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Inter-generational consequences for growing *Caenorhabditis elegans* in liquid

Itamar Lev[1], Roberta Bril[1], Yunan Liu[1], Lucila Inés Ceré[1] and Oded Rechavi[1,2]

[1]Department of Neurobiology, Wise Faculty of Life Sciences and Sagol School of Neuroscience, Tel Aviv University, Tel Aviv 69978, Israel
[2]Allen Discovery Center at Tufts University, Medford, MA 02155, USA

IL, 0000-0002-9100-5100; OR, 0000-0001-6172-3024

In recent years, studies in *Caenorhabditis elegans* nematodes have shown that different stresses can generate multigenerational changes. Here, we show that worms that grow in liquid media, and also their plate-grown progeny, are different from worms whose ancestors were grown on plates. It has been suggested that *C. elegans* might encounter liquid environments in nature, although actual observations in the wild are few and far between. By contrast, in the laboratory, growing worms in liquid is commonplace, and often used as an alternative to growing worms on agar plates, to control the composition of the worms' diet, to starve (and synchronize) worms or to grow large populations for biochemical assays. We found that plate-grown descendants of M9 liquid medium-grown worms were longer than control worms, and the heritable effects were already apparent very early in development. We tested for the involvement of different known epigenetic inheritance mechanisms, but could not find a single mutant in which these inter-generational effects are cancelled. While we found that growing in liquid always leads to inter-generational changes in the worms' size, trans-generational effects were found to be variable, and in some cases, the effects were gone after one to two generations. These results demonstrate that standard cultivation conditions in early life can dramatically change the worms' physiology in adulthood, and can also affect the next generations.

This article is part of the theme issue 'Developing differences: early-life effects and evolutionary medicine'.

1. Introduction

You could not step twice into the same river (Heraclitus)

In comparison with Mendelian inheritance, which entails faithful transmission of discrete packets of information which do not blend or dilute [1], heritable epigenetic data are 'soft', or 'fluid', as they change as a function of time, and in response to physiological processes [2].

Generally, mechanisms that allow trans-generational epigenetic effects, namely transmission of information to progeny not exposed to the original trigger, are still poorly understood [3]. However, in *Caenorhabditis elegans*, substantial understanding has been gained regarding the molecular mechanisms that allow transmission of responses via small RNAs across multiple generations (reviewed in [4]). Further, many studies with *C. elegans* have demonstrated that different environmental challenges leave a trace in the progeny, for example, viral infections [5–7], starvation [8–11], high temperatures [12–14], high osmolality [15,16] and exposure to toxins [15]. Such stressors can lead to short- or long-term gene expression alterations and physiological changes. The inheritance of some of these responses is associated with changes in heritable small RNAs [8,13,14] and/or is correlated with alteration in histone modifications (for example, of H3K4me [15,17]).

In the laboratory, *C. elegans* are commonly grown on agar plates, but when large numbers of worms are needed, worms are often grown in liquid media [18]. *Caenorhabditis elegans* are grown in liquid for other purposes as well, for example, to synchronize the worms' growth at the L1 stage [18], to control for the composition of the worms' diet [19] and sometimes for studying starvation [18].

Liquid media were also used to study how swim exercise affects the worm's physiology [20]. In addition to *C. elegans*, other nematodes, such as the entomopathogenic species *Steinernema* [21,22] and *Heterorhabditis* [23,24], are routinely grown in liquid culture, and recently, growth in liquid medium of different *Pristionchus* species was shown to generate striking phenotypic differences [25] (see also Discussion).

In nature, *C. elegans* worms are most often isolated from solid decomposing plant material. However, there are rare reports of isolation of *C. elegans* from freshwater as well [26]. In addition, it has been hypothesized, but not yet documented, that *C. elegans* could encounter liquid environments in the wild, in cases of excessive decomposition, especially of fruits [27].

Here, we report that growth of worms in liquid media changes the morphology of the liquid-grown worms and also the morphology of their plate-grown progeny. We found that plate-grown descendants of liquid-grown worms are on average longer than control worms. This morphological change is apparent early during the worms' life, and persists even in spite of experimental synchronization of the worms' development. In addition, the inheritance of this phenotype does not appear to depend on pheromone production or any single known epigenetic inheritance pathway, as we could not find a single mutant in which these inter-generational effects are cancelled. While the effect is robustly inherited to the immediate F1 progeny, the inheritance to the F2 and the F3 generations exhibits variability. These results demonstrate that commonplace cultivation conditions can dramatically change the worms' physiology in adulthood, and can also affect the next generations. We suggest that the growth and handling conditions of at least a few generations should be monitored before experimentation begins.

