical equivalence (ROPE), *i.e.*, differences of means within this interval are practically equal to no difference. The pairwise comparisons are based on Kruschke's BEST method (see **Materials and Methods**). Comparable numbers of mothers were used: RSC017(*E. coli*=40, *Novosphingobium*=47); RSC019 (*E. coli*=48, *Novosphingobium*=45); RS5405(*E. coli*=40, *Novosphingobium*=43) **(c)**. Developmental speed for the three genotypes on both conditions. Individual worms were staged 75 hours after mothers were killed; for RSC017(*E. coli*=320, *Novosphingobium*=301); RSC019 (*E. coli*=265, *Novosphingobium*=331); RS5405(*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.

Fig. 4: Costs of phenotype and plasticity in a spatially-homogeneous population: (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 (BA₁ to BA₅). **(b-c)** Population dynamics of RSC017 and RS5405 on *E. coli* and *Novosphingobium*. Note that the end point of 1000 steps represent an arbitrary endpoint, which roughly corresponds to 10 generations. Food will be gone long before this end point, as evident by the production of dauer larvae. Simulations based on empirical data demonstrate the differential response of the strains on both food conditions. The number of adults and dauer larvae for RSC017 in *E. coli* relative to their counts on

Novosphingobium are 1.05 and 1.09, respectively. For RS5405, 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 BA₁ and higher fecundity of RS5405 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. **(d)** Standard with-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 hours. **(e-f)** 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 RSC017 and RS5405 on *E. coli* and *Novosphingobium*. In both conditions, the non-plastic strain RS5405 drives RSC017 into extinction. Simulations in (b), (c) start with 50 YAs of a strain. The initial food supply, $S_0 = 10^{12}$. 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 Materials and Methods for more information). On *Novosphingobium*, $\gamma_{YA} = 0.13$ for RSC107 and $\gamma_{YA} = 0.4$ for RS5405 to account for the change in the developmental speed observed in the experiment. The same transition probabilities and survival are used for the rest of the simulations. Simulations in (e) and (f) start with 50 YAs of each strain. 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 predation rates are used in the subsequent simulations.

Fig. 5: Costs of phenotype and plasticity in a spatially-structured population: **(a)** A structured population, consisting of 12 localities are arranged in a line. Each simulation starts with 50 YAs of RSC017 on the first locality and 50 YAs of RS5405 on the 12th locality. At each step, ω dauer larvae migrate from population i to j if j has more food than i . **(b-c)** The frequency of RSC017 adults (YA, BA_{*i*}, OA) (b) and dauer larvae (c) across 12 localities with interaction (i.e., predation) after 1000 steps. As previously noted, the end point of 1000 steps represent an arbitrary endpoint, which roughly corresponds to 10 generations. Food will be gone long before as evident by the production of dauer larvae. **(d-e)** The frequency of RSC017 adults (YA, BA_{*i*}, OA) (d) and dauer larvae (e) across 12 localities assuming no interaction between the two strains. In this scenario, the frequency of RSC017 in the metapopulation is a function of developmental speed and fecundity only. 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 the entire metapopulation.

Materials and Methods

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 [40]. 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 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 [22]. 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 [16]. 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, RS1113= 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 [56]. 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 (*SI Appendix*; Fig. S1a).

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 (*SI Appendix*; Fig. S1b). The time point of 75hrs was chosen to capture the transition rate from juvenile stages to adulthood [57,58].

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 [59]. 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 μ l 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[59]. 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 (SI Appendix; Fig. S1c).

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 (Fig. 4a). The Markov chain is used to construct a stage-structured population model (for more on this approach to modelling population dynamics, see[60,61]). The projection matrix for this chain is:

$$\mathbf{A}_{B,S} = \begin{bmatrix} \text{E} & \text{J2} & \text{D} & \text{J3} & \text{J4} & \text{YA} & \text{BA}_1 & \dots & \text{BA}_5 & \text{OA} \\ 1 - \gamma_E & 0 & 0 & 0 & 0 & 0 & F_1 & \dots & F_5 & 0 \\ \gamma_E & (1 - \gamma_{J2})(1 - \gamma_{J2}^*) & 0 & 0 & 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & \gamma_{J2}^* & 1 - \gamma_D & 0 & 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & \gamma_{J2} & 0 & 1 - \gamma_{J3} & 0 & 0 & 0 & \dots & 0 & 0 \\ 0 & 0 & \gamma_D & \gamma_{J3} & 1 - \gamma_{J4} & 0 & 0 & \dots & 0 & 0 \\ 0 & 0 & 0 & 0 & \gamma_{J4} & 1 - \gamma_{YA} & 0 & \dots & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \gamma_{YA} & 1 - \gamma_{BA_1} & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \dots & \vdots & \vdots \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \dots & 1 - \gamma_{BA_5} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \dots & \gamma_{BA_5} & \delta_{OA} \end{bmatrix},$$

where γ_i is the probability of transition from developmental stage i into the next developmental stage. In the case of J2, γ_{J2} and γ_{J2}^* are the probabilities for $J2 \rightarrow J3$ and $J2 \rightarrow D$, respectively. Note that as long as food is available ($S_t > 0$), $J2 \rightarrow D$ 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 $J2 \rightarrow D$

is no longer zero ($\gamma_{J2}^* = 0.1$). The five breeding adult stages (BA₁ to BA₅) 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 RSC017 and RS5405 are listed in **Table 3**.

