Temporally regulated cell migration is sensitive to variation in body size

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Reviewer 1

Evidence, reproducibility and clarity

Dubois and colleagues analysed the sensitivity to stochastic, environmental and genetic perturbations of the position of a long range migrating developmental system in C. elegans (the QR neuroblast and its neuronal progeny, QR.pax) which is known to be controlled by a clock mechanism and not by spatial cues. They show that both temperature and body size alters the mean final position of the QR.pax while starvation at hatching alters the variance but not the mean of its final position. Further, to explain the effect of body size on the final position the authors build a simple mathematical model which predicts that the final position of the cell is partially compensated by a change in speed. This prediction is then experimentally validated.
The authors analysed a wide panel of natural isolates and found substantial intra-species variation in final position which is not explained by variation in body size.
Overall the findings are interesting and the methods that include a nice combination of quantitative biology and mathematical modelling are appropriate. My points are therefore only minors.

1- Do the authors control for maternal age in their experiments? Maternal age has been recently reported to have a major phenotypic impact in C. elegans, notably it increases body size at hatch and resistance to L1 starvation. Does maternal age impact QR.pax final position consistently with the increase in body size? Does controlling for maternal age reduce the variance in the QR.pax final position induced by starvation?

2- Starvation. the authors avoid bleaching in their starvation protocol which could induce side effects however the way those experiment are performed has also the potential to induce a lot of variation in the amount of starvation the larvae are subjected to. By letting a population on an exhausted plate for two more days after food exhaustion the author traduce a substantial variation in starvation. After food exhaustion in fact there will be plenty of eggs in the plate and larvae keep hatching for the next 10-12 hours at 20C. Coupled with the fact that some larvae might have had access to minimal amount of food as the authors themselves explain in the materials and methods section. This increased variation without change in the mean final position might therefore be a consequence of variable level particularly if the true response to starvation is non-monotonic. What happens if instead the authors better control the amount of starvation for example by starving larvae that hatches in a short window (1-2h)?

3- Jargon. I find that the abstract is heavy on jargon. It would help to increase the readability (and readership) if the authors could for example avoid the term QP.pa and QR.pax in the abstract especially because they only explain them in the introduction. Or, alternatively, explain them in the abstract first.

Significance

Understanding sensitivity/robustness of a long range migrating systems during development is an important topic. The migration of the system is not coordinated by spatial cue but by a timing mechanism therefore the system final position is bound to be sensitive to changes in body size. The finding that the system intrinsic sensitivity to body size is only compensated partially compensated by a change of speed is interesting. As well as the finding that the within species variation in the system final position is not correlated to variation in body size means that widespread compensation has evolved and it will be interesting to analyze the genetic bases of such compensation.

Despite not being the first study to quantitatively analyse long range migration robustness to stochastic noise in a developmental system, the combined analysis of robustness to stochastic robustness with robustness to both environmental and genetic variation across a wide panel of natural isolates is quite rare if not unique.

This paper should be interesting to a wide audience from developmental to evolutionary and quantitative biologists.

My expertise in is genomics, quantitative and systems biology and C. elegans development.

Reviewer 2

Evidence, reproducibility and clarity

The manuscript by Dubois et al, titled "Sensitivity to perturbations of a cell migration under temporal regulation" reports characterizations of long-range neuronal cell migrations (specifically QR and its descendants) across wild type C. elegans isolates, environmental perturbations, and body size variants. This is a particularly interesting exploration, as final position of QR descendants is regulated by a temporal event (MIG-1 expression) and not by positional cues.

This manuscript demonstrates that final positioning of QR descendants is sensitive to environmental conditions and genetic variation of C. elegans wild isolates. This work combines a descriptive study with mathematical modeling to assess the extent and the potential causes that may compensate for cell positioning under conditions of variable body size. The manuscript draws several interesting conclusions regarding the robustness of cell migration against various perturbations.
The experiments are adequately replicated and the data are analyzed with appropriate statistical analyses.

This reviewer has no major comments but believes addressing the following minor comments could improve the manuscript.

Minor comments:
1."Robustness to environmental perturbations" Page 8, lines 203-205. It was unclear whether the authors ensured that the larvae were not any older than late L1. It is clear that they ensured that younger larvae were not scored, and this reviewer assumes that older larvae were also not scored, due to the potential variability that can be introduced by scoring older aged larvae and the fact that the timing of migration may have occurred during higher food availability. A statement to the upper "age limit" on the larvae would clarify the matter.