2. Results

Even in the laboratory, *C. elegans* often encounters different environments, for example, worms are frequently starved or grown at slightly stressful temperatures, either accidentally or intentionally. It is very common to synchronize or control the speed of the worms' growth by starving them or by switching the growth temperature. Similarly, in many cases, researchers are forced to grow temperature-sensitive worms at low temperatures, or to cultivate the worms at high temperatures to avoid transgene silencing [28]. Contaminations with different pathogens are very common in *C. elegans* cultures, and while this has not been studied in detail, such infections could also leave heritable imprints [29]. As described above, worms are often grown in liquid in the laboratory (the standard liquid used is M9 buffer [18]). In the course of conducting many experiments with liquid-grown worms, we noticed that after growth in liquid medium, the worms exhibit a different morphology in comparison with plate-grown worms. We first set out to characterize this phenomenon, and then to examine whether the effects could be inherited (figure 1*a*).

(a) Progeny of liquid-grown worms are longer

We noticed that worms that grow in liquid become narrow, and that their body length becomes more variable in comparison with plate-grown worms. These observations are in accordance with a previous observation [18], and a recent study showing that worms grown in axenic (chemically defined and bacteria-free) liquid medium (specifically the

'Habitation and Reproduction' medium used by NASA when growing worms in space [19]) are also narrower and longer than plate-grown worms [30].

We found that the worms' concentration in liquid affects their size ($p < 0.0001$, one-way ANOVA), and that while in low concentrations, the liquid-grown worms are longer (figure 1*b,c*); in very high concentrations, liquid-grown worms become shorter (perhaps in this condition, not enough food is available). As starvation is known to generate heritable effects in *C. elegans* [8–11], in all the subsequent experiments, we grew worms in liquid in low concentrations (maximum 0.05 worms per microlitre, see Methods). In this concentration, liquid-grown worms are approximately 22% longer than plate-grown worms ($p < 0.0001$, Sidak's multiple comparisons test, figure 1*b,c*).

Next, we tested whether plate-grown progeny of liquid-grown worms differ in their body length (figure 1*a,b*). Interestingly, we found that the F1 progeny of liquid-grown worms were on average approximately 8% longer than control worms ($p < 0.0001$, two-way ANOVA, figure 1*b,d*). The heritable effect was very robust, repeated across many biological repeats, and was independent of the concentration of the parent worms in the liquid medium (electronic supplementary material, figure S1; in addition, experiments were replicated by multiple different experimenters).

When placed in liquid media, worms change their movement, to a swim-like behaviour, which involves continuous 'thrashing'. Previously, this swim-like behaviour was used to study the effects of exercise on the worm's physiology [20]. We wondered whether this energetically demanding behaviour could be involved in the morphological change found in the liquid-grown worms and their plate-grown progeny. To test this, we examined the morphology of liquid-grown immobile *unc-119* mutants across generations. We found that liquid-grown *unc-119* mutants do not become longer in liquid ($p < 0.0001$, two-way ANOVA, electronic supplementary material, figure S2A,B). While experimentation with such *Unc* mutants is challenging (the mutants exhibit higher length variability, and do not grow well in liquid), the plate-grown progeny of liquid-grown mutants were nevertheless longer than controls ($p < 0.0001$, two-way ANOVA, electronic supplementary material, figure S2C). These results suggest that swimming in liquid is not required for the inter-generational effect.

Recently, Çelen *et al.* analysed the transcriptome of worms that grew in a special axenic liquid medium [31]. They found that the genes that were upregulated in axenic medium were enriched with genes related to cuticle formation and morphology. Further, some transcriptional changes were also inherited by plate-grown F1 progeny. The authors did not examine whether the morphology of the progeny of the worms that grew in axenic medium changed; however, it is possible that these transcriptional changes correspond to the inherited morphological changes that we observe in the progeny of M9 liquid-grown worms.