Transition probabilities			
	<i>E. coli</i>	<i>Novosphingobium</i>	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			
	<i>E. coli</i>	<i>Novosphingobium</i>	
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 3: 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. RSC017 specific values is indicated by * and RS5405 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 ($\mathbf{N}$) for transition matrix $\mathbf{U}$, where $\mathbf{N} = (\mathbf{I} - \mathbf{U})^{-1}$ (Caswell, 2019). 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 [57, 58]. We adjusted the probability of $YA \rightarrow BA_1$ 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:

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

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:

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

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 highly plastic strain, RSC017, the expected number of predatory adults equals the number of RSC017 adults in the population multiplied by the probability of developing the predatory mouth form on a given bacterial diet.

Population dynamic

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

$$\mathbf{n}_i(t + 1) = \mathbf{A}_t \mathbf{n}_i(t) - \mathbf{K}_i(t) - \mathbf{E}_i(t) \quad , \quad (3)$$

where $K_i(t)$ is the number of J2 individuals of strain i that were killed at time t and $E_i(t)$ is the number of dauer larvae that emigrated from the population at time t .

Structured population in one dimension

In order to investigate the effect of dispersal on the population dynamics, 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 $\omega = 0.1$.

Estimating the predation parameter

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

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

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.

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 Eu mouth morph, we assumed

the number of observed Eu worms in a sample of n worms follows the likelihood function $y \sim \text{Bernnoully}(\theta)$, 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 [62,63]: 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, $\text{Unifrom}(1,300)$. 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 [64]. All statistical analyses were conducted with PyMC3 version 3.9.3 in Python 3.7.1, 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., $\hat{R} = 1$ [65]. The code used to analyze the data 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.

Data availability

The software used to run all simulations and conduct all the data analysis was written in Python 3.7.1. For reproducibility, the code and the raw experimental data are available at (https://github.com/Kalirad/cost_of_plasticity).

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Supplementary figures captions

Fig. S1: 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 predation setup. (Adopted from Lightfoot et al, 2019).

Fig. S2: 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 three selected *P. pacificus* wild isolates on *Novosphingobium* (c), respectively.

Fig. S3: 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 negative-binomial model was fitted using PyMC3 and the credible estimate for $\beta_0 = 2.19$ ($1.81 \geq 95\%HDI \leq 2.56$) and $\beta_1 = 1.41$ ($1 \geq 95\%HDI \leq 1.82$). 95% HDP region indicates the highest posterior density. The orange dots are the mean estimate sampled from the posterior predictive distribution.

Fig. S4: 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. S5: The effect of interaction on the pattern of dauer larvae dispersal: We measured the total number of RSC017 and RS5405 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 5. The first locality (1) was the starting locality for RSC017, and the last locality (12) was the starting locality for RS5405. 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. S6: The interplay between developmental speed and fecundity in RSC017: 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 RSC017 by assuming no change in developmental speed. The difference in the steady-state counts of A and D stages on *E. coli* versus *Novosphingobium* compared to Fig. 4b supports this hypothesis.

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(Figure 1)

(Figure 2)

(Figure 3)

(Figure 4)

(Figure 5)

a

b

c

(Supplementary Figure 1)

(Supplementary Figure 2)

(Supplementary Figure 3)

(Supplementary Figure 4)

(Supplementary Figure 5)

(Supplementary Figure 6)

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	Experimental setup	Condition
RSS348	La Reunion, Trois Bassins (TB)/C	44	33	99,75	105,939939	29,03576383	30,79259184	intra-genotype cost of phenotype	<i>E. coli</i>
RSAL13	La Reunion, Trois Bassins (TB)/C	54	41	68,18518519	72,92682927	25,92738341	29,5375949	intra-genotype cost of phenotype	<i>E. coli</i>
RSAB22	Mauritius, Sugarcane Institute (MU)/C	50	49	121,5833333	140,0169492	45,12216625	40,81180188	intra-genotype cost of phenotype	<i>E. coli</i>
RSAB45	Mauritius, Lakaz Chamarel Med pla (MU)/A	55	54	148,6363636	160,8333333	38,48056924	40,74344489	intra-genotype cost of phenotype	<i>E. coli</i>
RSC019	La Reunion, Colorado (CO)/C	54	53	98,2037037	140,8490566	36,15030448	39,93330797	intra-genotype cost of phenotype	<i>E. coli</i>
RSC033	La Reunion, Grand Etang Lake-3 (GE)/C	54	47	97,92592593	125,5744681	36,31302685	48,02433472	intra-genotype cost of phenotype	<i>E. coli</i>
RSD029	La Reunion, Nez de Boeuf (NB)/B	46	54	143,3478261	146,7407407	31,13784221	32,59365906	intra-genotype cost of phenotype	<i>E. coli</i>

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	Experimental 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 plasticity	<i>E. coli</i>
RS4076	La Reunion, Nez de Boeuf (NB)/ B	40	0	148,3	0	148,3	42,70002702	0	42,70002702	inter-genotype cost of plasticity	<i>E. coli</i>
RSS405	La Reunion, Trois Bassins (TB)/ C	40	0	149,625	0	149,625	45,37348571	0	45,37348571	inter-genotype cost of plasticity	<i>E. coli</i>
RSC017	La Reunion, Colorado (CO)/ C	0	40	0	186,525	186,525	0	33,05394775	33,05394775	inter-genotype cost of plasticity	<i>E. coli</i>

