Article

C. elegans sperm and oocytes differentially transmit diet-induced adaptations to their progeny

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Non-genetic inheritance allows organisms to transmit recently acquired adaptive information to progeny to maximize fitness in response to environmental change. While *Caenorhabditis elegans* exhibits persistent multigenerational responses to transient stress, how non-genetic mechanisms respond to multigenerational environmental change remains largely unexplored. As a bacterivore exposed to diverse microbes, *C. elegans* offers a powerful model to study adaptation to a persistent environmental change. We measured reproductive fitness via hermaphrodite self-brood size and found that novel diets often caused 20–45% brood-size reductions compared to controls. However, animals adapt to the new diet producing normal-sized broods within 5 to 10 generations. However, these adapted animals often became maladapted to their previous diet but could re-adapt within a similar timeframe. This rapidity and reversibility, which was also observed in genetically identical isogenic lines, strongly suggest a non-genetic mechanism. Phenotypic analyses of maladapted animals revealed a correlation between specific diets and germline defects primarily affecting either sperm or oocytes. Crosses between differently adapted parents demonstrated that sperm primarily transmitted sperm adaptations and oocytes primarily transmitted oocyte adaptations. Together, these results highlight the critical role of non-genetic inheritance as a flexible and heritable mechanism enabling organisms to rapidly adapt to unpredictable environmental change.

The ability of organisms to transmit acquired information about recent experiences across generations challenges traditional views of inheritance and adaptation[1]. Recent studies in *Caenorhabditis elegans*, a leading model for studying non-genetic inheritance, have documented that transient environmental stresses like starvation, hypoxia, temperature fluctuations, and pathogen exposure can drive heritable phenotypic changes[2–10]. However, by experimental design, these and other studies have focused on heritable responses – or "memory"– to transient environmental stresses. A critical dimension for heritable responses that remains under-studied involves adaptive responses to ongoing, ecologically relevant stimuli, such as growth temperature and shifts in bacterial food sources[11].

In natural environments, *C. elegans* populations undergo boom-and-bust cycles of growth and dispersion driven by fluctuations in bacterial abundance, rendering dietary shifts both ecologically significant and experimentally amenable for investigation[12]. Recent work has begun to explore how microbial environments influence heritable phenotypes. For example, Burton et al. [13] demonstrated that maternal exposure to *Pseudomonas vranovensis* enhances progeny resistance to infection via maternal activation of the cysteine synthases CYSL-1 and CYSL-2[13]. This study provides compelling evidence for heritable, diet-induced, pathogen-related adaptations in *C. elegans*, even in the absence of direct exposure of the offspring to the microbe. Similarly, Palominos et al.[14] demonstrated a remarkable transgenerational

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adaptive response, where *C. elegans* exposed to pathogenic bacteria for two generations transmitted information via the maternal germ line that induced offspring to enter diapause by forming dauer larvae, a developmental strategy that effectively prevents subsequent infection through mouth closure[14]. This study represents an elegant example of how persistent ecological threats can trigger complex heritable survival strategies beyond simple immune priming. While these studies demonstrate how nematodes can transmit ecologically relevant information across generations, altering developmental trajectories in anticipation of continuing environmental challenges[15], broader investigations into persistent ecological pressures, such as long-term shifts in food sources, remain sparse.

A critical conceptual gap persists between studies of transgenerational epigenetic inheritance (TEI) and the phenotypic inheritance of adaptive physiological tuning. While TEI emphasizes the fidelity and persistence of specific non-genetic inheritance signals across generations, heritable adaptive responses highlight the capacity for dynamic, context-dependent adjustments that enhance fitness in fluctuating environments[15]. This distinction underscores the need to understand not just how non-genetic information is faithfully transmitted, but how it can be flexibly modulated in response to persistent ecological pressures.

We previously demonstrated that exposing *C. elegans* to a transitory environmental change for two consecutive generations, rather than just one, increased the magnitude and perdurance of the heritable non-genetic response[11]. This finding led us to hypothesize that a selected function of heritable non-genetic mechanisms may be to enable adaptation to current environmental conditions. In the laboratory, *C. elegans* is typically cultured on the *Escherichia coli* strain OP50, a uracil auxotroph[16]. While *E. coli* is not recovered from wild-collected *C. elegans*, it is generally assumed to be a neutral food source. In contrast, the common soil bacterium *Bacillus subtilis* is less pathogenic to *C. elegans* and therefore may more accurately reflect the nematode's natural interactions with environmental microbes[17]. To further explore the diversity of bacteria encountered by wild *C. elegans* and their ecological significance, researchers have characterized both gut-associated and environmental microbiota, identifying hundreds of distinct bacterial species[18–22]. Among these, 12 gut-colonizing species were selected as phylogenetically representative of the *C. elegans* microbiome and assembled as a model Microbiome Resource (CeMbio)[22]. We have used representatives of the CeMbio to investigate adaptation to changing food sources in *C. elegans*.

Here we investigate adaptation to new bacterial diets over multiple generations (multigenerational adaptation). To investigate multigenerational adaptation in *C. elegans*, we shift to and maintain worms on new bacterial diets while measuring individual brood sizes at each generation. We find that worms exposed to new bacterial diets often exhibit initially reduced fecundity, associated with maladapted germ cells. Worms maintained on the new food gradually recover their reproductive capacity over 5-10 generations – indicative of heritable adaptive tuning. Notably, this adaptation often incurs a fitness cost: return to the previous diet is often associated with reduced fecundity and a similar period of re-adaptation. Phenotypic analysis indicates food-specific germ cell dysfunction and reciprocal crosses between differently adapted animals reveal that oocytes and sperm transmit distinct adaptive information. This flexible and robust non-genetic adaptation likely contributes to the broader landscape of adaptation mechanisms that enable organisms to thrive in variable ecosystems.

Results

Growth on novel bacteria reduces self-brood size
We assessed reproductive fitness by measuring hermaphrodite self-brood size as a primary metric to evaluate multigenerational adaptation. Specifically, we measured the total self-brood size of individual wild-type hermaphrodites grown on four different bacterial species;

E. coli (the lab strain OP50 [OP]), the soil bacterium *B. subtilis* (BS), and two members of the *C. elegans* model microbiome, *Pseudomonas berkeleyensis* (strain MSPm1 [PB]) and *Sphingobacterium multivorum* (strain BIGb0170 [SM])[22]. Wild-type (N2) adults, adapted to OP for greater than 10 generations at 25 °C, were treated with bleach to isolate generation 0 (G0) aseptic embryos, which were then placed on each bacterial species as their sole food source (Fig. 1a). We selected 25 °C to shorten the overall duration for multigenerational studies. To avoid any maternal effects from prior OP growth, brood size measurement began with the G1 progeny. For each food condition, single larval stage 4 (L4) G1 animals were transferred to individual plates seeded with each bacterial species. Because it is difficult to count embryos and larvae in thick bacterial lawns, we waited until the progeny reached L4 or early adulthood to count them. Since only adult progeny can reproduce, counting adult offspring serves as an effective measure of reproductive fitness.

We found that G1 animals maintained on OP or grown on BS produced ~300 adult self-progeny, while animals grown on SM and PB produced average total brood sizes ranging from 70 to 125 (Fig. 1b). Analysis of G0 embryo hatching rates revealed that over 90% of the eggs laid by animals grown on OP, BS, and PB successfully hatched, whereas fewer than 50% of the eggs from SM-grown animals hatched (Fig. 1c). Thus, reduced egg production primarily accounts for the decreased brood size in PB-grown animals, whereas in SM-grown animals, reduced egg viability accounted for one-third of the decreased brood, with diminished egg production accounting for the remainder.