2.Throughout the methods section, it would be useful to include the genotypes (when appropriate) next to the strain names so that the reader does not have to refer to the strain list each time (one example is on page 9, line 243). Similarly, strains generated in the course of this study should also

be listed with the strain name under "C. elegans strains". For example, the newly generated strains ayIs9 sma-1(e30) and lon-1(e185); ayIs9 on page 9 lines 243-244 should include strain names.

3.Page 13, lines 336-337. The sentence is either missing commas or needs to be rephrased.

Comment 4 relates to order of Figure panel discussions in text.

4a.Figure 1 panels A, and B are not referred to specifically. The first reference to Figure 1 panels is to Figure 1B in Methods, then Figure 1D, followed by a reference to Figure 1C,D. It would be beneficial to point the reader to specifically to Figure 1 panels A, B in the introduction when both describing the QR migration as well as mentioning other migrating neurons.

4b.Figure 1E is referred to after Figure 1F. This causes confusion as the reader expects to learn more about starvation-induced changes based on Figure 1 flow of data, but the text does not follow. Perhaps the paragraph on Page 14 lines 369 etc. could be moved up to match the Figure panels or the panels rearranged to follow the text.

4c.There are other instances of Figure panels shown but not referenced, for example: Figure S1B. The authors may want to work through the entire manuscript to ensure appropriate Figure panel referencing.

5. Page 3, line 89. "Figure 1.D" is a typo

6. Figure 1 panel C. It would be useful to have a scaled down (and perhaps simplified) version of panel B as part of panel C to orient the reader on the range of neuronal positions within the animal body. While the numerical positions are provided, having to refer back to panel B is somewhat cumbersome.

7. Page 13 line 345: "which is likely to reflect the fact that it only migrates at a short range" -perhaps should be changed to "which likely reflects the fact that it only migrates a short range"

8. Page 13 line 251 should be "implies".

9. Page 15, line 423, fln-1 allele name should be italicized.

10. Page 18, line 505. I am not sure if "better known animals" is an entirely accurate way to say it, perhaps "better known examples from other model organisms" or something along those lines. I am also not entirely convinced by the analogy. Cell positioning through migration does not seem quite the same as a homeotic transformation, where cell fates are drastically affected, to this reviewer. However, the displacements are very dramatic. (Please note that this reviewer is not necessarily asking for authors to change their wording, just cautions on the extent of the analogy.)

11. Figure 2C and related discussions on modeling. While may be beyond the scope of this study, having additional body size variants may be needed to confidently assess the predictive power of this model.

12. While temperature had only a mild effect on QR descendants positioning, could the authors include a short discussion (a couple of sentences) on how or why temperature may be affecting final QR.pax positions? Perhaps rate of migration vs. rate of overall animal development? In addition, it would be interesting to what effect temperature fluctuations during the time of migration might have on final cell positions, as this more egregious environmental assault and something that wild isolates likely experience.

13. The order of the Supplemental Figures 2 and 3 must have been switched during the upload, creating some momentary confusion for this reviewer.
Legend for Supplementary figure 3 should include description of the individual panels. Legend for Supplementary figure 2 is lacking references to panels E-G.

14. Figure 2 legend: the authors refer to the bottom portion of panel D as panel E and provide a legend for F (even though there is no F). Either add the panel labeling or fix the Figure legend.

Significance

This manuscript provides conceptual advances to the field of cell migration and to the field of developmental robustness faced with environmental and genetic perturbations. As such, this work is of interest to a broad audience spanning multiple fields and organismal systems.
This manuscript demonstrates that final positioning of QR descendants is sensitive to environmental conditions and genetic variation of C. elegans wild isolates. This work combines a descriptive study with mathematical modeling to assess the extent and the potential causes that may compensate for cell positioning under conditions of variable body size. In addition, the report includes characterizations of egg and body sizes that would be of practical use to researchers within the field of C. elegans development.

This reviewer's expertise is within C. elegans development, cell migration, and does not include mathematical modeling and.

Reviewer 3

Evidence, reproducibility and clarity

Summary:

In this article Dubois et al. analyzed the robustness of a neuroblast (QR) migration process to various perturbations in the C. elegans model organism. It was previously established that the end of migration, and therefore final position, is determined by a timing mechanism rather than a positional cue. They first analyzed the effect of perturbations of the size of the animal on the final position by combining genetic manipulations and mathematical modeling.
Interestingly, they showed that the effect of body size changes are partially compensated by a change in migrating cell velocity. They also showed that changes in temperature and starvation affect respectively the mean and the variance of the final position. Finally, they observed that the cell final position varies among different C. elegans wild isolates.