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	Experimental setup	Condition
RSS4054	La Reunion, Trois Bassins (TB)/ C	43	0	193,0465116	0	193,0465116	33,0273721	0	33,0273721	Cost of phenotype	<i>Novosphingobium</i>
RSC017	La Reunion, Colorado (CO)/ C	47	0	136,2553191	0	136,2553191	27,9887152	0	27,9887152	Cost of phenotype	<i>Novosphingobium</i>
RSC019	La Reunion, Colorado (CO)/C	45	0	157,3777778	0	157,3777778	37,0321046	0	37,0321046	Cost of phenotype	<i>Novosphingobium</i>

(Table Supplementary 1)

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	<i>E. coli</i>	25,3816371	Eu	intra-genotype cost of phenotype
RS5348	41,90909091	Day 2	15,71522	44	<i>E. coli</i>	42,0141262	Eu	intra-genotype cost of phenotype
RS5348	22,79545455	Day 3	12,53011	44	<i>E. coli</i>	22,852586	Eu	intra-genotype cost of phenotype
RS5348	6,431818182	Day 4	10,78414	44	<i>E. coli</i>	6,44793803	Eu	intra-genotype cost of phenotype
RS5348	3,29545	Day 5	7,79558	44	<i>E. coli</i>	3,30370927	Eu	intra-genotype cost of phenotype
RS5348	23,42424242	Day 1	9,87747	33	<i>E. coli</i>	22,1110036	St	intra-genotype cost of phenotype
RS5348	44,9393	Day 2	11,41528	33	<i>E. coli</i>	42,4198574	St	intra-genotype cost of phenotype
RS5348	24,93939394	Day 3	15,51801	33	<i>E. coli</i>	23,5412108	St	intra-genotype cost of phenotype
RS5348	8,363636364	Day 4	11,01368	33	<i>E. coli</i>	7,89474384	St	intra-genotype cost of phenotype
RS5348	4,272727273	Day 5	11,39976	33	<i>E. coli</i>	4,03318435	St	intra-genotype cost of phenotype
RSA645	36,83636364	Day 1	11,11349	55	<i>E. coli</i>	24,7828746	Eu	intra-genotype cost of phenotype
RSA645	63,34545455	Day 2	14,95081	55	<i>E. coli</i>	42,617737	Eu	intra-genotype cost of phenotype
RSA645	39,54545455	Day 3	22,02593	55	<i>E. coli</i>	26,6055046	Eu	intra-genotype cost of phenotype
RSA645	6,036363636	Day 4	10,45619	55	<i>E. coli</i>	4,06116208	Eu	intra-genotype cost of phenotype
RSA645	2,872727273	Day 5	14,02650	55	<i>E. coli</i>	1,93272171	Eu	intra-genotype cost of phenotype
RSA645	36,74074074	Day 1	10,71621	54	<i>E. coli</i>	22,8439839	St	intra-genotype cost of phenotype
RSA645	75,22222222	Day 2	16,58104	54	<i>E. coli</i>	46,7702936	St	intra-genotype cost of phenotype
RSA645	44,92592593	Day 3	22,62979	54	<i>E. coli</i>	27,9332182	St	intra-genotype cost of phenotype
RSA645	2,018518519	Day 4	5,06371	54	<i>E. coli</i>	1,25503742	St	intra-genotype cost of phenotype
RSA645	1,925925926	Day 5	12,64143	54	<i>E. coli</i>	1,1974669	St	intra-genotype cost of phenotype
RSA622	12,4	Day 1	6,99927	50	<i>E. coli</i>	10,1987663	Eu	intra-genotype cost of phenotype
RSA622	53,86666667	Day 2	18,98391	50	<i>E. coli</i>	44,304318	Eu	intra-genotype cost of phenotype
RSA622	46,46666667	Day 3	28,50600	50	<i>E. coli</i>	38,2179575	Eu	intra-genotype cost of phenotype
RSA622	6	Day 4	9,42949	50	<i>E. coli</i>	4,93488691	Eu	intra-genotype cost of phenotype
RSA622	2,85	Day 5	9,37329	50	<i>E. coli</i>	2,34407128	Eu	intra-genotype cost of phenotype
RSA622	14,37288136	Day 1	7,74351	49	<i>E. coli</i>	10,2651011	St	intra-genotype cost of phenotype
RSA622	65,42372881	Day 2	20,17271	49	<i>E. coli</i>	46,725578	St	intra-genotype cost of phenotype
RSA622	48,96610169	Day 3	27,42071	49	<i>E. coli</i>	34,9715531	St	intra-genotype cost of phenotype
RSA622	6,898305085	Day 4	10,12626	49	<i>E. coli</i>	4,92676431	St	intra-genotype cost of phenotype
RSA622	4,355932203	Day 5	14,28860	49	<i>E. coli</i>	3,11100351	St	intra-genotype cost of phenotype
RSC019	22,61111111	Day 1	9,51546	54	<i>E. coli</i>	23,024703	Eu	intra-genotype cost of phenotype
RSC019	33,18518519	Day 2	22,06515	54	<i>E. coli</i>	33,7921931	Eu	intra-genotype cost of phenotype
RSC019	32,01851852	Day 3	13,87340	54	<i>E. coli</i>	32,6041863	Eu	intra-genotype cost of phenotype
RSC019	6,611111111	Day 4	9,97434	54	<i>E. coli</i>	6,73203847	Eu	intra-genotype cost of phenotype
RSC019	3,777777778	Day 5	11,58344	54	<i>E. coli</i>	3,84687913	Eu	intra-genotype cost of phenotype
RSC019	19,52830189	Day 1	7,97015	53	<i>E. coli</i>	13,8647019	St	intra-genotype cost of phenotype
RSC019	58,88679245	Day 2	19,53071	53	<i>E. coli</i>	41,8084394	St	intra-genotype cost of phenotype