We also observed numerous oocytes on plates seeded with PB, suggesting a potential fertilization defect[23,24]. To determine whether this defect stemmed from issues with sperm or oocytes, we crossed wild-type OP-grown adult males to G0 hermaphrodites that had not laid a fertilized egg for two days, indicating either depletion of self-sperm and/or dysfunctional oocytes. Most OP-, BS-, and PB-grown G0 hermaphrodites produced cross-progeny (Fig. 1d), indicating the presence of fertilizable oocytes. This suggests that the reduced brood size of PB-grown animals is likely due to fewer functional sperm. Conversely, SM-grown hermaphrodites rarely produced cross-progeny, indicating an absence of functional oocytes. Consequently, we conclude that dysfunctional oogenesis may contribute to the reduced brood size observed in SM-grown animals. Additionally, SM- and PB-grown worms produced 15-20 times more males than OP grown animals (Fig. 1e). In *C. elegans*, X-chromosome non-disjunction leads to the production of XO male animals, a phenomenon that can be exacerbated by stressful growth conditions[25]. This suggests that the transition to these two bacterial foods may induce germline stress in the worms.

The induction of germline stress may indicate a pathogen response. To assay immune and stress responses we used an *irg-Sp::gfp* reporter, which indicates innate immune activation via the p38 MAPK pathway[26] and *hsp-6p::gfp*, which responds to mitochondrial stress and protein homeostasis disruption[27]. These experiments differ from typical pathogen response assays, as we expose animals from hatching to adulthood, rather than focusing on an acute response. Relative to animals maintained on OP, only the OP to SM transition (OP→SM) triggered a significant increase in *hsp-6p::gfp* expression in both biological replicates (Fig. S1a). These results suggest that SM may be a mild pathogen under these growth conditions. Consistent with prior reporting[22], we noted a developmental delay in SM grown animals reaching adulthood. In contrast, stress and immune signaling levels of OP→BS and OP→PB transitioned animals were similar to the OP→OP control, indicating these diets do not significantly challenge worm homeostasis.

In summary, switching from growth on OP to growth on SM or PB leads to an increase in self-progeny males and reduced fecundity. The reduced fecundity from growth on SM is also associated with
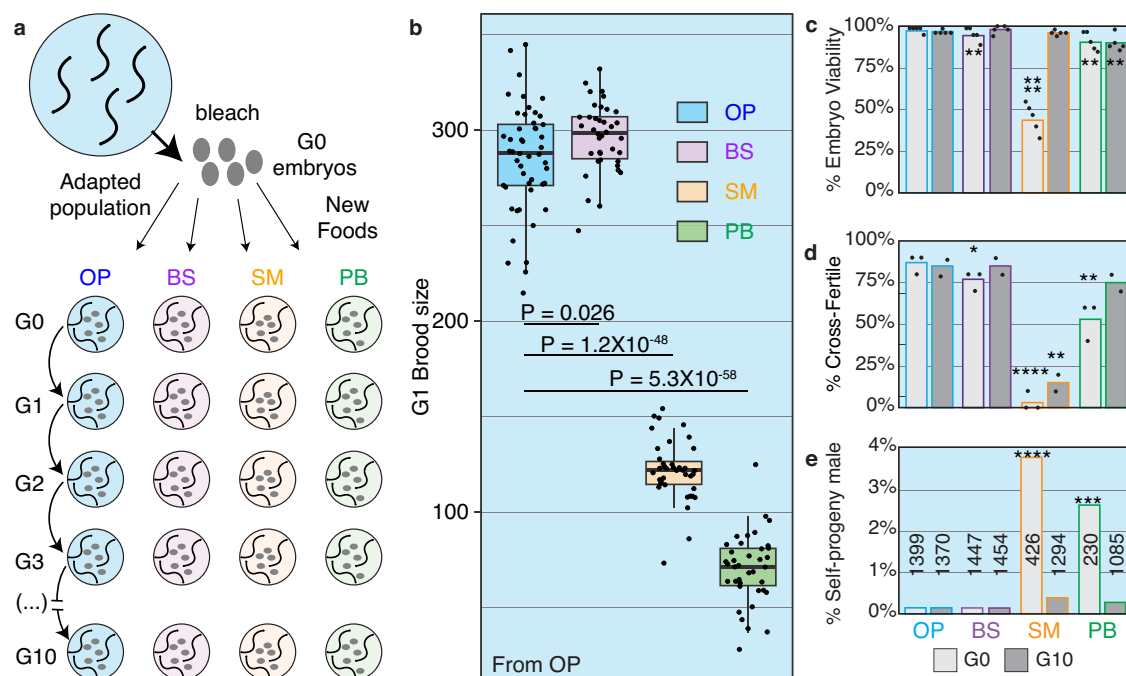
Fig. 1 | Introduction to a new food reduces self-brood size and causes germ cell dysfunction. a Schematic of the experimental design. Bleached embryos from OP-adapted hermaphrodites were placed on plates seeded with the indicated bacteria. Ten individual G0 L4 hermaphrodites were then individually plated on 10 separate plates for each of the four foods. At each subsequent generation, a single early-brood L4 hermaphrodite was selected as the founder of the next generation. All progeny on all plates were counted. **b** Quartile box plots (whiskers extend up to 1.5* interquartile range (IQR)) of self-brood size of G1 wild-type (N2) hermaphrodites previously adapted to growth on OP and newly transferred to the indicated bacterial foods (OP $n = 50$, BS n = 38, SM $n = 40$, PB n = 39). *P* values were calculated by Welch's 2-sided t-test. **c** The percentage of 50 embryos from G0 ($n = 5$) and G10 ($n = 5$) hermaphrodites grown on each food that hatched. One-tailed t-test compared to OP grown worms. **d** The percentage of 10 self-sperm-depleted G0 ($n = 3$) or G10 ($n = 2$) hermaphrodites grown on the indicated food that produced cross-progeny when mated with OP grown wild-type males. In one G0 SM and one G10 BS experiment only 9 hermaphrodites were assayed. *P* values were calculated by one-tailed t-test compared to OP grown worms. **e** The percent of self-progeny males from the sum of all progeny (inset numbers) produced by five G0 or G10 animals grown on the indicated food. *P* values were calculated by Fisher exact test, compared to OP grown worms. Approximate statistical significance is indicated on some panels. * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001. Source data and numerical *P* values are provided as a Source Data file.

embryonic lethality and a decrease in functional oocytes, whereas the reduced fecundity associated with growth on PB appears to be caused by a reduction in functional sperm.

Multi-generational growth on PB and SM leads to adaptation

To determine whether and how worms adapt to growth on SM or PB, we counted the complete broods of hermaphrodites grown on each of the four bacterial strains for 10 consecutive generations (Fig. 2a). For each food, nine to ten single L4 hermaphrodites were transferred to individual plates, establishing independent lines. At each generation, a single early-laid L4 from each line was selected as the founder for the next generation. Three independent replicates were performed over the course of a year. Our results show that animals continuously cultured on SM and PB exhibited increased average brood sizes in subsequent generations, eventually plateauing by the fifth or sixth generation at ~250 progeny per hermaphrodite (Fig. 2a; see Fig. S2 for individual replicate plots).

Consistent with the increased brood size, SM-adapted hermaphrodites (G10 +) produced progeny with higher hatching rates (Fig. 1c) and normal levels of male self-progeny (Fig. 1e). Both SM- and PB-adapted G10+ worms also demonstrated improvements in cross-fertilization rates (Fig. 1d). In contrast, animals maintained on OP or adapted to BS showed no substantial changes in brood size (Fig. 2a). Furthermore, animals maintained on these four bacterial foods for 15 to 20 generations showed no further change in self-brood size (Fig. S3a). Based on these findings, we conclude that OP-adapted *C. elegans* are initially maladapted to growth on PB and SM, but through multigenerational growth on each bacterial species, they eventually adapt (Fig. 3).