(b) The change in the morphology of the progeny of liquid-grown worms is apparent early during development, and persists even when the progeny's development is synchronized

It is possible that the progeny of the liquid-grown worms is longer because these worms develop faster. To test this
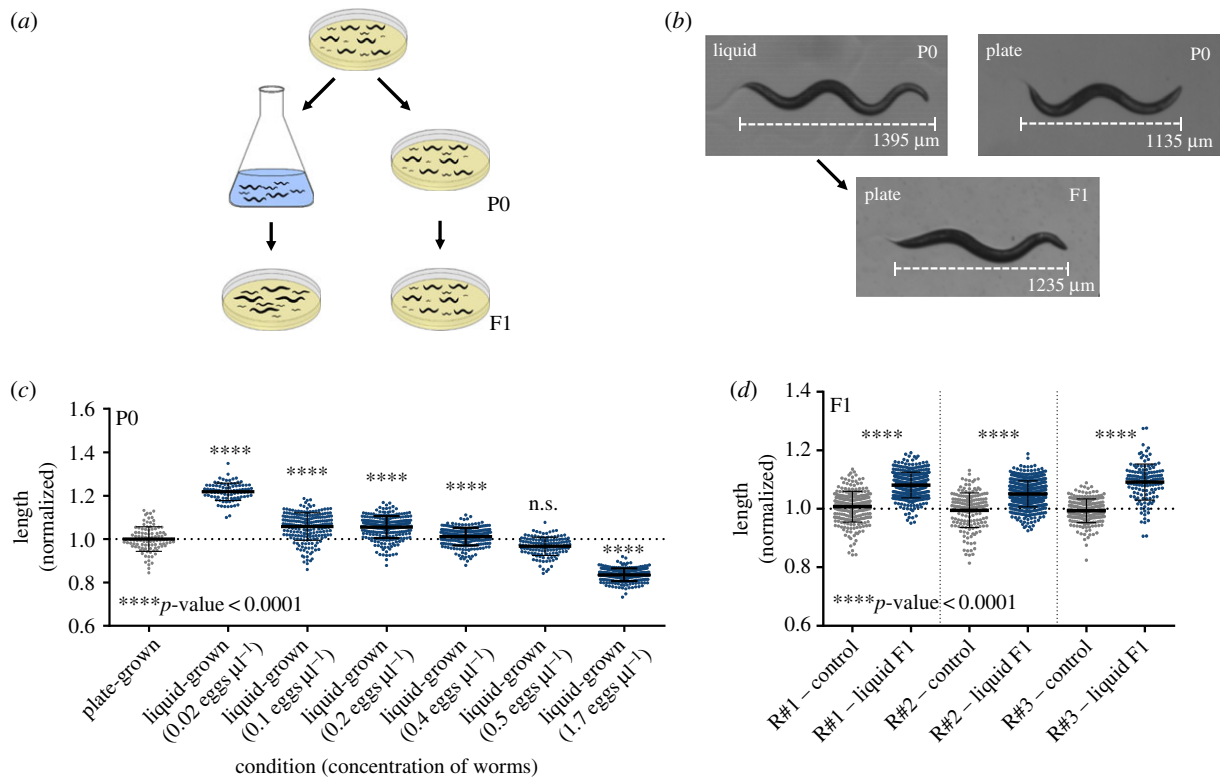
Figure 1. Progeny of liquid-grown worms have longer morphology. (*a*) Experimental scheme of inter-generational inheritance of growth in liquid medium. Eggs were produced from isogenic worm lines and placed either on plates or in liquid medium. When the parental P0 worms reached adulthood, they were placed on plates to lay their eggs. The body length of their resulting F1 adult progeny was measured. (*b*) Representative photographs of adult plate-grown (P0), liquid-grown (P0) or plate-grown progeny (F1) of liquid-grown worms. The differences in length between the photographed worms match the average length differences found between the examined populations. A scale bar that shows the total *stretched* length of the worm is presented. (*c*) Liquid-grown worms are longer when grown in low concentration. The normalized lengths of the parental plate-grown, and liquid-grown worms are shown. Worms were grown in liquid in different concentrations (*x*-axis). The measured length was normalized to that of the plate-grown worms. The *p*-value was determined using one-way ANOVA. n.s., not significant. (*d*) Plate-grown progeny of worms grown in liquid medium are longer. The length of F1 plate-grown progeny of liquid-grown worms and progeny of plate-grown controls are shown. The measured length was normalized to that of the corresponding control worms. Data from three independent biological repeats are presented ($N > 150$ worms per group). The *p*-value was determined using two-way ANOVA with the two categorical variables: (i) biological condition (growth in liquid or on plates) and (ii) biological repeat (independent experiments conducted on separate groups of animals, on different days). Both factors have a significant effect on the measured worm length ($p < 0.0001$). In addition, an interaction effect was found ($p < 0.0001$). The biological condition explained far more variance than the biological repeat or the interaction (32.44 compared with 2.07 or 1.69%, respectively). Error bars represent standard deviations.