Major comments:

This is a high quality study. The conclusions are well supported by the experiments. The method, data and replicability/statistics are adequately reported.

Minor comments:
-The authors compare the variability of QR migration to that of other neurons (BDU, ALM, CAN, HSN). Is it known that the final position of these neurons is determined by positional cues rather than a timing mechanism ?

-QR.pax final position distribution looks unimodal in well fed animals but bimodal afterstarvation (figure 1). Could the authors comment on that ? Any possible explanation ?

-Line 365 : The temperature shifts QR.pax final position toward the anterior as thetemperature decreased, with a value of 0.04 relative position unit per degree (Figure 1F).

The authors should precise that this is in N2.
- Figure S2 : several panels (B, C...) are not mentioned in the main text. The authors should comment on these panels in the text.

-Line 423 : ok2611 should be in italic.

-Line 482 : « We did not find any correlation between QR.pax final position and the absolute value of the sampling latitude (t = -0.94, p=0.35). ». This sentence is unclear to me, the author should explain a bit more.

-Line 880 : « (E) » should be suppressed.

-Line 883 : « F) » should be replaced by « E) ».

-Line 922 : « C-D) » should be replaced by « C-G) ».

-Figure 1C : x-axis label « QR.pax final position », « QR.pax» should be suppressed as the position of other cells is also presented in the graph.

-Figure S1D : the authors should label the AVM projection.

Significance

While the mechanisms that guide cell migrations have been extensively studied, how cells decide to stop their migration and how robust the final cell position is to perturbations has been less characterized. In this study, the authors analyzed an original case of cell migration (QR neuroblast of C. elegans) where the stop decision is based on an intrinsic timing mechanism rather than a more classic positional cue. How this time-based mechanism may affect the precision of the final cell position is unknown. In this study, the authors characterized the robustness of this cell migration mechanism to body size variations, environmental perturbations or natural genetic variations. In particular they observed that the effect of body size variations are partially compensated by changes in migrating cell velocity. This study will be of high interest not only for the field of cell migration but also for people interested in the robustness and precision of developmental processes.

Author response to reviewers' comments

We thank the reviewers for their general enthusiasm and their useful suggestions. We answer these suggestions below in blue font when we have already taken them into account by text and figure changes. Those in green correspond to proposed experiments for the revision.

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

Dubois and colleagues analysed the sensitivity to stochastic, environmental and genetic perturbations of the position of a long range migrating developmental system in C. elegans (the QR neuroblast and its neuronal progeny, QR.pax) which is known to be controlled by a clock mechanism and not by spatial cues. They show that both temperature and body size alters the mean final position of the QR.pax while starvation at hatching alters the variance but not the mean of its final position. Further, to explain the effect of body size on the final position the authors build a simple mathematical model which predicts that the final position of the cell is partially compensated by a change in speed. This prediction is then experimentally validated. The authors analysed a wide panel of natural isolates and found substantial intra-species variation in final position which is not explained by variation in body size. Overall the findings are interesting and the methods that include a nice combination of quantitative biology and mathematical modelling are appropriate. My points are therefore only minors.

1- Do the authors control for maternal age in their experiments? Maternal age has been recently reported to have a major phenotypic impact in C. elegans, notably it increases body size at hatch and resistance to L1 starvation. Does maternal age impact QR.pax final position consistently with the increase in body size? Does controlling for maternal age reduce the variance in the QR.pax final position induced by starvation?

Indeed, it has been shown that maternal age affects body size at hatching and resistance to L1 starvation (Perez et al 2017 in Nature). For all the experiments of this study, we avoided to use

synchronized population of mothers. The scored larvae were coming from populations of mothers with heterogeneous ages.

Considering the comment from Reviewer #1, we propose to measure the effect of the maternal age on QR.pax final position in N2 and the three wild isolates used in this study. We will compare the distribution of the trait among larvae coming from one, two and three days old mothers. According to the effect of the body size on QR.pax final position, we expect to have a more anterior distribution in the progeny coming from young adults (larvae supposedly smaller) and a more posterior distribution coming from old adults (larvae supposedly longer). We also may have to measure Pharynx-to-Rectum size at hatching in parallel to ensure that the maternal age affects larval sizes in all the strains.