Maladaptation to novel bacterial food is common

The above results show that adapted animals can thrive on any of the four bacterial foods. As a result, we were able to repeat the adaptation experiment, starting with animals previously adapted to BS, SM, or PB (Fig. 2, Fig. 3 and Fig. S1 and S2).

The results show that maladaptation is common, observed in nine of the 12 bacterial food transition conditions (Fig. 3). In eight of those nine cases, animals maintained on the new bacteria adapted within nine generations, producing normal-sized broods and restored germ cell phenotypes (Fig. 2). In the other three cases, adapted animals were immediately co-adapted to the new food, but curiously in no case was co-adaptation reciprocal (Fig. 3). To avoid potential parental effects of prior food exposure, we considered only G1 and later generations in the above analysis. To detect any potential parental effects, we measured G0 brood sizes, and in only two cases did we see evidence that a "parental" effect delayed maladaptation; BS-adapted animals transferred to OP (BS→OP) G0 broods were significantly larger than G1 broods (Fig. S4) and SM→PB G0 and G1 broods were larger than G2 broods (Fig. 2 and Fig. S4).

Although co-adaptation was not reciprocal, we observed two cases of reciprocal maladaptation, or mutual maladaptation. First, OP→PB and PB→OP animals were maladapted for all four tested phenotypes: brood size, production of self-progeny males, reduced embryo viability, and reduced cross-fertilization rates (Fig. 1c-e, Fig. 2a, d, k-m, Fig. 3). Second, SM→BS and BS→SM animals were maladapted for three of four tested phenotypes; both transfers produced animals that exhibited reduced brood size, reduced embryo viability, and diminished oocyte availability (Fig. 2b, c, e-j, Fig. 3).Thus, *C. elegans* appears unable to be co-adapted for growth on both OP and PB or
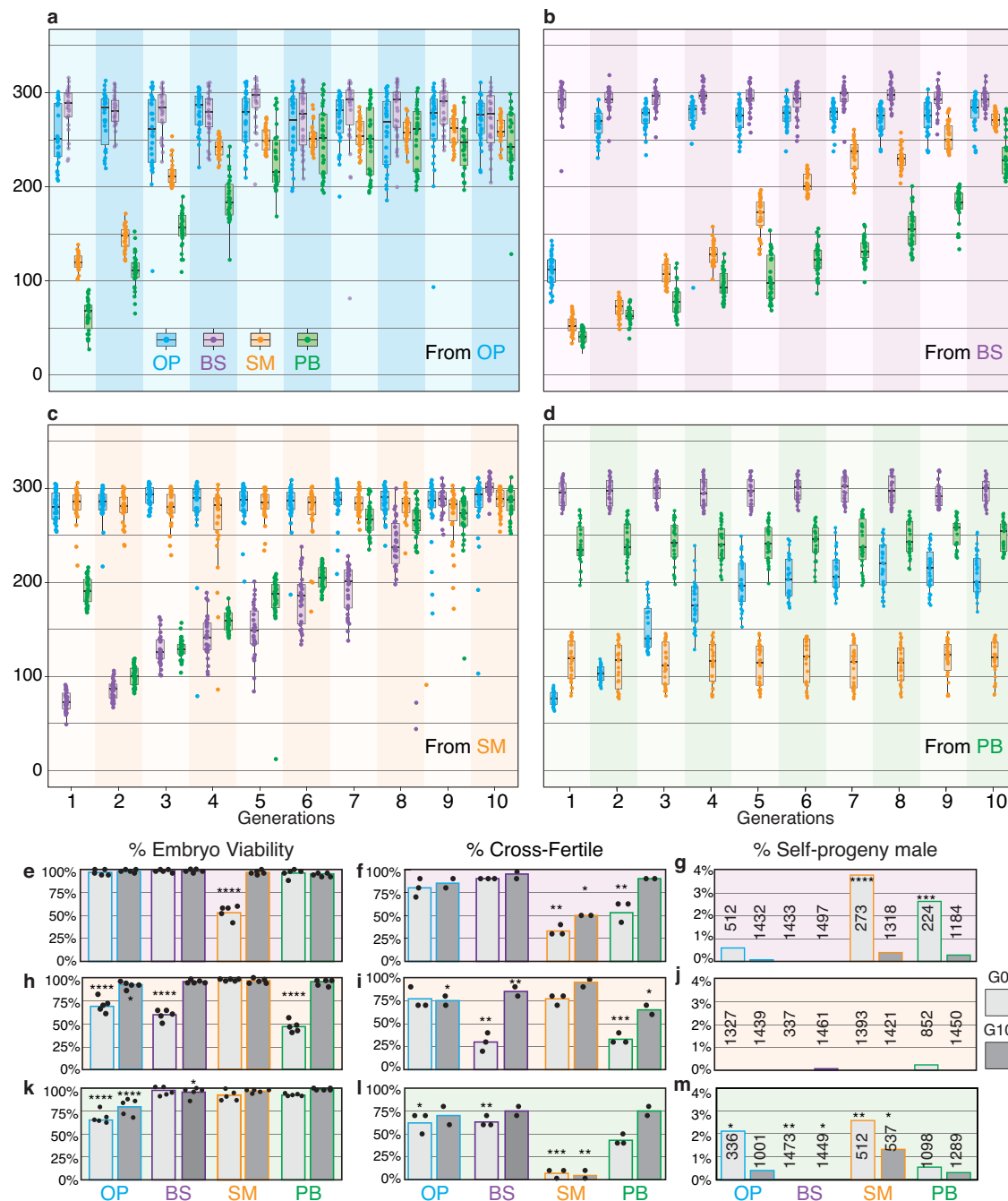
Fig. 2 | Multigenerational adaptation of animals previously adapted to OP, BS, SM and PB bacteria. a–d Quartile box plots (whisker 1.5*IQR) of brood size of animals previously adapted to (**a**), OP (light blue background), **b** BS (light purple), **c** SM (light orange), and (**d**) PB (light green), and then transferred to each of the indicated new bacterial foods. Three biological replicates, $n = 10$ complete broods per each food for each replicate (except $n = 9$ for SM- > BS, rep 3 G9, G10 and SM- > SM rep 3, G10). **e**, **h**, **k** The percentage of 50 embryos from G0 ($n = 5$) and G10 ($n = 5$) hermaphrodites grown on each food that hatched. One-tailed t-test compared to "same-to-same" grown worms. **f**, **i**, **l**, The percentage of 10 self-sperm-depleted G0 ($n = 3$) or G10 ($n = 2$) hermaphrodites grown on the indicated food that

produced cross-progeny when mated with OP grown wild-type males. In two G0 (BS- > BS, PB- > OP) and two G10 (BS- > OP BS- > OP) experiments only 9 hermaphrodites were assayed. P values were calculated by one-tailed t-test compared to "same-to-same" grown worms. **g**, **j**, **m**, The percent of self-progeny males from the sum of all progeny (inset numbers) produced by five G0 or G10 animals grown on the indicated food. P values were calculated by Fisher exact test, compared to "same-to-same" grown worms. Approximate statistical significance is indicated on some panels. * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001. Source data and numerical P values are provided as a Source Data file.

growth on both BS and SM, adaptation to one bacterial strain results in maladaptation to the other.