possibility, we monitored the worms' length throughout development. To our surprise, we found that the progeny of liquid-grown worms were already longer as eggs ($p < 0.0001$, two-way ANOVA, figure 2*a*). The progeny of the liquid-grown worms were also longer immediately after hatching, at the L1 stage (figure 2*b*; electronic supplementary material, figure S3; $p = 0.022$, see Statistical analysis (§4g)), they maintained the elongated morphology throughout development, and their rate of development was identical to the control group (figure 2*b*; electronic supplementary material, figure S3; $p = 0.7806$, see Statistical analysis (§4g)). Moreover, the effect persisted in adults, even when we synchronized the worms' development at the L1 stage by starvation (for 12 h, see Methods; $p < 0.0001$, two-way ANOVA, figure 2*c*). In summary, the elongated morphology of the F1 progeny of liquid-grown worms does not stem from differences in the rate of development.

(c) Progeny of liquid-grown worms are longer even in mutants defective in chemosensation and pheromone biosynthesis

In response to various environmental changes, *C. elegans* alter the composition of the molecular social cues (or pheromones)

that they secrete [32,33]. In turn, the secreted pheromones greatly affect the worms' physiology [34,35]. For example, in response to crowded culture conditions, *C. elegans* secrete pheromones that cause starved larval worms to arrest their development and enter the dauer stage (an alternative developmental stage during which worms can endure harsh conditions) [35]. We examined whether sensation of pheromones or other secreted molecules could be the factor that transforms the progeny of the liquid-grown worms. To test this, we conducted experiments with mutants defective in pheromone production in general (*daf-22*) and dauer pheromones sensation specifically (*srg-36/37*). We also examined mutants that are generally defective in chemosensation (*che-2*).

DAF-22 is a fatty acid β-oxidase important for the production of short-chain ascarosides [36] and dauer pheromones [37]. SRG-36/37 are two redundant G-protein-coupled receptors for the dauer pheromone ascaroside C3 [38]. Mutations in these genes that confer resistance to dauer formation were identified in two independent long-term liquid-grown strains (in worms that were grown in liquid for many decades [38]). We found that liquid-grown *daf-22* and *srg-36/37* mutants generate a functional inter-generational response and, similarly to wild-type worms,
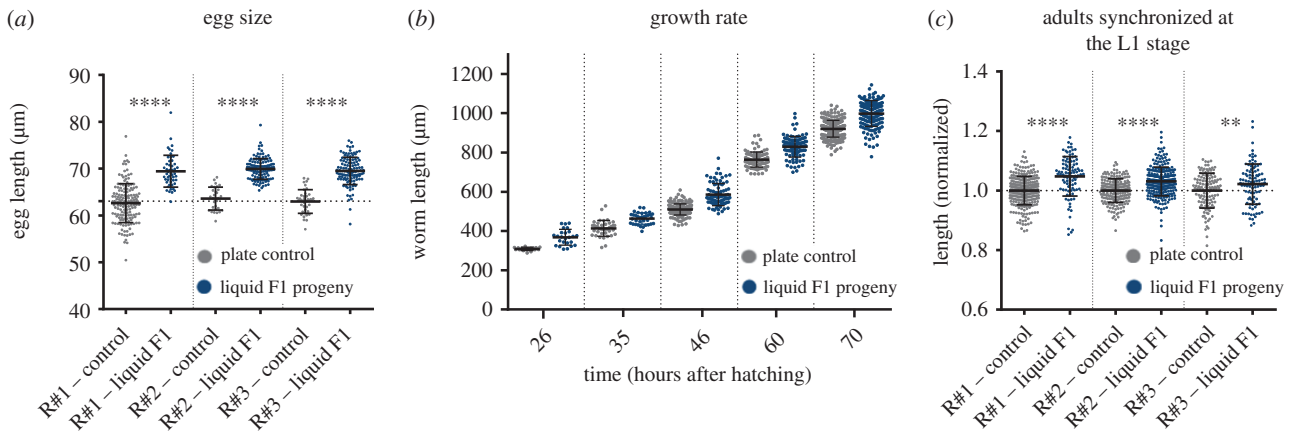
Figure 2. Progeny of liquid-grown worms are already longer early in development. (*a*) The length of eggs laid by liquid-grown worms and plate control worms. Data from three independent biological repeats are presented ($N > 30$). ****$p < 0.0001$, two-way ANOVA (biological condition is significant: $p < 0.0001$, biological repeat and interaction effect factors are not significant: $p = 0.8642$ and $p = 0.2045$, respectively). Sidak's multiple comparisons test p-values are presented. (*b*) The length of progeny of liquid-grown worms and control worms is presented at different stages of the worms' development. (*c*) The length of adult worm progeny of liquid-grown and plate-grown controls, 72 h after being released from starvation-induced developmental arrest at the L1 stage. Data from three independent biological repeats are presented ($N > 100$). ****$p < 0.0001$, **$p = 0.0066$. Two-way ANOVA (biological condition is significant: $p < 0.0001$, biological repeat and interaction effect factors are also significant: $p = 0.0093$. These factors explain 0.81% of the variance, while the biological condition explains 7.96% of the variance). Sidak's multiple comparisons test p-values are presented. Error bars represent standard deviations.
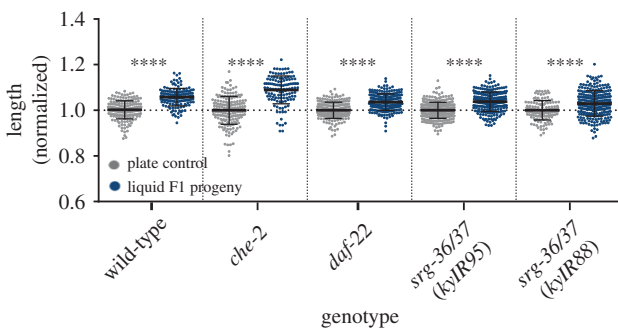