RSC019	48,05660377	Day 3	19,27475	53	<i>E. coli</i>	34,119223	St	intra-genotype cost of phenotype
RSC019	8,773584906	Day 4	9,97434	53	<i>E. coli</i>	6,22906899	St	intra-genotype cost of phenotype
RSC019	5,603773585	Day 5	14,41999	53	<i>E. coli</i>	3,97856664	St	intra-genotype cost of phenotype
RSC011	17,53658537	Day 1	8,553062495	41	<i>E. coli</i>	10,4278463	highly st	inter-genotype cost of plasticity
RSC011	76,24390244	Day 2	23,9629923	41	<i>E. coli</i>	45,3372009	highly st	inter-genotype cost of plasticity
RSC011	62,26829268	Day 3	27,51064081	41	<i>E. coli</i>	37,026831	highly st	inter-genotype cost of plasticity
RSC011	8	Day 4	14,91978552	41	<i>E. coli</i>	4,75707034	highly st	inter-genotype cost of plasticity
RSC011	4,12195122	Day 5	13,14000594	41	<i>E. coli</i>	2,45105149	highly st	inter-genotype cost of plasticity
RSA076	10,675	Day 1	7,230233886	40	<i>E. coli</i>	7,1982468	highly Eu	inter-genotype cost of plasticity
RSA076	54,375	Day 2	16,02031963	40	<i>E. coli</i>	36,6655428	highly Eu	inter-genotype cost of plasticity
RSA076	57,875	Day 3	21,06624728	40	<i>E. coli</i>	39,0256237	highly Eu	inter-genotype cost of plasticity
RSA076	23,2	Day 4	20,65491808	40	<i>E. coli</i>	15,6439649	highly Eu	inter-genotype cost of plasticity
RSA076	2,175	Day 5	4,684330125	40	<i>E. coli</i>	1,46662171	highly Eu	inter-genotype cost of plasticity
RSC017	22,65	Day 1	6,290204024	40	<i>E. coli</i>	12,1431444	highly st	inter-genotype cost of plasticity
RSC017	68,45	Day 2	10,19684797	40	<i>E. coli</i>	36,6974936	highly st	inter-genotype cost of plasticity
RSC017	57,05	Day 3	15,19606899	40	<i>E. coli</i>	30,5857124	highly st	inter-genotype cost of plasticity
RSC017	33,4	Day 4	18,09348941	40	<i>E. coli</i>	17,9064469	highly st	inter-genotype cost of plasticity
RSC017	4,975	Day 5	5,757882	40	<i>E. coli</i>	2,66720279	highly st	inter-genotype cost of plasticity
RS5405	19,8	Day 1	5,302152441	40	<i>E. coli</i>	13,2696386	highly Eu	inter-genotype cost of plasticity
RS5405	60,3	Day 2	13,61597855	40	<i>E. coli</i>	40,4120813	highly Eu	inter-genotype cost of plasticity
RS5405	43,025	Day 3	18,94457165	40	<i>E. coli</i>	28,8346567	highly Eu	inter-genotype cost of plasticity
RS5405	19,4878049	Day 4	16,20667795	40	<i>E. coli</i>	13,0604105	highly Eu	inter-genotype cost of plasticity
RS5405	6,6	Day 5	4,684330125	40	<i>E. coli</i>	4,42321288	highly Eu	inter-genotype cost of plasticity
RSC017	11,67391304	Day 1	6,09208721	47	<i>Novosphingobium</i>	8,58419751	on <i>E. coli</i> highly St	cost of plasticity
RSC017	62,53191489	Day 2	17,5261929	47	<i>Novosphingobium</i>	45,9816949	on <i>E. coli</i> highly St	cost of plasticity
RSC017	47,12765957	Day 3	13,86835155	47	<i>Novosphingobium</i>	34,6544588	on <i>E. coli</i> highly St	cost of plasticity
RSC017	13,93617021	Day 4	12,39460657	47	<i>Novosphingobium</i>	10,2477068	on <i>E. coli</i> highly St	cost of plasticity
RSC017	0,723404255	Day 5	2,337800226	47	<i>Novosphingobium</i>	0,53194203	on <i>E. coli</i> highly St	cost of plasticity
RSC019	16,86666667	Day 1	6,652408996	45	<i>Novosphingobium</i>	10,6020394	on <i>E. coli</i> unbiased	cost of plasticity
RSC019	66,35555556	Day 2	16,87541993	45	<i>Novosphingobium</i>	41,709736	on <i>E. coli</i> unbiased	cost of plasticity
RSC019	61,86666667	Day 3	20,0086345	45	<i>Novosphingobium</i>	38,8881129	on <i>E. coli</i> unbiased	cost of plasticity
RSC019	13,44444444	Day 4	15,10953275	45	<i>Novosphingobium</i>	8,45090096	on <i>E. coli</i> unbiased	cost of plasticity
RSC019	0,55555556	Day 5	1,778093406	45	<i>Novosphingobium</i>	0,34921078	on <i>E. coli</i> unbiased	cost of plasticity
RS5405	16,88372093	Day 1	8,555752742	43	<i>Novosphingobium</i>	8,76315975	on <i>E. coli</i> highly Eu	cost of plasticity
RS5405	80,76744186	Day 2	16,65595448	43	<i>Novosphingobium</i>	41,9207353	on <i>E. coli</i> highly Eu	cost of plasticity
RS5405	77,34146341	Day 3	20,45382126	43	<i>Novosphingobium</i>	40,1425493	on <i>E. coli</i> highly Eu	cost of plasticity
RS5405	16,27906977	Day 4	13,30471735	43	<i>Novosphingobium</i>	8,44932759	on <i>E. coli</i> highly Eu	cost of plasticity
RS5405	1,395348837	Day 5	3,193293082	43	<i>Novosphingobium</i>	0,72422808	on <i>E. coli</i> highly Eu	cost of plasticity