Unlike all other transfers, PB→SM worms did not rapidly adapt when maintained on SM (Fig. 2d). Even by G10, PB→SM animals continued to produce self-progeny males and failed to show any increase in cross-fertilization rates, representing the only instance where these

phenotypes did not adapt (Fig. 2k-m). Strikingly, PB-adapted *hsp-6::gfp* and *irg-5::gfp* animals transferred to SM exhibited strong GFP expression (Fig. S1), suggesting activation of mitochondrial stress and innate immune pathways. Importantly, G15 and G20 PB→SM worms showed a progressive increase in brood size, indicating slow adaptation to SM growth (Fig. S3).

First Food	Second Food	Generations to Adapt	Embryo Viability	Oocyte Viability	Reduced Sperm	Male Progeny	irg-5p ::gfp	hsp-6p ::gfp	Maladapted	Co-adapted
OP	OP	NA	97%	++++	NA	No	1.0			
	BS	0	94%	++++	NA	No	1.6	1.1		
	SM	5	44%	+	?	Yes	1.6	1.5		
	PB	5	90%	++	Yes	Yes	1.7	1.1		
BS	OP	2	97%	++++	NA	No	1.4	1.0		
	BS	NA	98%	++++	NA	No	1.0			
	SM	10	54%	++	Yes	Yes	1.4	0.8		
	PB	10	96%	+++	Yes	Yes	1.4	0.6		
SM	OP	0	68%	+++	NA	No	0.5	1.8		
	BS	10	59%	++	Yes	No	0.6	1.5		
	SM	NA	97%	+++	NA	No	1.0			
	PB	7	46%	++	Yes	No	0.6	1.1		
PB	OP	5	65%	+++	Yes	Yes	1.0	1.3		
	BS	0	98%	+++	NA	No	1.1	1.0		
	SM	>10	92%	+	?	Yes	2.3	2.3		
	PB	NA	99%	++	NA	Low	1.0			

Fig. 3 | Summary of adaptation phenotypes. "Generations to Adapt" refers to the first generation after which brood size appears to plateau (no or minimal significant increase, see Source Data file). "Embryo Viability" is the percentage of G0 embryos that successfully hatched. "Oocyte Viability" is a representation of the percentage of G0 sperm-depleted hermaphrodites that produced cross progeny - ++++ > 80%, +++ 60-80%, ++ 20-60%, + < 20%. "Reduced Sperm" indicates whether sperm-depleted hermaphrodites produced cross progeny. "Male Progeny" indicates yes (> 2%), low (> 0.5%), or no increased production of self-progeny males. *hsp-6p::gfp* & *irg-5p::gfp* show the average fold change in gfp expression level relative to adapted-to-adapted control for each reporter (Fig. S1). Fold-change values that were statistically significant in both biological replicates are shown in red font. Maladapted and Co-adapted columns present a schematized version of the results presented in Fig. 2 for ease of comparison.

Overall, these results indicate that in mono-culture conditions, *C. elegans* rapidly adapts to its new bacterial environment, often at the expense of growth on even recently encountered bacteria. Notably, other than PB→SM, which showed a statistically significant increase in the expression of both the immune and mitochondrial stress sensors in both biological replicates, there is no correlation between expression of the pathogen sensors and maladaptation or adaptation (Fig. 3).

Maladaptation and adaptation in pure isogenic lines
The rapidity and reversibility of the observed adaptation indicate a non-genetic mechanism. Furthermore, because *C. elegans* hermaphrodites are self-fertile, the N2 strain is nearly isogenic, therefore it is unlikely that selection for genetic variation underlies the observed adaptation. To fully determine whether sequence variation is required for adaptation, we generated several pure isogenic lines following the methodology of Artiles et al. and selected two lines for further analysis[28]. These lines were adapted to OP and then transferred to
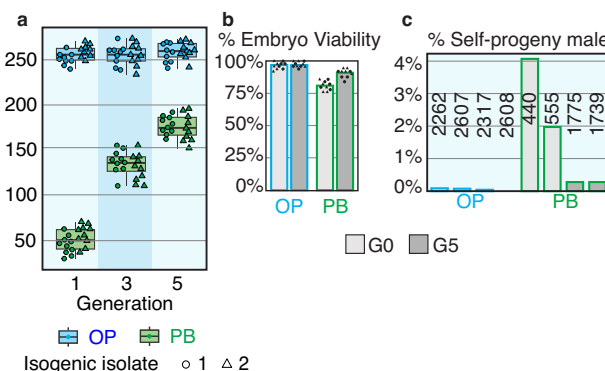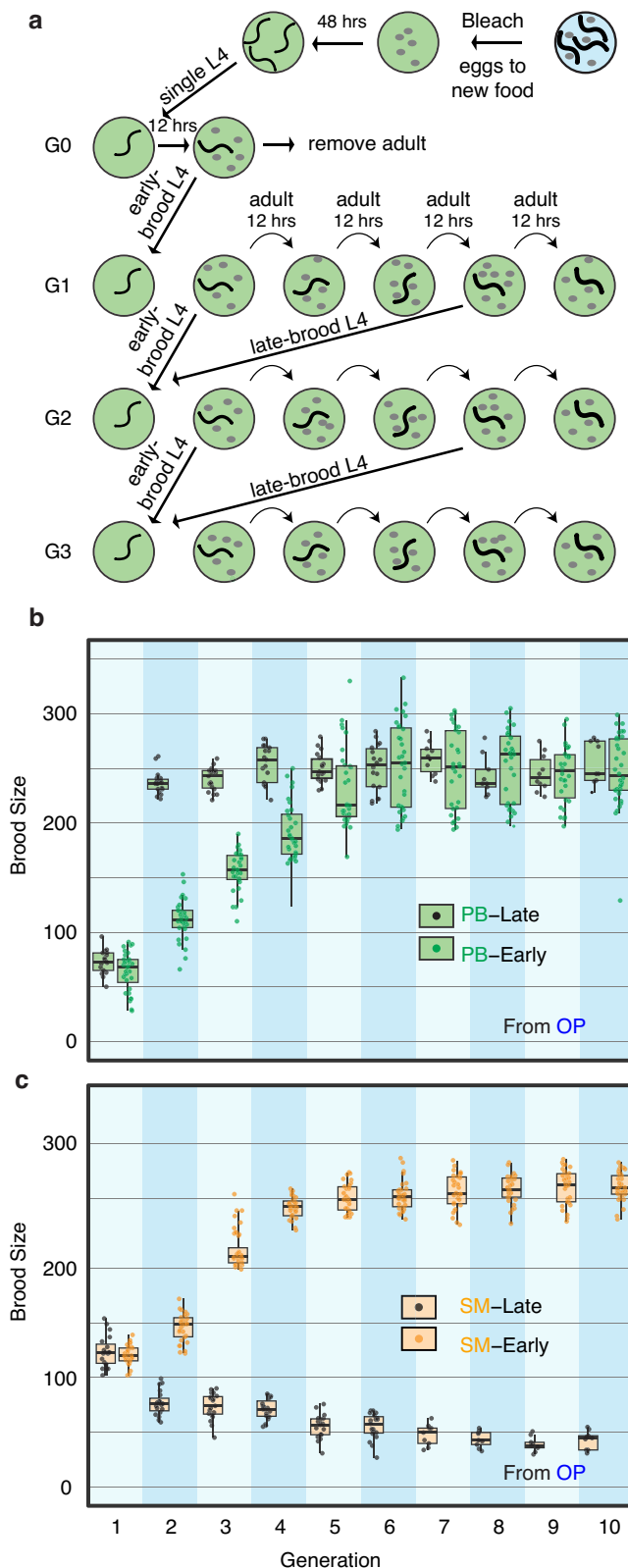