Figure 3. Progeny of liquid-grown worms are longer even in mutants defective in chemosensation and pheromone biosynthesis. The normalized lengths of progeny of liquid-grown mutants defective in genes involved in dauer pheromone synthesis (*daf-22*), pheromone sensation (*srg-36/37*), stress response (*daf-16*) and chemosensation (*che-2*) are presented. $N > 100$ per group. ****$p < 0.0001$, one-way ANOVA, Sidak's multiple comparisons test. Error bars represent standard deviations.

plate-grown progeny of the liquid-grown mutants were longer than controls (figure 3). Thus, pheromones are probably not involved in this inter-generational effect.

In *che-2* mutants the cilia of the sensory neurons are severely defective [39]. Accordingly, these mutants show defects in osmotic pressure avoidance [40], and are defective in chemotaxis to NaCl [39,40] and to many odorants [41]. We found that the progeny of liquid-grown *che-2* mutants are also elongated, similarly to wild-type worms. Therefore, sensation of secreted molecules does not appear to mediate the inter-generational response that we describe here (figure 3).

DAF-16 is a transcription factor playing a pivotal role in the insulin/IGF-1 pathway, regulating stress responses, stress survival, longevity, fat metabolism, immunity and dauer formation [42]. DAF-16 was further implicated in inter-generational responses to stress [16]. Surprisingly, plate-grown progeny of liquid-grown *daf-16* mutants were elongated, similarly to wild-type worms (figure 3). This suggests that the

DAF-16-dependent stress response is not involved in the liquid-induced heritable effect.

(d) Progeny of liquid-grown worms are longer even in mutants defective in inheritance of small RNAs and chromatin modifications