(Table Supplementary 2)

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	<i>E. coli</i>
RSC017	72	0	0	3	6	63	0	9	0	0	4,166667	8,333333	87,5	0	<i>E. coli</i>
RSC017	66	1	1	1	8	55	0	10	1,515152	1,515152	1,515152	12,12121	83,33333	0	<i>E. coli</i>
RSC017	62	0	0	1	0	61	0	9	0	0	1,612903	0	98,3871	0	<i>E. coli</i>
RSC017	50	0	0	0	3	47	0	10	0	0	0	6	94	0	<i>E. coli</i>
RS5405	69	0	0	0	13	56	0	10	0	0	0	18,84058	81,15942	0	<i>E. coli</i>
RS5405	51	0	0	0	10	41	0	10	0	0	0	19,60784	80,39216	0	<i>E. coli</i>
RS5405	71	0	1	0	12	58	0	10	0	1,408451	0	16,90141	81,69014	0	<i>E. coli</i>
RS5405	56	0	1	0	7	48	0	10	0	1,785714	0	12,5	85,71429	0	<i>E. coli</i>
RS5405	59	0	0	1	6	52	0	10	0	0	1,694915	10,16949	88,13559	0	<i>E. coli</i>
RSC019	54	0	0	0	28	26	0	10	0	0	0	51,85185	48,14815	0	<i>E. coli</i>
RSC019	59	0	3	0	16	40	0	10	0	5,084746	0	27,11864	67,79661	0	<i>E. coli</i>
RSC019	47	0	1	1	23	22	0	10	0	2,12766	2,12766	48,93617	46,80851	0	<i>E. coli</i>
RSC019	53	0	2	0	9	42	0	10	0	3,773585	0	16,98113	79,24528	0	<i>E. coli</i>
RSC019	52	1	1	0	15	35	0	10	1,923077	1,923077	0	28,84615	67,30769	0	<i>E. coli</i>
RSC011	68	0	0	1	31	36	0	10	0	0	1,470588	45,58824	52,94118	0	<i>E. coli</i>
RSC011	98	0	1	1	33	63	0	9	0	1,020408	1,020408	33,67347	64,28571	0	<i>E. coli</i>
RSC011	41	0	0	0	21	20	0	10	0	0	0	51,21951	48,78049	0	<i>E. coli</i>
RSC011	56	0	0	0	17	39	0	9	0	0	0	30,35714	69,64286	0	<i>E. coli</i>
RSA076	50	0	1	1	38	10	0	10	0	2	2	76	20	0	<i>E. coli</i>
RSA076	59	0	1	4	44	10	0	9	0	1,694915	6,779661	74,57627	16,94915	0	<i>E. coli</i>
RSA076	70	0	0	1	53	16	0	9	0	0	1,428571	75,71429	22,85714	0	<i>E. coli</i>
RSA076	53	0	0	0	26	27	0	9	0	0	0	49,0566	50,9434	0	<i>E. coli</i>
RSC017	72	0	0	0	5	55	12	10	0	0	0	6,944444	76,38889	16,66667	<i>Novosphingobium</i>
RSC017	87	0	0	0	2	67	18	10	0	0	0	2,298851	77,01149	20,68966	<i>Novosphingobium</i>
RSC017	65	0	0	0	6	40	19	9	0	0	0	9,230769	61,53846	29,23077	<i>Novosphingobium</i>
RSC017	77	0	0	2	3	47	25	10	0	0	2,597403	3,896104	61,03896	32,46753	<i>Novosphingobium</i>
RS5405	124	0	0	0	3	46	75	10	0	0	0	2,419355	37,09677	60,48387	<i>Novosphingobium</i>
RS5405	131	0	0	1	1	21	108	10	0	0	0,763359	0,763359	16,03053	82,44275	<i>Novosphingobium</i>
RS5405	126	0	0	0	0	13	113	10	0	0	0	10,31746	89,68254		<i>Novosphingobium</i>
RS5405	108	0	0	0	4	12	92	10	0	0	0	3,703704	11,11111	85,18519	<i>Novosphingobium</i>
RSC019	86	0	0	0	0	72	14	10	0	0	0	0	83,72093	16,27907	<i>Novosphingobium</i>
RSC019	73	0	0	0	2	46	25	10	0	0	0	2,739726	63,0137	34,24658	<i>Novosphingobium</i>
RSC019	83	0	0	2	3	43	35	10	0	0	2,409639	3,614458	51,80723	42,16867	<i>Novosphingobium</i>
RSC019	89	0	0	0	0	48	41	10	0	0	0	0	53,93258	46,06742	<i>Novosphingobium</i>