The last part of the comment relates to the effect of the maternal age on QR.pax variance after starvation. The answer can be related to the comment below.

2- Starvation. the authors avoid bleaching in their starvation protocol which could induce side effects however the way those experiment are performed has also the potential to induce a lot of variation in the amount of starvation the larvae are subjected to. By letting a population on an exhausted plate for two more days after food exhaustion the author traduce a substantial variation in starvation. After food exhaustion in fact there will be plenty of eggs in the plate and larvae keep hatching for the next 10-12 hours at 20C. Coupled with the fact that some larvae might have had access to minimal amount of food as the authors themselves explain in the materials and methods section. This increased variation without change in the mean final position might therefore be a consequence of variable level particularly if the true response to starvation is non-monotonic. What happens if instead the authors better control the amount of starvation for example by starving larvae that hatches in a short window (1-2h)?

Reviewer #1 suggests to control the amount of starvation by comparing larvae with the same age (from 1-2h). As our method to induce starvation is less precise but closer to natural conditions, it is of interest to understand and quantify the true response to starvation.

We propose to perform another experiment, as the Reviewer #1 suggested, by letting hermaphrodites lay eggs for 1-2h in plates without food. Hatched larvae will be maintained starved for two days before food provisioning and scoring as described in the methods.
Related to the first comment above about the maternal age, we can even compare the response to starvation of population coming from mothers of different ages.

3- Jargon. I find that the abstract is heavy on jargon. It would help to increase the readability (and readership) if the authors could for example avoid the term QP.pa and QR.pax in the abstract especially because they only explain them in the introduction. Or, alternatively, explain them in the abstract first.
The abstract has been modified to introduce QR.pax a bit better, and to use the term less often.

Reviewer #1 (Significance (Required)):

Understanding sensitivity/robustness of a long range migrating systems during development is an important topic. The migration of the system is not coordinated by spatial cue but by a timing mechanism therefore the system final position is bound to be sensitive to changes in body size. The finding that the system intrinsic sensitivity to body size is only compensated partially compensated by a change of speed is interesting. As well as the finding that the within species variation in the system final position is not correlated to variation in body size means that widespread compensation has evolved and it will be interesting to analyze the genetic bases of such compensation.

Despite not being the first study to quantitatively analyse long range migration robustness to stochastic noise in a developmental system, the combined analysis of robustness to stochastic robustness with robustness to both environmental and genetic variation across a wide panel of natural isolates is quite rare if not unique.

This paper should be interesting to a wide audience from developmental to evolutionary and quantitative biologists.

My expertise in is genomics, quantitative and systems biology and C. elegans development.

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

The manuscript by Dubois et al, titled "Sensitivity to perturbations of a cell migration under temporal regulation" reports characterizations of long-range neuronal cell migrations (specifically QR and its descendants) across wild type C. elegans isolates, environmental perturbations, and body size variants. This is a particularly interesting exploration, as final position of QR descendants is regulated by a temporal event (MIG-1 expression) and not by positional cues.

This manuscript demonstrates that final positioning of QR descendants is sensitive to environmental conditions and genetic variation of C. elegans wild isolates. This work combines a descriptive study with mathematical modeling to assess the extent and the potential causes that may compensate for cell positioning under conditions of variable body size. The manuscript draws several interesting conclusions regarding the robustness of cell migration against various perturbations.

The experiments are adequately replicated and the data are analyzed with appropriate statistical analyses.

This reviewer has no major comments but believes addressing the following minor comments could improve the manuscript.

Minor comments:

1."Robustness to environmental perturbations" Page 8, lines 203-205. It was unclear whether the authors ensured that the larvae were not any older than late L1. It is clear that they ensured that younger larvae were not scored, and this reviewer assumes that older larvae were also not scored, due to the potential variability that can be introduced by scoring older aged larvae and the fact that the timing of migration may have occurred during higher food availability. A statement to the upper "age limit" on the larvae would clarify the matter.
The pattern of division of the seam cells ensured that not older nor younger animals were used to measure QR.pax final position.

We changed the text on p. 9 to make this clear:
"We scored QR.pax position six to eight hours later at a precise developmental timepoint: only when V seam cells divided once, Pn cells migrated ventrally, QR.pap reached the dorsal part of the animal and QR.paa the ventral part. This ensured that larvae were phenotyped at the same age."