To examine whether the heritable changes in body length depend on inheritance of small RNAs or histone modifications, we examined mutants that are defective in production or inheritance of small RNAs and histone modifications. The nuclear argonautes HRDE-1 and NRDE-3 carry heritable small RNAs, and are required for the inheritance of dsRNA-induced silencing [43,44]. While NRDE-3 is expressed in somatic tissues and is important for inter-generational inheritance of somatic RNA interference [45], HRDE-1 is expressed in the germline and is required for the trans-generational inheritance (lasting multiple generations) of exogenous, dsRNA-derived small interfering RNAs (siRNAs) and endogenous siRNAs that target germline genes [43]. The amplified siRNAs that are carried by the NRDE-3 and HRDE-1 argonautes are produced by RNA-dependent RNA polymerases (RdRPs), such as RRF-1. RRF-1 is involved in the inheritance of Piwi-interacting RNA (piRNA)-triggered endogenous siRNAs and viral-derived siRNAs [5,46]. We found that *hrde-1*, *nrde-3* and *rrf-1* mutants, as well as additional mutants defective in siRNA biogenesis (*rde-1*, *rde-4*, *rrf-3* and *mut-16*), are not required for the inheritance of the elongated morphology (figure 4). Previously, histone-H3-lysine-9 (H3K9) methylations were implicated in trans-generational inheritance of small RNAs [47–50]. H3K9 mono- and di-methylations are deposited by the MET-2 methyltransferase [51], while H3K9me3 is deposited by the SET-25 and SET-32 methyltransferases [51,52]. We found that *met-2*, *set-25* and *set-32* mutants also inherit the elongated morphology from their liquid-grown parents (figure 4). To conclude, the inter-generational inheritance of this phenotype does not appear to depend on the canonical epigenetic inheritance pathways that we examined.
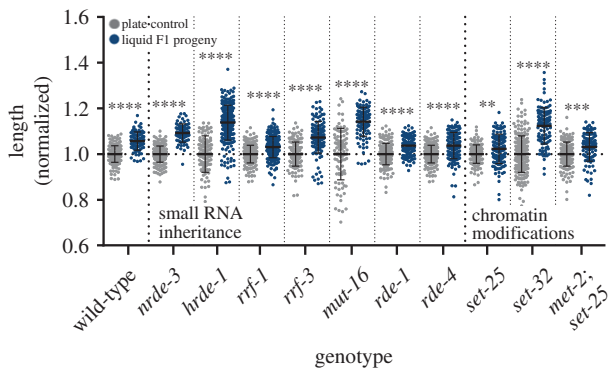
Figure 4. Mutants defective in inheritance of small RNAs and histone methylations are also longer, if they derive from ancestors that were grown in liquid. The normalized lengths of progeny of liquid-grown worms mutated in genes involved in small RNA biogenesis and inheritance (*hrde-1*, *nrde-3*, *rrf-1*, *rrf-3*, *rde-1*, *rde-4* and *mut-16* mutants) or chromatin methylation (*set-25*, *set-32* and *met-2; set-25* mutants) are presented. $N > 100$ per group. ****$p < 0.0001$, ***$p = 0.0001$, **$p = 0.0084$, one-way ANOVA, Sidak's multiple comparisons test. Error bars represent standard deviations.

(e) Unlike the inter-generational effects, trans-generational inheritance of the morphological changes exhibits variability

Previously, certain environmental stresses, such as L1 starvation [8], starvation during dauer arrest [11] and exposure to high temperatures [12], were shown to induce trans-generational effects that persist for more than two generations. To test whether the elongated body morphology can be inherited for more than one generation, we measured the body length of plate-grown worms that derive from liquid-grown parents in the F1, F2 and F3 generations. In contrast with the inter-generational changes to the worms' size which were robustly inherited by the F1 generation, the trans-generational effect in the F2 and F3 generations was variable. While in some experiments we did observe trans-generational heritable changes, in other cases the effect was gone after one to two generations (figure 5).

3. Discussion

In this manuscript, we show that growth in liquid medium under laboratory conditions changes the morphology of *C. elegans* worms and the morphology of their progeny. As briefly described above, there are rare reports of isolation of *C. elegans* from freshwater habitats [26]. There are also phenotypes which are specific to worms that grow in liquid. For example, infection of *C. elegans* in liquid by the pathogenic bacterium *Leucobacter celer* was found to induce aggregation of worms (termed 'worm stars') [53]. Whether the morphological changes that we describe are adaptive in natural circumstances remains to be determined.

In addition to *C. elegans*, other nematodes are routinely cultured in liquid cultures. Entomopathogenic nematodes such as *Steinernema* [21,22] and *Heterorhabditis* [23,24] are grown in liquid cultures as a means of mass production of a biocontrol agent. However, liquid-grown entomopathogenic nematodes were found to have lower dauer stage recovery rates and pathogenicity [21,54,55], and much effort was invested in optimizing the different aspects of their mass production in liquid cultures

[22]. Recently, a different nematode model organism, *Pristionchus pacificus*, was found to exhibit altered, narrow and elongated morphology when grown in liquid cultures [25]. Interestingly, the dimorphic mouth-form of *P. pacificus* was found to respond to growth in liquid medium; however, the response was not heritable [25]. Several other *Pristionchus* species, but not all, also showed mouth-form alteration in response to growth in liquid culture [25].