(Table Supplementary 3)

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

(Table Supplementary 4)

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

(Table Supplementary 5)

		mean	sd	hdi_2.5%	hdi_97.5%	mcse_mean	mcse_sd	ess_mean	ess_sd	ess_bulk	ess_tail	r_hat
RS5348	group1_mean	106.35	5.54	95.36	117.27	0.03	0.02	44288.58	44288.58	44459.22	29104.65	1.0
	group2_mean	100.24	4.55	91.47	109.22	0.02	0.01	52011.46	51888.25	52073.74	31707.78	1.0
	group1_std	28.79	3.49	22.40	35.90	0.02	0.01	40484.90	40044.28	40107.94	29067.03	1.0
	group2_std	30.94	4.21	23.41	39.55	0.02	0.01	46105.51	43578.48	48035.44	29783.35	1.0
	ν _minus_one	39.55	30.60	2.45	100.24	0.15	0.12	40548.75	34484.50	38321.99	29693.67	1.0
	difference of means	6.10	7.16	-8.09	20.04	0.03	0.03	47002.57	32385.34	47019.65	31337.94	1.0
	difference of stds	-2.15	5.34	-12.93	8.16	0.02	0.02	47861.82	24887.63	48419.06	30915.78	1.0
	effect size	0.20	0.24	-0.26	0.67	0.00	0.00	48479.04	33778.71	48479.04	32474.91	1.0
RSA113	group1_mean	72.43	4.71	63.33	81.79	0.02	0.02	38494.22	38338.84	38625.12	29630.08	1.0
	group2_mean	67.47	3.73	60.26	74.88	0.02	0.01	35369.72	35122.37	35454.78	29608.96	1.0
	group1_std	25.04	2.96	19.45	31.09	0.02	0.01	31425.22	31425.22	30631.53	23335.99	1.0
	group2_std	28.73	3.79	21.65	36.37	0.02	0.01	36190.61	36190.61	35353.63	26825.76	1.0
	ν _minus_one	30.29	27.80	1.58	85.69	0.16	0.12	31626.48	29183.47	26499.70	22546.26	1.0
	difference of means	4.96	5.99	-6.71	16.77	0.03	0.02	38678.71	29701.85	38709.34	30238.24	1.0
	difference of stds	-3.69	4.54	-12.89	4.85	0.02	0.02	50760.35	31125.47	51249.34	30618.26	1.0
	effect size	0.18	0.22	-0.24	0.63	0.00	0.00	38403.69	29573.92	38385.19	30436.70	1.0
RSA622	group1_mean	140.37	5.49	129.50	151.01	0.02	0.02	53904.73	53863.07	54013.50	31536.61	1.0
	group2_mean	122.04	6.06	110.26	134.06	0.03	0.02	58497.36	58301.66	58554.71	30948.75	1.0
	group1_std	45.27	4.45	37.00	54.15	0.02	0.01	47137.68	45665.99	48520.77	31873.18	1.0
	group2_std	40.73	4.15	33.05	49.16	0.02	0.01	50387.56	48675.23	51706.14	30854.68	1.0
	ν _minus_one	47.21	33.10	4.63	112.85	0.16	0.12	45293.25	35808.20	50484.83	30000.31	1.0
	difference of means	18.33	8.21	2.48	34.48	0.03	0.03	56920.94	49831.68	56926.73	32492.54	1.0
	difference of stds	4.54	6.00	-7.28	16.38	0.03	0.02	52755.12	31794.16	53112.82	31238.64	1.0
	effect size	0.43	0.19	0.06	0.81	0.00	0.00	57735.40	51414.98	57704.25	32517.12	1.0
RSA645	group1_mean	161.32	5.51	150.61	172.23	0.03	0.02	29696.94	29696.94	29727.32	27777.68	1.0
	group2_mean	148.03	5.07	138.15	158.13	0.03	0.02	33176.32	33172.21	33210.03	28720.70	1.0
	group1_std	34.82	4.74	25.39	43.98	0.03	0.02	20351.06	20351.06	19906.60	15117.07	1.0
	group2_std	37.28	5.16	27.08	47.63	0.04	0.03	20961.56	20961.56	20823.39	15012.76	1.0
	ν _minus_one	19.10	21.76	0.68	62.98	0.14	0.10	24942.00	24942.00	16744.65	15051.39	1.0
	difference of means	13.29	7.52	-1.45	28.13	0.04	0.03	30697.45	29006.35	30708.63	28439.65	1.0
	difference of stds	-2.46	5.80	-13.42	9.28	0.03	0.03	44305.30	24135.03	44477.83	30354.09	1.0
	effect size	0.37	0.22	-0.06	0.79	0.00	0.00	26926.55	24017.49	27090.24	24570.85	1.0
RSC019	group1_mean	141.00	5.67	130.12	152.28	0.03	0.02	50926.85	50814.60	51097.71	32148.50	1.0
	group2_mean	98.42	5.19	87.89	108.29	0.02	0.01	61677.20	61401.01	61936.33	31875.30	1.0
	group1_std	36.45	3.83	29.50	44.20	0.02	0.01	47860.74	46007.86	49607.05	32207.17	1.0
	group2_std	39.51	4.37	31.21	48.15	0.02	0.01	46458.58	45334.95	47074.59	30847.29	1.0
	ν _minus_one	43.53	32.06	3.20	106.10	0.16	0.12	41438.60	34412.42	43317.58	30879.68	1.0
	difference of means	42.58	7.71	27.33	57.55	0.03	0.02	55793.15	54133.14	55808.36	33027.60	1.0
	difference of stds	-3.06	5.73	-14.29	8.30	0.03	0.02	51630.58	27138.29	51822.58	31195.47	1.0
	effect size	1.12	0.22	0.71	1.57	0.00	0.00	53821.11	53231.61	53714.18	32412.45	1.0
RSC033	group1_mean	126.02	7.35	111.40	140.26	0.03	0.02	49886.01	49757.38	49939.36	31496.47	1.0
	group2_mean	97.68	5.18	87.51	107.80	0.02	0.02	56855.55	56855.55	56953.60	32201.96	1.0
	group1_std	36.52	3.84	29.23	44.06	0.02	0.01	48872.02	47041.67	50632.76	32242.19	1.0
	group2_std	48.48	5.43	38.49	59.32	0.02	0.02	50161.88	47803.66	52465.20	31056.12	1.0
	ν _minus_one	47.32	33.00	4.31	112.04	0.15	0.12	45438.58	34962.37	52086.13	30450.19	1.0
	difference of means	28.34	9.00	11.31	46.58	0.04	0.03	51251.75	48144.35	51301.57	32921.36	1.0
	difference of stds	-11.97	6.61	-24.87	1.11	0.03	0.02	52766.04	41823.87	53399.79	31224.45	1.0
	effect size	0.66	0.21	0.24	1.08	0.00	0.00	51295.43	49900.73	51222.00	32243.60	1.0
RSD029	group1_mean	150.47	3.86	142.66	157.81	0.02	0.02	26840.09	26840.09	27461.50	25228.92	1.0
	group2_mean	148.00	4.01	140.03	155.70	0.02	0.02	26725.97	26725.97	26946.35	24328.41	1.0
	group1_std	22.01	4.20	14.13	30.23	0.03	0.02	21833.11	21833.11	21714.85	24084.62	1.0
	group2_std	23.59	4.42	15.33	32.34	0.03	0.02	20004.84	20004.84	19970.91	24013.22	1.0
	ν _minus_one	4.07	5.98	0.23	10.89	0.05	0.03	17423.34	17423.34	18718.25	18563.22	1.0
	difference of means	2.47	5.38	-8.12	13.04	0.03	0.03	30095.83	22559.26	30149.17	25893.37	1.0
	difference of stds	-1.58	4.60	-10.67	7.46	0.02	0.02	43416.62	23634.96	43374.54	30787.33	1.0
	effect size	0.11	0.24	-0.34	0.59	0.00	0.00	30840.64	23145.28	30856.97	27514.64	1.0