2. Throughout the methods section, it would be useful to include the genotypes (when appropriate) next to the strain names so that the reader does not have to refer to the strain list each time (one example is on page 9, line 243). Similarly, strains generated in the course of this study should also be listed with the strain name under "C. elegans strains". For example, the newly generated strains ayIs9 sma-1(e30) and lon-1(e185); ayIs9 on page 9 lines 243-244 should include strain names.
The genotype is now included next to the strain names. Furthermore, *ayIs9 sma-1(e30) and lon-1(e185); ayIs9* now have a strain name, JU4128 and JU4129, respectively.

Correction in the text related to the strain names and genotypes: On p. 6, we added:

"To visualize the Q lineage in body size mutants, we generated the strains JU4128 (genotype *ayIs9 sma-1(e30)*) and JU4129 (genotype *lon-1(e185); ayIs9*) by crossing NH646 males carrying the *ayIs9[Pegl-17::gfp + dpy-20(+)]* transgene with hermaphrodites of strain CB30 carrying *sma-1(e30)* and strain CB185 carrying *lon-1(e185)*, respectively."

The text on p. 10was modified with the new strain names:

"The strains JU4128 of genotype *ayIs9 sma-1(e30)* and JU4129 of genotype *lon-1(e185); ayIs9* were used to estimate cell velocity of QR"

p. 10:
"[…]the strain GOU174 (genotype *casIs35*[*Pgcy-32*::mCherry, *unc-76(+)*] X; *zdIs5*[*Pmec- 4*::gfp, *lin-15(+)*]I) with a GFP marker[…]"

3. Page 13, lines 336-337. The sentence is either missing commas or needs to be rephrased.
Correction in the text on p. 14:
"Using N2 and three genetically distinct wild isolates, we first compared the variance in QR.pax final position to that of other neurons that migrate during embryogenesis: BDU, ALM, CAN and HSN (Sulston et al., 1983; Hedgecock et al., 1987) (Figure 1D)".

Comment 4 relates to order of Figure panel discussions in text.

4a.Figure 1 panels A, and B are not referred to specifically. The first reference to Figure 1 panels is to Figure 1B in Methods, then Figure 1D, followed by a reference to Figure 1C,D. It would be beneficial to point the reader to specifically to Figure 1 panels A, B in the introduction when both describing the QR migration as well as mentioning other migrating neurons.
Figure panels are now properly referenced.

4b.Figure 1E is referred to after Figure 1F. This causes confusion as the reader expects to learn more about starvation-induced changes based on Figure 1 flow of data, but the text does not follow. Perhaps the paragraph on Page 14 lines 369 etc. could be moved up to match the Figure panels or the panels rearranged to follow the text.
The paragraph on temperature has been moved after that on starvation. The order of the paragraph now matches with the order of the figure and the reference. The order in the Introduction has also been modified.

4c.There are other instances of Figure panels shown but not referenced, for example: Figure S1B. The authors may want to work through the entire manuscript to ensure appropriate Figure panel referencing.
Both Reviewer#2 and Reviewer#3 suggested to rework references of the figure panels in the manuscript (especially for supplementary figures) to make it clearer and more consistent. The text has been modified accordingly and all the figures and panels are now referenced.

5. Page 3, line 89. "Figure 1.D" is a typo
Corrected in the text.

6. Figure 1 panel C. It would be useful to have a scaled down (and perhaps simplified) version of panel B as part of panel C to orient the reader on the range of neuronal positions within the animal body. While the numerical positions are provided, having to refer back to panel B is somewhat cumbersome.
Figure 1 has been modified. The scale of panel B has been changed. Ex-panel D showing the migration of other neurons during embryogenesis has been moved and renamed panel C. Ex-panel C is now panel D. This new configuration of the figure allows the scale of the panel B to be aligned with the panel D as the reviewer suggested.

7. Page 13 line 345: "which is likely to reflect the fact that it only migrates at a short range" - perhaps should be changed to "which likely reflects the fact that it only migrates a short range"
Corrected in the text.