During the course of this study, we could not identify mutants in which epigenetic pathways are affected that are defective for the inter-generational inheritance of the elongated morphology. It is possible that such mutants do exist, and could be detected in more comprehensive screens. Alternatively, the inter-generational effects that we examined could transmit via many different molecular 'agents' that do not necessarily affect epigenetics. Pheromone production and sensation do not seem to be required for this effect, as mutants in the relevant pathways also exhibited the heritable liquid-induced effect. Changes in other molecules, such as metabolites or hormones, which are not necessarily self-propagating or 'amplifiable' (in contrast with heritable small RNAs), could mediate such short-term inter-generational inheritance as well.

Previously, environmental changes such as starvation and temperature shifts, both common in the laboratory, were shown to change the physiology of the *C. elegans* worms and of their progeny. Together with growth in liquid, these manipulations should be taken into consideration when designing experiments with *C. elegans*. It is reasonable to assume that many other subtle characters are affected in the progeny of liquid-grown worms (for example, behaviour). To control for such changes, the worms' growth conditions should be monitored for several generations before experimentation.

Inter-generational and trans-generational inheritance present a unique challenge: to compare between different experiments, the experimenter must keep track of the history of the culture, to make sure different experiments begin from the same starting point. There are currently no standard protocols for achieving this goal. We do not know yet how to 're-set' epigenetic inheritance. Previously, we used CRISPR/cas9 to deactivate HRDE-1, a factor that is important for small RNA inheritance, consequently erasing small RNA memory. This led to the re-setting of the accumulated sterility of chromatin mutants (Mrt phenotype) [49]. Since not all heritable effects are necessarily small-RNA-mediated, other re-setting strategies should be devised in parallel.

The same principles that were discussed above with regard to inter- and trans-generational epigenetic inheritance in *C. elegans* could apply also to other organisms, and even to experiments conducted with cell lines. Several years ago, a controversy arose regarding the mislabelling of many cell lines [56]. While this is certainly a serious problem, it is worth noting that even when the correct cell line is used, cells in culture acquire mutations [57] (and differ in ploidy), and moreover, non-genetic inheritance could create variability between cultures in different laboratories. Indeed, the 'generation time' of the culture is a factor, as cells that have been passaged many times can differ from recently thawed stocks [58,59]. RdRPs, which are crucial for small RNA inheritance in *C. elegans*, are not known to be conserved in humans [4]; however, even if this mechanism is missing in other organisms, epigenetic memories could be transmitted to daughter cells via different feedback mechanisms [60,61]. For example, in
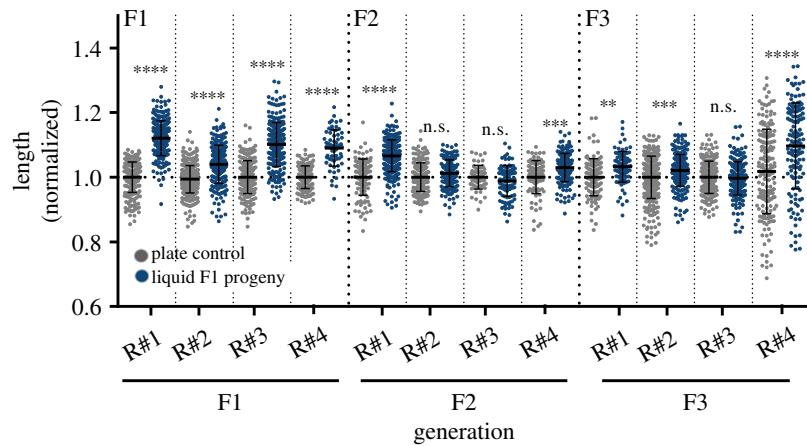
Figure 5. The liquid-induced longer morphology is not always inherited trans-generationally (in some experiments the effects peter out after one generation). The normalized lengths of progeny of liquid-grown worms and progeny of plate-grown controls in the F1, F2 and the F3 generations are presented. The measured length is normalized to the corresponding control worms of the matching generation and biological repeat. Data from four independent biological repeats are presented ($N > 150$ worms per group). ****$p < 0.0001$, ***$p = 0.0003$ for F3 R#3 ***$p = 0.0010$ for F2 R#4, **$p = 0.0015$, n.s. not significant. One-way ANOVA, Sidak's multiple comparisons test. Error bars represent standard deviations.

human cells, genes known to be induced by interferon-γ (IFN-γ) were found to exhibit a more robust induction in cells that have previously experienced IFN-γ [62,63]. Importantly, this effect was maintained for four to seven cell divisions, and was associated with H3K4 dimethylation [63]. Therefore, while the causative agents are not always characterized, it is clear that some information that is not encoded in the DNA sequence can persist for long durations. This type of inheritance should be taken into account in the experimental design.