Fig. 4 | Maladaptation and adaptation in isogenic lines. a Quartile box plots (whisker 1.5*IQR) of brood size of two isogenic strains previously adapted to OP and then transferred to OP or PB. $n = 9$ for isogenic line 1 OP→OP brood measurements and $n = 10$ per generation for all others. **b** The percentage of 50 embryos from each G1 ($n = 10$) and G5 ($n = 10$) hermaphrodites from each isogenic strain grown on each food that hatched. **c** The percent of self-progeny males from the sum of all progeny produced by five G0 or G5 isogenic strain 1 (left) or 2 (right) hermaphrodites grown on the indicated food. For each food at each indicated generation, five complete broods were counted (inset numbers). Source data and numerical *P* values are provided as a Source Data file.

either OP or PB for brood size measurements at generations 1, 3, and 5 (Fig. 4a). Although the brood size of both isogenic strains was reduced on both foods compared to N2 (Fig. 1b), both isogenic lines were maladapted to PB at generation 1 and both showed significant increases in brood size at generations 3 and 5, comparable to adaptation rates observed for N2 (Fig. 2). We also determined that embryo viability increased between generations 1 and 5 and that the fraction of self-progeny males decreased significantly from generation 1 to generation 5 (Fig. 4b, c). Thus, both maladaptation and adaptation of brood size, embryo viability, and meiotic chromosome segregation can occur in fully isogenic lines.

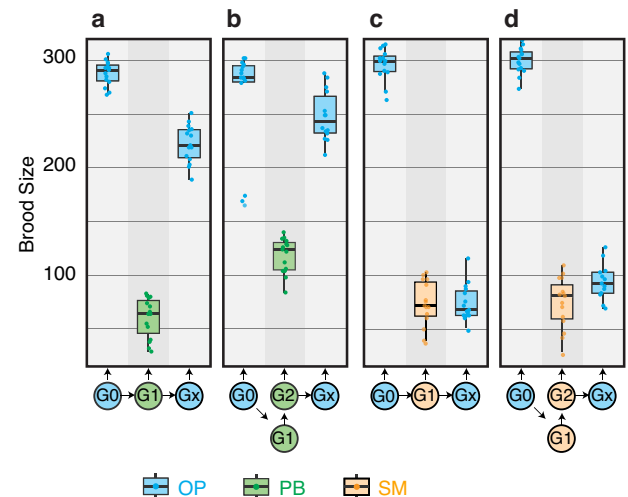**Reproductive exposure time determines adaptation rate**
Adaptation to novel foods occurred over a remarkably similar time-scale (4–9 generations; Fig. 2). To determine whether this time frame reflects generational exposure time (number of generations) or cumulative exposure time to the new environmental condition, we altered the experimental design to focus on differences in within-generation exposure time (Fig. 5a). Rather than selecting an *early*-laid L4 as the founder of each successive generation, we instead selected a *late*-laid (end of day 2 egg lay) L4 animal as the founder of each successive generation. For OP→PB animals, we found that this change resulted in full adaptation by G2, strongly supporting the hypothesis that total exposure time is more critical than the number of exposed generations (Fig. 5b).

To compare the total exposure time to full adaptation of the two experimental designs, we first determined that the brood size of G0 OP→ PB animals is similar to the brood size of G1 OP→PB animals, indicating that there is no apparent maternal effect (Fig. S4). Consequently, the total exposure time to full adaptation for the early-laid L4 transfer condition is five early laid generational exposures (GE) or 5X GE. The total exposure time to full adaptation for late-laid L4 transfer condition is 1 GE (G0 to G1) plus 48 h for the late-laid L4 transfer to G2. Thus, 5X GE = 1X GE + 48 h, and GE is therefore ~12 h and the total exposure time to full adaptation is ~60 h. A 12-h GE suggest that the critical exposure period for reproductive fitness is limited to a portion of the life cycle. Since the first eggs are laid about four hours after the adult molt, the critical period likely begins about eight hours earlier in the mid-L4 stage when spermatogenesis begins. This is consistent with
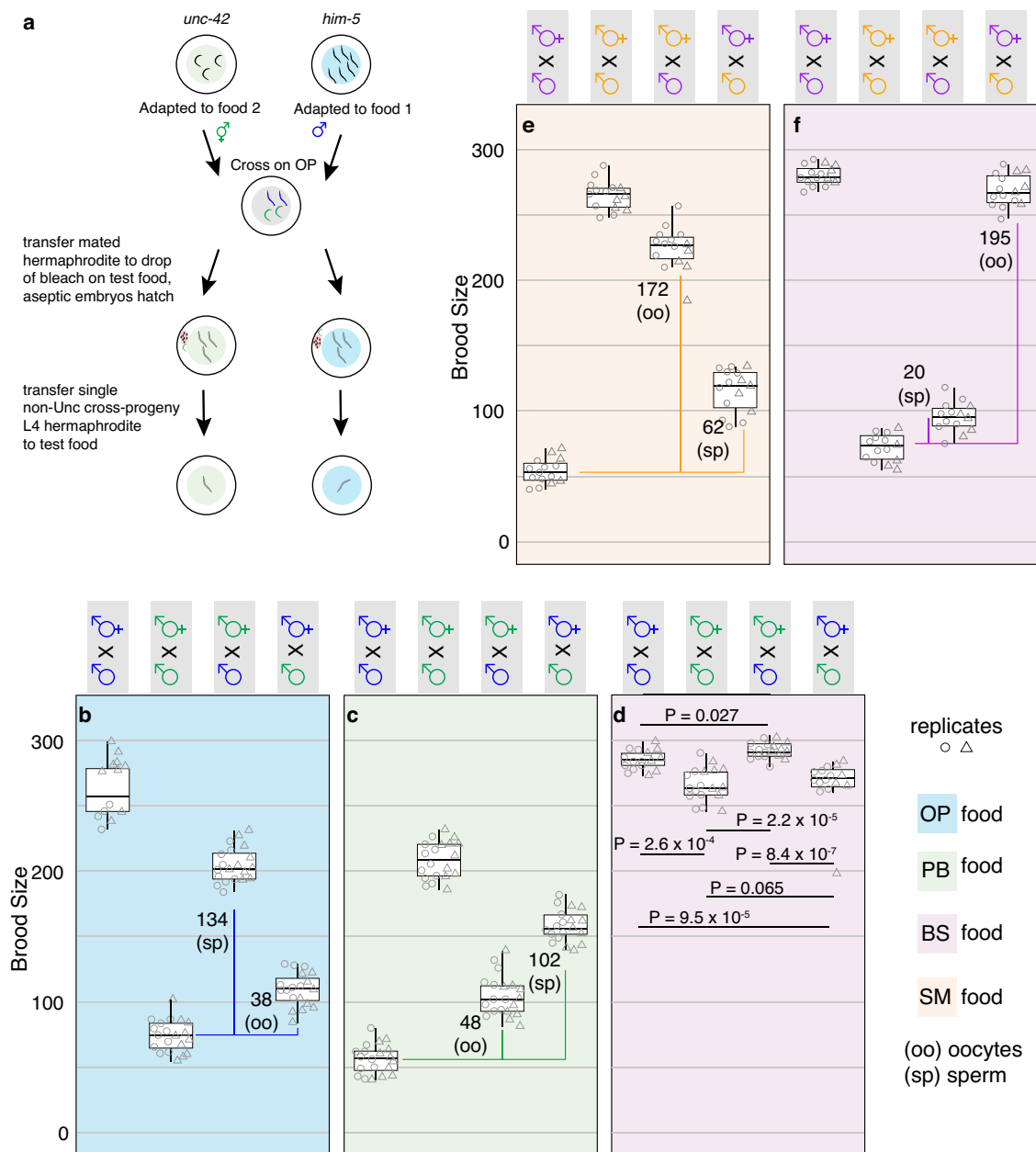
Fig. 6 | Differential dynamics of heritable adaptation. a, **b** Quartile box plots (whisker 1.5*IQR) of brood size of animals previously adapted to OP and then transferred to PB for **a** one or **b** two generations and then returned to OP.
c, **d** Quartile box plots (whisker 1.5*IQR) of brood size of animals previously adapted to OP that were then transferred to SM for (**c**) one or (**d**) two generations and then returned to OP. $n = 15$ complete broods per condition. Source data and numerical P values are provided as a Source Data file.