(Table Supplementary 6)

			mean	sd	hdi_2.5%	hdi_97.5%	mcse_mean	mcse_sd	ess_mean	ess_sd	ess_bulk	ess_tail	r_hat
RSC011	RSA076	group1_mean	173.11	6.60	159.80	185.68	0.05	0.03	20055.49	20055.49	20307.51	20799.06	1.0
		group2_mean	155.91	6.49	142.80	168.20	0.05	0.04	17058.45	17058.45	17493.96	19034.09	1.0
		group1_std	31.45	7.53	18.36	46.26	0.07	0.05	12754.75	12754.75	12672.96	17474.07	1.0
		group2_std	34.38	7.33	20.79	48.86	0.06	0.04	13767.41	13767.41	13532.92	16649.57	1.0
		ν _minus_one	8.20	14.79	0.05	34.74	0.12	0.08	16354.65	16354.65	11972.32	18171.42	1.0
		difference of means	17.20	8.49	0.42	33.67	0.05	0.04	26719.07	24856.96	26764.59	23583.41	1.0
		difference of stds	-2.93	7.24	-17.42	11.20	0.04	0.03	33344.50	21506.83	33410.45	26520.92	1.0
		effect size	0.54	0.28	0.00	1.08	0.00	0.00	23262.27	21803.22	23383.43	23893.89	1.0
RSC017	RS5405	group1_mean	187.83	5.50	177.48	199.00	0.03	0.02	47392.11	47378.63	47586.81	30652.88	1.0
		group2_mean	150.31	7.62	135.37	165.16	0.04	0.03	42081.11	41894.70	42248.44	29312.61	1.0
		group1_std	45.66	5.78	35.00	57.12	0.03	0.02	38915.14	37575.96	40104.00	29175.40	1.0
		group2_std	32.20	4.34	24.20	41.10	0.02	0.02	35056.14	35056.14	34412.62	25372.71	1.0
		ν _minus_one	35.59	29.43	1.88	93.97	0.16	0.11	35502.55	33664.83	31016.51	24524.15	1.0
		difference of means	37.52	9.37	19.23	56.05	0.04	0.03	46285.23	44490.64	46385.14	31252.22	1.0
		difference of stds	13.46	7.06	0.05	27.66	0.03	0.03	43853.56	36052.82	44634.26	30176.78	1.0
		effect size	0.96	0.26	0.44	1.44	0.00	0.00	42294.03	41242.60	42318.11	31393.10	1.0
RSC011	RS5405	group1_mean	169.51	6.90	155.66	182.67	0.03	0.02	40465.22	40465.22	40583.91	29967.73	1.0
		group2_mean	150.30	7.55	135.60	165.28	0.03	0.02	48130.69	47929.46	48267.64	29891.28	1.0
		group1_std	45.59	5.74	34.96	57.04	0.03	0.02	38202.31	37327.33	38795.11	29926.13	1.0
		group2_std	41.75	5.71	30.84	53.37	0.03	0.02	31156.75	31156.75	30539.75	20989.48	1.0
		ν _minus_one	35.52	29.67	1.85	94.59	0.16	0.11	36030.15	34366.06	29709.41	22856.79	1.0
		difference of means	19.21	10.20	-1.17	38.88	0.05	0.04	45381.95	41240.13	45437.15	30700.41	1.0
		difference of stds	3.85	7.81	-11.31	19.54	0.04	0.03	44107.74	26317.30	44324.06	30590.77	1.0
		effect size	0.44	0.24	-0.02	0.91	0.00	0.00	42592.21	39285.06	42523.00	30975.96	1.0
RSC017	RSA076	group1_mean	190.62	5.57	179.83	201.50	0.04	0.03	20170.80	20170.80	20191.90	25430.51	1.0
		group2_mean	155.19	6.61	141.84	167.54	0.05	0.03	18740.82	18740.82	19094.64	22424.67	1.0
		group1_std	32.92	7.41	19.17	47.02	0.06	0.04	14327.45	14327.45	13994.00	17528.73	1.0
		group2_std	27.97	5.20	17.79	37.89	0.04	0.03	16279.51	16279.51	15866.95	14238.72	1.0
		ν _minus_one	10.19	16.65	0.07	41.27	0.12	0.09	18361.16	18361.16	12963.22	19656.19	1.0
		difference of means	35.44	7.80	20.43	51.10	0.05	0.03	27442.44	26584.28	27506.64	26097.59	1.0
		difference of stds	4.95	6.76	-8.40	18.32	0.04	0.03	28478.22	27589.53	28333.95	24675.94	1.0
		effect size	1.19	0.33	0.56	1.84	0.00	0.00	18013.75	16708.00	18856.68	18107.95	1.0