8. Page 13 line 251 should be "implies".
Corrected in the text.

9. Page 15, line 423, fln-1 allele name should be italicized.
Corrected in the text.

10. Page 18, line 505. I am not sure if "better known animals" is an entirely accurate way to say it, perhaps "better known examples from other model organisms" or something along those lines. I am

also not entirely convinced by the analogy. Cell positioning through migration does not seem quite the same as a homeotic transformation, where cell fates are drastically affected, to this reviewer. However, the displacements are very dramatic. (Please note that this reviewer is not necessarily asking for authors to change their wording, just cautions on the extent of the analogy.)
We changed to "better known examples from other model organisms" as suggested. The word "homeotic" has been removed.

11. Figure 2C and related discussions on modeling. While may be beyond the scope of this study, having additional body size variants may be needed to confidently assess the predictive power of this model.
We agree in principle but, as explained in Methods, we could not find other appropriate mutants to use:
First, most body size mutations in *C. elegans* affect body size at late larval stages and in adults. They do not affect body size at the L1 stage and thus cannot be used here.
Second, there are mutations leading to a smaller body size at hatching, especially by affecting the cuticle. Unfortunately, in these mutants, the pattern of the seam cells is distorted and the scale to measure QR.pax final position cannot be used.
Third, longer animals than wild type are particularly difficult to find. Even the difference between the long mutant *lon-1(e185)* and the N2 wild type is subtle at the L1 stage.

Fourth, as described in Methods, we screened for L1 body size mutants without gross cell migration defect (in QR.pax, ALM, BDU, CAN or HSN). This is the reason why several mutants were excluded from the analysis as detailed in the method section, and why we added the tetraploid animals.

12. While temperature had only a mild effect on QR descendants positioning, could the authors include a short discussion (a couple of sentences) on how or why temperature may be affecting final QR.pax positions? Perhaps rate of migration vs. rate of overall animal development? In addition, it would be interesting to what effect temperature fluctuations during the time of migration might have on final cell positions, as this more egregious environmental assault and something that wild isolates likely experience.

Growth temperature might affect many traits during development in *C. elegans*. We added in Discussion, p: 21:
"Concerning growth temperature, many biochemical and thus developmental processes are likely to be affected, including the growth speed of the worm and embryo size (Gutteling et al., 2007). Under high temperature, the slightly posterior position of QR.pax might reflect the fact that the growth rate of the larvae is increased but not QR migration. Furthermore, the gene expression profile varies with temperature (Gomez-Orte et al., 2017). Another hypothesis is that higher temperature can lead to a precocious (or higher) expression of *mig-1* that stops QR.pa migration. The diversity of reactions that may be affected by temperature makes it difficult at this point to make hypotheses regarding the mechanism."
Besaus the effect of temperature is small, and the biohcmeical lmechanism by which tempertaure acts are unclear, we did not try further treatments, such as alternating high and low temperatures. These would indeed be relevant with a 12-hour 'circadian' rhyhtm.

13. The order of the Supplemental Figures 2 and 3 must have been switched during the upload, creating some momentary confusion for this reviewer.
Legend for Supplementary figure 3 should include description of the individual panels. Legend for Supplementary figure 2 is lacking references to panels E-G.
We apologize for this confusion. The legends have now been corrected.
! We are unable to switch the figures uploaded in July on the website. !

14. Figure 2 legend: the authors refer to the bottom portion of panel D as panel E and provide a legend for F (even though there is no F). Either add the panel labeling or fix the Figure legend.
Corrected in the text.

Reviewer #2 (Significance (Required)):

This manuscript provides conceptual advances to the field of cell migration and to the field of

developmental robustness faced with environmental and genetic perturbations. As such, this work is of interest to a broad audience spanning multiple fields and organismal systems. This manuscript demonstrates that final positioning of QR descendants is sensitive to environmental conditions and genetic variation of C. elegans wild isolates. This work combines a descriptive study with mathematical modeling to assess the extent and the potential causes that may compensate for cell positioning under conditions of variable body size. In addition, the report includes characterizations of egg and body sizes that would be of practical use to researchers within the field of C. elegans development.

This reviewer's expertise is within C. elegans development, cell migration, and does not include mathematical modeling.

Reviewer #3 (Evidence, reproducibility and clarity (Required)):

Summary:

In this article Dubois et al. analyzed the robustness of a neuroblast (QR) migration process to various perturbations in the C. elegans model organism. It was previously established that the end of migration, and therefore final position, is determined by a timing mechanism rather than a positional cue. They first analyzed the effect of perturbations of the size of the animal on the final position by combining genetic manipulations and mathematical modeling. Interestingly, they showed that the effect of body size changes are partially compensated by a change in migrating cell velocity. They also showed that changes in temperature and starvation affect respectively the mean and the variance of the final position. Finally, they observed that the cell final position varies among different C. elegans wild isolates.