Unexpectedly, late-brood OP→SM transferred animals failed to adapt, producing a significantly reduced G2 brood that remains low through G10 (Fig. 5c). One possibility is the extended exposure may exacerbate the maladapted state or may even cause genetic damage, perhaps related to chromosomal segregation defects associated with the high incidence of self-progeny males and reduced embryonic viability observed in OP→SM animals (Fig. 1c, e). We also note that OP→ SM induced expression of the mitochondrial stress reporter *hsp-6p::gfp*, suggesting that accumulated stress responses may interfere with adaptation. While the underlying cause remains a mystery, this outcome does highlight the dramatic differences in how exposure to the different bacterial species can affect adaptation.

Differential retention of prior adaptation state

OP-adapted animals transferred to SM and PB produced significantly reduced brood sizes (Fig. 1b and S4), while the reverse transfers PB→OP produced maladapted progeny and SM→OP produced co-adapted progeny (Fig. 2). Thus, our expectation is that adapting OP→SM animals will maintain OP-adaptation information, while adapting OP→PB animals will quickly eliminate OP-adaptation information. To determine how a one- or two-generation exposure to SM and PB differentially affect retention of OP-adapted information, we aseptically transferred G1 and G2 embryos back to OP and measured their brood sizes (Fig. 6). We found that G1 and G2 PB-maladapted animals returned to OP

the cross-fertilization analysis (Fig. 1d), which indicates that half the tested OP→PB G0 animals produced additional progeny when supplied with functional sperm. However, because spermatogenesis ends prior to adulthood, the post-adult adaptation to growth on PB (Fig. 4b, late transfer) indicates that mature sperm and/or the adult germline (oocytes) can adapt and/or can receive and transmit adaptation information to progeny.

Fig. 7 | Sperm and oocytes differentially transmit heritable adaptation information to progeny. a Schematic of experimental design for crosses within (controls) and between (experimental) fully adapted animals. All crosses were performed on OP. Mated hermaphrodites are transferred to a drop of bleach on a plate seeded with each test bacterial strain The bleach dissolves the adult and all associated bacteria. The embryos survive the bleach, hatch, and crawl to the food. Prior to adulthood, single cross-progeny (non-Unc) hermaphrodites are placed on individual plates to count full broods. **b–d** Quartile box plots (whisker 1.5*IQR) of brood sizes of the indicated cross-progeny between OP- and PB-adapted *him-5* males and *unc-42* hermaphrodites on the indicated bacterial foods. Two independent replicates, with $n = 10$ cross progeny per condition ($n = 9$ for PB male crossed to OP hermaphrodite in (**c**), replicate 2 and (**d**) replicate 2. **d** P values for replicate 1 only, were calculated by Welch's t-test, 2 sided. Source data and numerical P-values are provided as a Source Data file. **e, f** Quartile box plots (whisker 1.5*IQR) of brood size of the indicated cross progeny between BS- and SM-adapted *him-5* males and *unc-42* hermaphrodites grown on the indicated bacterial foods. Two independent replicates, with $n = 10$ cross progeny per condition replicate 1 ($n = 9$ for BS male crossed to SM hermaphrodite in panel f replicate 1), $n = 5$ for replicate 2. The lines indicate samples to be compared to evaluate the contribution of sperm and oocytes to cross progeny adaptation. The inset numbers are the difference in mean brood size for sperm (sp) and oocyte (oo) transmitted adaptation information. Source data and numerical P values are provided as a Source Data file.

produced nearly normal brood sizes on OP (Fig. 6a, b), indicating transgenerational retention of OP-adapted information. In contrast, G1 and G2 SM-maladapted animals returned to OP produced small broods on OP (Fig. 6 c, d), indicating near complete loss of OP-adapted information after only a single generation of growth on SM. Because SM-adapted animals are co-adapted to growth on OP (Fig. 2c), this result suggests that co-adaptation to these two foods may occur concurrently during adaptation to SM.

The apparent transgenerational persistence of OP-adaptation on PB and the rapid loss of such information on SM indicate that multigenerational adaptation is not necessarily reciprocally linked to decay of prior transgenerational non-genetic information.

Sperm and oocytes differentially transmit adapted phenotypes
The analysis presented above indicates that heritable non-genetic information for efficient growth on at least four independent bacterial

species is transmitted between multiple generations (Figs. 2, 4). To investigate whether this information is primarily transferred via sperm or oocytes, we set up crosses between differently adapted populations and asked to which food is the cross progeny better adapted. We noted that transfer between two pairs of bacteria resulted in both reciprocal maladaptation-adaptation phenotypes and similar effects on germ cells: OP→PB, PB→OP (primarily sperm defective), and BS→SM, SM→BS (primarily oocyte defective) (Figs. 2, 3). Therefore, we set up crosses between animals adapted to these pairs of bacterial foods.

We first adapted *him-5* and *unc-42* strains to all four bacteria. *him-5* mutants produce self-progeny males, and *unc-42* is used to distinguish self-progeny (Unc) from cross-progeny (non-Unc). We then recapitulated the adapted and maladapted phenotypes observed for self-progeny broods by crossing males and hermaphrodites adapted to the same food to each other. The results demonstrate that the mutants and mating procedures did not interfere with our assays (Fig. 7b, c, e, f). Taking advantage of the observation that both PB and OP adapted animals produce normal broods when transferred to BS, we also counted cross progeny brood sizes on BS, where all four classes of cross progeny produced adapted-sized broods demonstrating that there is no incompatibility caused by crossing differently adapted parents (Fig. 7d). We then compared the fertility of cross progeny of OP and PB adapted animals to the fertility of fully maladapted cross progeny on either OP or PB food. On OP food (Fig. 7b) the cross progeny of OP-adapted males and PB-adapted hermaphrodites produced larger broods than the cross progeny of OP-adapted hermaphrodites and PB-adapted males. On PB food (Fig. 7c) we observed reciprocal results; the cross progeny of PB-adapted males and OP-adapted hermaphrodites produced larger broods than the cross progeny of PB-adapted hermaphrodites and OP-adapted males.

To quantitively compare the contribution of food-specific adaptation information by sperm and oocytes we calculated the difference in cross progeny mean brood size on each food. To compare the contribution of OP-adapted males and hermaphrodites to their cross progeny, we determined that the average increase in cross-progeny brood size from maladapted (PB to PB) parents to that of cross progeny from OP-adapted males to PB-adapted hermaphrodites was 134 (Fig. 7b). The cross progeny of the reciprocal cross, PB-adapted males crossed to OP-adapted hermaphrodites, produced a mean brood size difference of 38 (Fig. 7c). Thus, the cross progeny of OP-adapted males produced 3.5 times more self-progeny than do the cross progeny of OP-adapted hermaphrodites. Similar calculations show that the cross progeny PB-adapted males produce 2.1 times more self-progeny than do the cross progeny of PB-adapted hermaphrodites.