4. Methods

(a) Cultivation of worms

Standard culture techniques were used to maintain *C. elegans* on nematode growth medium (NGM) plates seeded with OP50 *Escherichia coli* bacteria. Extreme care was taken to avoid contamination or starvation, and contaminated plates were discarded from the analysis. For inheritance experiments, worms were synchronized either by a standard sodium hypochlorite bleaching technique or by letting adult worms lay eggs on OP50-seeded plates for 2 h.

(b) Growth in liquid medium

Worm eggs were seeded into flasks with 20 ml of M9 buffer (including 1 mM MgSO$_4$ [18]) supplemented with 200 μl penicillin–streptomycin–nystatin (penicillin G sodium salt: 10 000 units ml^{-1}, streptomycin sulfate: 10 mg ml^{-1}, nystatin: 1250 units ml^{-1}) and 20 μl cholesterol (5 mg ml^{-1}) and were fed with 200 μl of concentrated OP51 *E. coli* bacteria (the bacteria were grown overnight in LB buffer + 100 μg ml^{-1} streptomycin, pelleted and resuspended in 1 ml of M9 buffer). The flasks were shaken (120 r.p.m.) at 20°C.

(c) L1 arrest experiments

Adult worms were washed using M9 buffer [18]. After removal of the buffer supernatant, an approximately 100 μl pellet of worms was treated for 5 min with 1 ml of bleach solution (2 ml of 5% sodium hypochlorite, 7.5 ml double distilled water, 0.5 ml NaOH 10 M). Next, 0.5 ml of M9 buffer was added and the solution was centrifuged (8000 r.p.m., 1 min). The resulting egg pellet was washed three times by removing the supernatant, adding fresh M9 buffer and centrifugation of 8000 r.p.m. for 1 min to re-pellet

the eggs. Around 400 eggs were left to hatch and arrest in the larval L1 stage on fresh plates for approximately 12 h. Next, larval worms were transferred using M9 buffer to NGM plates seeded with OP50 bacteria. Worms were photographed 72 h later.

(d) Strains used in this study

YY720: *hrde-1(tm1200)* III., YY158: *nrde-3(gg66)* X., RB798: *rrf-1(ok589)* I., GW638: *set-25(n5021)* III.; *met-2(n4256)* III., VC967: *set-32(ok1457)* I., MT17463: *set-25(n5021)* III., WM27: *rde-1(ne219)* V., WM49: *rde-4(ne301)* III., YY13: *rrf-3(mg373)* II., GR1823: *mut-16(mg461)* I., CF1038: *daf-16(mu86)* I. CB1033: *che-2(e1033)* X., DR476: *daf-22(m130)* II., EG6699: *ttTi5605* II.; *unc-119(ed3)* III., CX13249: *srg-37; srg-37(kyIR88)* X., CX13591: *srg-36; srg-37(kyIR95)* X.

(e) Worm length measurements

Worms were placed on empty NGM plates and photographed using a ScopeTek DCM310 binocular camera. The worms' length was measured using WormSizer [64].

(f) Egg length measurements

Worms were allowed to lay eggs on an empty NGM plate, and the laid eggs were photographed using a ScopeTek DCM310 binocular camera. Egg length was measured using the particle analysis tool of the ImageJ software.

(g) Statistical analysis

Two-way ANOVA was used to compare the effect of two categorical variables on the length of the worms' progeny (this statistical analysis is relevant to the experiments shown in figures 1*d* and 2*a,c* and in electronic supplementary material, figures S2B,C): (i) biological condition (growth in liquid or on plates) and (ii) biological repeat (independent experiments conducted on separate groups of animals, on different days). One-way ANOVA was used to compare the effect of growth in liquid medium on worm length between different genetic backgrounds (figures 3 and 4), between different food regimes (figure 1*c*; electronic supplementary material, figure S1) or between conditions across generations (figure 5). In cases of multiple comparisons between genotypes conditions, Sidak's multiple comparison test was applied. Linear regression analysis and a statistical method equivalent to analysis of covariance was used to compare the growth rates of progeny of liquid-grown

worms and control worms throughout development (figure 2b; electronic supplementary material, figure S3). Biological repeats are defined as independent experiments conducted on separate populations of animals, on different days. Statistical tests were performed using GraphPad Prism software version 6.

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