Major comments:

This is a high quality study. The conclusions are well supported by the experiments. The method, data and replicability/statistics are adequately reported.

Minor comments:

-The authors compare the variability of QR migration to that of other neurons (BDU, ALM, CAN, HSN). Is it known that the final position of these neurons is determined by positional cues rather than a timing mechanism ?

We added in the Introduction:
" These neurons migrate during embryogenesis. In *C. elegans*, the Wnt ligand EGL-20 forms a gradient from the tail to the anterior part of the animal and drives QR migration as well as that of HSN. One mechanism for their final positioning is the homogeneous concentration or undetectable level of the guiding cue (attractive or repulsive). Many evidences now show that HSN stops when EGL-20 concentration is low. More precisely, the location of the CAN neurons defines the final position of HSN (Forrester and Garriga, 1997) by sequestering EGL- 20 protein in its vicinity (Modzelewska et al., 2013). As for CAN, BDU and ALM, the mechanism of the their final positioning and even the guiding cues are not totally uncovered. CAN neurons are probably guided by several molecular cues at the same time (Silhankova and Korswagen, 2007) and more specifically by EGL-17/FGF signaling (Fleming et al., 2005). The end of their migration may be related to a specific location in the animal at given concentrations of the different molecules. A timing mechanism cannot however be ruled out concerning the final position. Nonetheless, those cells migrate during embryogenesis a much smaller distance than QR. This is another reason to expect a smaller variance in their position compared to that of QR.pax, and thus they constitute a good comparison. "

-QR.pax final position distribution looks unimodal in well fed animals but bimodal after starvation (figure 1). Could the authors comment on that ? Any possible explanation ?
Addition in the text on p.15 lines:
“The distribution of QR.pax final position after starvation may appear bimodal. This can be directly related to the proportion of QR.pa location observed during starvation (see Methods).”

-Line 365: « The temperature shifts QR.pax final position toward the anterior as the temperature decreased, with a value of 0.04 relative position unit per degree (Figure 1F). » The authors should precise that this is in N2.
Corrected in the text.

- Figure S2: several panels (B, C...) are not mentioned in the main text. The authors should comment on these panels in the text.
Panels are now commented in the text.

-Line 423 : ok2611 should be in italic.
Corrected in the text.

-Line 482 : « We did not find any correlation between QR.pax final position and the absolute value of the sampling latitude (t = -0.94, p=0.35). ». This sentence is unclear to me, the author should explain a bit more.

Addition in the text p. 19;
"As the temperature of growth affects (slightly) the final position of QR.pax, we wondered whether the temperature of origin of the wild isolates could have explained a part of the natural variation. The only information available is the latitude and longitude of the sampling location. We used the sampling latitude (in absolute value) of each strain as a proxy for the natural habitat temperature. We did not find a correlation between QR.pax final position and the absolute value of the sampling latitude (t=-0.94, p=0.35)."

-Line 880: « (E) » should be suppressed.
Corrected in the text.

-Line 883: « F) » should be replaced by « E) ».
Corrected in the text.

-Line 922: « C-D) » should be replaced by « C-G) ».
Corrected in the text.

-Figure 1C: x-axis label « QR.pax final position », « QR.pax» should be suppressed as the position of other cells is also presented in the graph.
Corrected in the Figure 1D (former 1C, cf comment 6 from Reviewer #2).

-Figure S1D: the authors should label the AVM projection.
AVM projection label has been added to Figure S1D.

Reviewer #3 (Significance (Required)):
While the mechanisms that guide cell migrations have been extensively studied, how cells decide to stop their migration and how robust the final cell position is to perturbations has been less characterized. In this study, the authors analyzed an original case of cell migration (QR neuroblast of C. elegans) where the stop decision is based on an intrinsic timing mechanism rather than a more classic positional cue. How this time-based mechanism may affect the precision of the final cell position is unknown. In this study, the authors characterized the robustness of this cell migration mechanism to body size variations, environmental perturbations or natural genetic variations. In particular they observed that the effect of body size variations are partially compensated by changes in migrating cell velocity. This study will be of high interest not only for the field of cell migration but also for people interested in the robustness and precision of developmental processes.