We observed a similar pattern for the cross progeny of BS- and SM-adapted animals (oocyte defective), only here the hermaphrodites primarily transferred the adaptive information (Fig. 7f, g). That is, the cross progeny of BS-adapted males crossed to SM-adapted hermaphrodites produced larger broods on SM than did the cross progeny of SM-adapted males crossed to BS-adapted hermaphrodites (Fig. 7f). Similarly, the cross progeny of SM-adapted males crossed to BS-adapted hermaphrodites produced larger broods on BS than did the cross progeny of BS-adapted males crossed to SM-adapted hermaphrodites (Fig. 7g). Calculations as described above show that the cross progeny of BS-adapted hermaphrodites produce 9.5 times more self-progeny than do the cross progeny of BS-adapted males. Similarly, the cross progeny of SM-adapted hermaphrodites produced 3.8 times more self-progeny than do the cross progeny of SM-adapted males. Therefore, in both pairs of crosses the germ cell type that appears to be defective in maladapted animals and that adapts after multi-generational exposure, transmits more food-specific adaptation information to their cross progeny.

We also noted that the cross progeny of OP-adapted males produced larger broods on BS than do the cross progeny of PB-adapted males (Fig. 7d). This difference correlates with the persistent

difference in fully adapted self-progeny brood sizes on OP and PB, suggesting that sperm dysfunction on PB grown worms contributes to the incomplete adaptation of worms to PB growth conditions (Fig. 2, S3).

Discussion

Our investigation into reproductive fitness in *C. elegans* during transitions between ecologically relevant food sources revealed two major discoveries that add to our understanding of non-genetic heritable adaptation. First, *C. elegans* is frequently maladapted to bacterial species they had not recently encountered (Fig. 2). Second, these maladapted animals adapt to the new food within five to ten generations (Fig. 2). Our analysis also showed that the rate of adaptation is proportional to exposure time, not generational time (Fig. 5), and that adaptation is not dependent on DNA sequence variation (Fig. 4). We identified dysfunctional sperm and oocytes as the proximal cause of reduced fertility, with the specificity and severity of germ cell dysfunction influenced by the interplay of past and current food environments. Crosses between animals fully adapted to different foods shows that this non-genetic heritable food and germ cell-specific information can be transmitted by both sperm and oocytes, and in the four combinations analyzed, sperm primarily transmitted information that enables cross progeny to grow on food that disrupts sperm function, and oocytes primarily transmitted information that enables cross progeny to grow on food that disrupts oocyte function (Fig. 7).

Adaptation to a new food often resulted in maladaptation to the previous food and other not recently encountered foods (Fig. 3). These observations imply that maladaptation is a cost associated with maximizing reproductive fitness (the benefit) on the previous food. Thus, we infer that during evolution, the average period of adapted growth must be long enough to bear the cost of future maladaptation. Co-adaptation, where fertility remained normal on both the new and previous food, was observed in only three instances, and it was never reciprocal. For example, animals adapted to OP and SM maintained normal brood sizes on OP (Fig. 2); however, OP-adapted animals were maladapted to SM (Fig. 1), indicating that the OP- and SM-adapted states are distinct.

We also investigated whether maladaptation is associated with established host pathogen response pathways (Fig. S1). We found that PB→SM induced expression of IRG-5 and HSP-6 reporters, and that this was the only example where transferred animals did not adapt within 10 generations (Fig. 2) but noted increased fecundity by G15 and G20 (Fig. S3). Gut commensal bacteria are known to modulate pathogenesis[29] and PB, SM, and BS are commensal gut-colonizing bacteria[22,30]. However, because bleaching embryos eliminates all prior microbiome bacteria, our results clearly demonstrate that commensal bacteria need not be present in the gut to modulate pathogenesis responses, indicating that pathogen susceptibility can be mediated by maternal metabolic effects[31–33].

We note that PB is also a likely mild pathogen; the transition to PB from each of the other three bacteria induced either IRG-5 or HSP-6 (Fig. 3, Fig. S1). Furthermore, *C. elegans* never completely or persistently adapted to growth on PB as the brood size of PB-grown worms plateaued around 250, although there is some prior experience-based variation; for example, the brood size of SM→PB animals peaks at ~300 before dropping to ~250 after 20 generations, while the brood size of BS→ PB animals plateau at ~220 before increasing to ~250 after 20 generations (Fig. 2 and S3). We also note that in the control crosses on BS (Fig. 7d) that OP adapted males produce more fecund cross progeny than PB adapted males with both OP and PB adapted hermaphrodites. Therefore, the apparent incomplete adaptation of worms grown on PB is associated with sperm-transmitted information, which from data presented in Fig. 1 and Fig. 2, is associated with reduced functional sperm. We speculate that *C. elegans* sperm simply cannot fully adapt to PB, further suggesting that this *Pseudomonas* species

may be a mild pathogen. These observations raise the issue of distinguishing between adapting to mild pathogens versus adapting to poor food sources; indeed, a mild pathogen is likely also a poor food choice. This suggests that the previously reported multigenerational adaptation to a pathogen[13] may also reflect food adaptation that results in a healthier host that can better resist the pathogen[34].

To further investigate adaptation states, we examined the fitness of OP→SM animals to grow on OP after just a single generation on SM and found them to be fully maladapted to OP (Fig. 6). This rapid loss of OP fitness suggests that the slow adaptation to SM is not due to the gradual replacement of OP-adaptation information. In contrast, analysis of the mutually maladapted OP and PB food pair showed the opposite effect: OP→PB animals, after one or two generations on PB, retained near-normal fecundity on OP (Fig. 6). These findings demonstrate that similar multigenerational adaptation phenotypes can result from distinct mechanisms of non-genetic inheritance. This complexity likely contributes to the varied, non-reciprocal relationships between different adapted states.

The stepwise increase in fertility observed in nearly every example of food adaptation is a strong indicator of a cumulative intergenerational effect. However, we cautiously note that the inverse of this stepwise change would appear to be multigenerational persistence of a prior state. For example, the persistence of OP adaptation while animals are adapting to PB can credibly be called TEI. Thus, the distinction between intergenerational and transgenerational effects may depend as much on context as on mechanism. At present no identified non-genetic heritable mechanism is excluded as a possible mediator of the adaptation we observe, such as non-coding RNAs, histone modifications, prions, feed-forward regulatory networks, physiological adaptation of mitochondria, or any combination thereof[7–9,35–39]. Our observation that germ-cell-specific adaptations are preferentially transmitted to progeny by the adapted germ cell type raises the possibility of selection of physiologically adapted germ cells at each generation that directly transmit the adaptation (phenotype) to their progeny.

Our study builds on recent findings reporting ecologically relevant, heritable, non-genetic adaptation to variable environmental conditions. Early reports highlighted that nutrition-responsive gene networks mediate multigenerational starvation resistance following a single generation of starvation[5]. In the nematode *Pristionchus pacificus*, a microRNA family inhibits food-type-induced heritable transitions in mouth morphology that can persist for many generations[40]. Mutations in heritable RNAi and chromatin-modifying pathways have effects on germline mortality that are modified by growth on diverse bacteria[41]. And perhaps directly relevant to our investigations is the demonstration of intergenerational induction of pathogen neutralizing activity in the progeny of exposed parents[13]. These observations prompt curiosity about the evolutionary pressures shaping these adaptive strategies. In natural environments, *C. elegans* populations experience a large, unpredictable variety of food sources[18], demanding rapid yet reversible adaptation mechanisms to sustain reproductive success. This capacity for non-genetic recalibration may serve as a protective buffer, safeguarding finite, inbred populations from genetic extinction.