(Table Supplementary 7)

			mean	sd	hdi_2.5%	hdi_97.5%	mcse_mean	mcse_sd	ess_mean	ess_sd	ess_bulk	ess_tail	r_hat
RSC017		group1_mean	136.68	4.03	128.76	144.61	0.02	0.02	30134.98	30089.30	30142.38	28043.68	1.0
		group2_mean	188.76	5.49	177.72	199.24	0.03	0.02	26691.97	26671.53	26779.34	27794.28	1.0
		group1_std	30.74	4.76	21.44	40.23	0.03	0.02	21145.26	21145.26	20600.88	16254.60	1.0
		group2_std	25.78	3.84	18.27	33.49	0.03	0.02	18301.01	18301.01	18205.07	14843.09	1.0
		ν _minus_one	22.33	24.57	0.54	72.34	0.16	0.11	23904.73	23904.73	15621.20	15418.15	1.0
		difference of means	-52.08	6.75	-65.89	-39.28	0.04	0.03	28356.32	28356.32	28331.43	27488.67	1.0
		difference of stds	4.96	5.19	-5.36	15.01	0.03	0.02	42592.26	31112.81	42895.42	30491.32	1.0
		effect size	-1.85	0.38	-2.63	-1.14	0.00	0.00	15278.14	14008.37	16916.90	15006.72	1.0
RSC019		group1_mean	159.43	5.56	148.20	170.08	0.03	0.02	35074.09	35074.09	35326.07	28533.97	1.0
		group2_mean	120.43	6.53	107.60	133.11	0.03	0.02	34973.10	34922.50	35048.27	29085.70	1.0
		group1_std	42.05	5.25	32.24	52.69	0.03	0.02	29378.54	29378.54	28916.58	23523.62	1.0
		group2_std	34.03	4.91	24.37	43.64	0.03	0.02	23215.59	23215.59	22619.31	19544.81	1.0
		ν _minus_one	24.41	25.24	0.87	74.82	0.16	0.11	25448.29	25448.29	19098.29	21041.60	1.0
		difference of means	38.99	8.48	22.60	55.81	0.04	0.03	36163.81	36163.81	36196.96	30852.16	1.0
		difference of stds	8.02	6.48	-4.88	20.75	0.03	0.03	42905.59	31911.02	43313.34	29541.96	1.0
		effect size	1.03	0.25	0.56	1.54	0.00	0.00	28231.13	28231.13	28293.33	27728.87	1.0
RS5405		group1_mean	193.22	5.12	183.27	203.41	0.02	0.02	47608.73	47608.73	47716.57	29737.23	1.0
		group2_mean	150.31	7.58	135.20	164.92	0.03	0.02	47988.22	47905.57	48091.29	29946.84	1.0
		group1_std	45.62	5.73	35.16	57.26	0.03	0.02	39810.10	38774.98	40479.30	30143.67	1.0
		group2_std	32.10	4.14	24.46	40.66	0.02	0.02	30237.53	30237.53	29904.20	24384.60	1.0
		ν _minus_one	35.76	29.71	1.76	95.44	0.16	0.12	35205.23	32927.22	32069.09	25974.07	1.0
		difference of means	42.91	9.15	24.64	60.53	0.04	0.03	47872.99	46705.32	47882.70	31455.53	1.0
		difference of stds	13.53	6.86	0.43	27.28	0.03	0.03	43146.27	37519.75	43490.71	31025.20	1.0
		effect size	1.09	0.25	0.59	1.58	0.00	0.00	46283.55	45517.30	46213.44	30409.33	1.0

(Table Supplementary 8)