Submission to Development

First decision letter

MS ID#: DEVELOP/2020/196949

MS TITLE: Sensitivity to perturbations of a cell migration under temporal regulation

AUTHORS: Marie-Anne Felix, Clément Dubois, Shivam Gupta, and Andrew Mugler

Thank you for transferring your paper to Development from Review Commons.

My apologies for the previous email. Instead of 'returning your paper' to you, I have marked it as 'under revision'. It doesn't change much except that you should select 'Submit Revision' in your 'Author Area' when you are ready to upload your files.

First revision

Author response to reviewers' comments

We have revised the manuscript according to our previous response letter.

Second decision letter

MS ID#: DEVELOP/2020/196949

MS TITLE: Sensitivity to perturbations of a cell migration under temporal regulation

AUTHORS: Clément Dubois, Shivam Gupta, Andrew Mugler, and Marie-Anne Félix

I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is positive and we would like to publish a revised manuscript in Development. Referee 3 has some very minor points that it would easiest to tidy up before formal acceptance. I have designated your manuscript as a Minor Revision and I would be grateful if you could address these points and resubmit your manuscript.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Reviewer 1

Advance summary and potential significance to field

Not applicable. Revised manuscript.

Comments for the author

I previously reviewed this study for Review Commons. In this revised version the authors addressed all my comments in a satisfactory manner. I therefore support publication of this revised manuscript in Development.

Reviewer 2

Advance summary and potential significance to field

I just reiterate my previous points: Understanding sensitivity/robustness of a long range migrating systems during development is an important topic. The migration of the system is not coordinated by spatial cue but by a timing mechanism therefore the system final position is bound to be sensitive to changes in body size. The finding that the system intrinsic sensitivity to body size is only partially compensated by a change of speed is interesting. As well as the finding that the within species variation in the system final position is not correlated to variation in body size means that widespread compensation has evolved and it will be interesting to analyse the genetic bases of such compensation.

Despite not being the first study to quantitatively analyse long range migration robustness to stochastic noise in a developmental system, the combined analysis of
stochastic robustness with robustness to both environmental and genetic variation across a wide panel of natural isolates is quite rare if not unique.

Comments for the author

The authors have addressed all my points and the paper improved substantially I am happy with this revised version

Reviewer 3

Advance summary and potential significance to field

The manuscript by Dubois et al, titled "Sensitivity to perturbations of a cell migration under temporal regulation" characterizes robustness of long-range neuronal cell migrations (specifically QR and its descendants) across wild type C. elegans isolates, environmental perturbations, and body size variants. The manuscript demonstrates that final positioning of QR descendants is sensitive to environmental conditions and genetic variation of C. elegans wild isolates. In addition, it reports that migrations are partially compensated in animals with different body size. This work is a well-executed investigation of the effects natural genetic variation and multiple environmental perturbations (including interruption of cell migration by developmental arrest) have on the cell migration robustness. The findings presented by this paper are novel.
In addition, the authors use mathematical modeling to assess the extent and the potential causes that may compensate for cell positioning under conditions of variable body size. Controlling for maternal age adds a nice dimension to an already excellent study and further support the conclusions that correlate body size with final migrating cell position.
This work results in several conceptual advances to the field of cell migration and to the field of developmental robustness. As such, this manuscript will be of interest to a broad audience spanning multiple fields and organismal systems. This is an important (yet infrequently done) exploration of how development is affected by ecologically and evolutionary relevant perturbations and is a key step towards understanding genotype-to-phenotype relationships.

(The experiments are adequately replicated and the data are analyzed with appropriate statistical analyses.)

Comments for the author

Minor comments:
Figure 3B. Could the cell positions be shown as gray dots, similar to panel A?
Line 511 has an extra line break.
Figure 1 legend: the title is missing a period.
Figure 1B. Perhaps mention the other cells since they are shown in the figure panel but not mentioned in the legend until C.

Second revision

Author response to reviewers' comments

- Figure 3B. Could the cell positions be shown as gray dots, similar to panel A?
We changed this.

- Line 511 has an extra line break.
Figure 1 legend: the title is missing a period.
Figure 1B. Perhaps mention the other cells since they are shown in the figure panel but not mentioned in the legend until C.
We changed this.

Third decision letter

MS ID#: DEVELOP/2020/196949

MS TITLE: Sensitivity to perturbations of a cell migration under temporal regulation

AUTHORS: Clément Dubois, Shivam Gupta, Andrew Mugler, and Marie-Anne Félix
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.