Methods

Worm Strain and Maintenance
The N2 strain of *Caenorhabditis elegans* was used in this study. Unless otherwise noted, worms were maintained at 25 °C on normal growth medium (NGM) plates seeded with *Escherichia coli* OP50[42]. The pathogen immune response reporters AY100, *acIs101* [p*DBO9.1(irg-5p::gfp)*; pRF4(*rol-6(su1006)*)][26] and the mitochondrial stress reporter SJ4100, *zcIs13* [*hsp-6p::GFP + lin-15*(+)][27] were adapted to each food at 25 °C for at least 10 generations. For mating assays, CB270, *unc-42(e270)* V and CB4088, *him-5 (e1490)* V were similarly adapted to each

food for at least 10 generations. PD2220, *ccTi1594; [mex-5p::GFP::gpr-1::smu-1 3'UTR + Cbr-unc-119(+)*, III: 680195], *umnIs27 [myo- 2::GFP + NeoR*, III: 8856215 (intergenic)] III was used to generate isogenic lines[28].

Bacterial strains
Escherichia coli, strain OP50, was obtained from the CGC. *Bacillus subtilis* strain RL1275 (*spoIIAC::erm* in a PY79 background, was a gift from Richard Losick). The *Caenorhabditis elegans* Microbiome (CeMbio) bacteria *Sphingobacterium multivorum*, strain BIGb0170[21] and *Pseudomonas berkeleyensis*, strain MSPm1[21] were obtained from the CGC.

Preparation of bacteria for seeding NGM plates
B. subtilis, S. multivorum, and *P. berkeleyensis* stored as −80 °C glycerol stocks were streaked to single colonies on LB plates and incubated at 25 °C for two to three days. *E. coli* was similarly streaked to single colonies on LB but incubated at 37 °C overnight. A full single colony was then used to inoculate 5 mL of LB, which was grown with aeration at 25 °C for 48–56 h, diluted to an OD[600] of 1.0, and 150 μL was spotted on each 35 mm NGM plates. Growth of liquid cultures for less than 48 h interfered with reproducibility. Seeded plates were left at room temperature (-22 °C) to dry and allow bacterial growth for three days and then the seeded plates were stored at 4 °C for less than three weeks. *B. subtilis* seeded plates were maintained at room temperature.

Preparing adapted worms
N2 animals were continuously propagated on NGM plates seeded on the select bacterial food by transferring L4 larvae every 2–3 days for 10 or more generations at 25 °C.

Preparing initial generation (G0) animals
In all cases, only bleach sterilized embryos were transferred to a new bacterial food source. To obtain bleach treated aseptic embryos, adult worms were washed from plates (M9 buffer), transferred to a 1.7 mL microcentrifuge tubes, centrifuged (2500 × *g*, 1 min), and the buffer removed leaving 100 μl. 0.5 mL of hypochlorite solution (0.2 mL NaClO [J.T. Baker], 0.1 mL 5 M KOH, 0.7 mL H₂O) was added and the tube vortexed for 2 min. The worms/eggs were pelleted as above, old hypochlorite solution removed, 500 μL of fresh hypochlorite solution added, vortexed for 2 min, and centrifuged as above. The embryos were then washed four times with 1 mL of M9 buffer, once with water, and placed on an appropriately seeded plate. For experiments presented in Fig. 4 and Fig. 7 gravid adults were picked into a 30-35 μL of hypochlorite solution on the unseeded portion of a seeded NGM plate. The hypochlorite solution dissolves the adults, and several hours later the hatched aseptic larvae crawl to the new bacteria on the plate.

Preparing first generation (G1) L4 larvae and brood counting procedures
L4 larvae from G0 embryos were transferred to plates with the same food source. After 24 h of egg-laying, the G0 adults were removed. 36–48 h later, 10 L4 G1 larvae were singled onto brood counting plates. After the onset of egg-laying, the adults were transferred to a fresh plate at 8–24-h intervals to maintain a synchronously developing cohort of G2 progeny. When G2 animals on the initial egg-lay plates reached the L4 stage, one L4 larva was selected as the founder for each of the 10 lines. As G2 progeny transitioned to late L4 or young adult stages, all individuals were counted and removed from the plate to prevent double counting. This process was repeated for at least 10 generations.

Late brood L4 transfers
The data in Fig. 5 was obtained by transferring a late brood L4 from eggs laid 48 h after the egg-laying adult was transferred from the first

egg-lay plate from generation G1 through G10 (replicate 1) or G6 (replicate 2).

Pathogen response assay

Aseptic embryos from adapted (G10 +) AY100 (*irg-5::gfp*) and SJ4100 (*hsp-6::gfp*) worms were spotted on plates seeded with each of the four bacteria and maintained at 25 °C for 2–2.5 days. 10 young adults were then picked to a 45-50 μL drop of 0.1 M sodium azide on a non-seeded NGM plate. The paralyzed worms were arranged into a group and imaged under transmitted light and gfp fluorescence conditions. All images were obtained under identical imaging and magnification conditions using a Basler acA3088-57 CCD camera. Collected images were manipulated (orientation, cropping, brightness and contrast) using Fiji/Image J. GFP Brightness and contrast settings were propagated across all images relative to the brightest image within an adapted worm cohort. Cropped images were imported into Adobe Illustrator to assemble figures.

Isogenic strains isolated from N2

N2 males were crossed to PD2220 hermaphrodites. Six chimeric F1 cross progeny (N2 derived P1 [germline] lineage) were identified by lack of germ line GFP expression and mosaic pharyngeal *myo-2*::GFP expression[28]. A single F2 from each chimeric F1 was selected as a founder isogenic line. Two lines, HC1295 and HC1296, were selected based on reproductive output (reproducible normal-sized self-broods) for subsequent experiments.

Hatching rate assay

To accurately measure hatching rates, for each food combination, 50 laid embryos from each of five hermaphrodites were hand-picked from thick bacterial lawns and grouped into five sets of 10 embryos per group in an unseeded area of a fresh plate. Unhatched embryos were counted the next day and the number of adults recorded after two days.

Cross Fertilization assay

To determine whether self-sterile hermaphrodites, after exhaustion of self-sperm, could produce cross progeny, we first confirmed that each hermaphrodite to be tested laid no eggs for 48 consecutive hours. We then placed individual confirmed self-sterile hermaphrodites on an OP50 seeded plate with ten N2 males grown on OP50 25 °C. Hermaphrodites that produced one or more cross-progeny were deemed to be sperm-limited for self-fertility. Animals that did not produce cross progeny were deemed to be non-fertile.

Mating assay

him-5(e1490) and *unc-42(e270)* mutants were adapted to each bacterial diet (OP, PB, BS, and SM) for 10+ generations. All crosses were conducted on 35 mm NGM plates seeded with 30 μL of OP50. Prior to mating, worms were transferred to unseeded plates for 15–20 min to minimize residual bacteria transfer to mating plates. For each cross, 6–8 *him-5* males and 1 *unc-42* hermaphrodite were placed together on an OP50 plate. 12–24 h later, the mated hermaphrodite was picked to a 35 μl spot of hypochlorite solution on a non-seeded portion of an appropriate test bacteria plate. Two days later individual non-Unc L4 cross progeny hermaphrodites were picked to individual plates seeded with the appropriate bacteria to determine brood size as described.

Supplementary Materials

Source Data.xlsx. The raw data and numerical *P* values associated with all figures. Supplementary Figs. 1–4.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The authors declare that all data generated or analyzed during this study are included in the published article and its supplementary information files. Source data are provided with this paper.

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Author contributions

A.B.P. and C.P.H. are responsible for conceptualization, development of methods, and reviewing and editing the manuscript. A.B.P. is responsible for performing the experiments. C.P.H. is responsible for visualization and writing the original draft.

Competing interests

The authors declare no competing interests.

Additional information

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