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RESEARCH NOTE

Quantitative trait loci mapping of dauer larvae development in growing populations of *Caenorhabditis briggsae* [version 1; peer review: 3 approved with reservations]

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Abstract

The life cycles of many free-living nematodes contain developmental switches that allow individuals to either develop directly to adulthood, or to arrest development as a stress resistant and long-lived dauer larval stage. Here, in a panel of *Caenorhabditis briggsae* recombinant inbred lines derived from the isolates HK104 x AF16, we use methodologies developed for *C.elegans* to map quantitative trait loci (QTLs) affecting the number of dauer larvae present at the point of food patch exhaustion. These analyses provide strong support for three QTLs and are suggestive of a further two.

Keywords

dauer larvae , *Caenorhabditis briggsae* , QTL , nematode , natural variation

Open Peer Review

Approval Status ? ? ?

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15 Dec 2015	view	view	view

1. **Erik C. Andersen**, Northwestern University, Evanston, USA
2. **Eric Haag**, University of Maryland, College Park, USA
3. **Christian Braendle**, University of Nice Sophia Antipolis, Nice, France

Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

Caenorhabditis elegans is an important model species, but there is a need to further understand its ecology and that of the other species with which it is associated¹⁻³. Active, growing, populations of *Caenorhabditis* nematodes are normally associated with nutrient and bacteria-rich substrates such as rotten fruit or very fresh compost^{1,3,4}. Growing populations can reach very large sizes, and worms would be expected to have optimized fitness (population growth and/or the production of dispersal stages) under such conditions. Appropriate development of growing worms as dauer larvae, the developmentally-arrested alternate third larval stage, is therefore likely to be central to genotype survival.

We have previously looked at dauer larvae development in growing populations of *C. elegans*⁵⁻⁷. These analyses identified both extensive variation between wild isolates in the number of dauer larvae present when a bacterial food patch is exhausted⁶ and large numbers of QTLs effecting this trait in introgression lines (ILs) produced from the isolates N2 and CB4856^{6,7}. Naturally, *C. elegans* has however been found to be associated with both *C. remanei* and *C. briggsae*^{1,3,8}. Here, using the methodologies developed for *C. elegans*, we have investigated dauer larvae formation in growing populations in a panel of *C. briggsae* recombinant inbred lines (RILs) produced from the isolates AF16 and HK104⁹.

Methods

The *C. briggsae* RILs were generated from reciprocal crosses between males and sperm-depleted hermaphrodites of isolates HK104 and AF16⁹ and were obtained from Asher Cutter (University of Toronto). Worms were maintained using standard methods¹⁰ and all assays were conducted at 20°C. Assays were performed as previously described⁶, with populations initiated from single fourth larval stage worms (L4s) grown from synchronized, arrested, L1s with 100 µl of a 20% w/v suspension of *Escherichia coli* in water and monitored daily until food exhaustion. At this point, the population size and the number of dauer larvae were determined^{5,6}. RILs were analyzed in three experimental blocks with AF16 and HK104 assayed in each block and five populations initiated per genotype. Treatments (genotypes) were randomized within blocks and plates were blind coded such that counts were performed without knowledge of worm genotype. Populations that failed to grow were discarded.

The RILs have previously been genotyped at 451 markers distributed across all six chromosome pairs⁹ and these data were used for this analysis. QTL mapping was performed by composite interval mapping (CIM) using QTL Cartographer v2.5¹¹. Genome-wide thresholds (0.05) were estimated based on 1000 permutations of the data and CIM analysis undertaken with a 1.0cM walk speed.

Results

Dataset 1. The number of dauer larvae and the population size at food exhaustion for replicate populations of the RILs and the respective AF16 and HK104 controls from three experimental blocks

<http://dx.doi.org/10.5256/f1000research.7546.d109088>

The AF16 and HK104 data are presented in Figure 1. The RIL data are used to calculate the trait values used in QTL mapping.

Dataset 2. Marker distances (from Ross et al., 2011) for informative markers in the RILs analysed

<http://dx.doi.org/10.5256/f1000research.7546.d109089>

Dataset 3. RIL genotypes (from Ross et al., 2011) and analysed trait values for the RILs

<http://dx.doi.org/10.5256/f1000research.7546.d109090>

In combination with the marker distances, these data were used for QTL mapping and the generation of the results shown in Figure 2 and Table 1.

Comparing the AF16 and HK104 controls between the experimental blocks indicates that the number of dauer larvae at food exhaustion varies extensively between blocks ($H = 11.06$, $p = 0.004$ and $H = 8.21$, $p = 0.007$, for AF16 and HK104, respectively: Figure 1A). This variation is much greater than that seen for population size at food exhaustion (Figure 1B). Such variability between experimental blocks has been observed before with this assay, and is likely to be due to variation in either the actual or perceived food quality between batches of bacteria⁶.

Figure 1. Dauer larvae formation is variable across experimental blocks. The A) number of dauer larvae, and B) population size, at food exhaustion in AF16 and HK104 controls in the three experimental blocks. Values from each plate are shown, with red bars indicating the median values.

Given the variation in dauer larvae formation between the experimental blocks we considered it unlikely that analysis of raw data would identify any QTLs, and this is what was observed (Figure 2A). Two approaches were therefore taken to determine if we could control for this variation. Firstly, we scaled the number of dauer larvae observed for each RIL either to the AF16 or HL104 controls within that experimental block (Figure 2A), e.g. the AF16 scaled value for a RIL was determined as $(\text{RIL mean} - \text{AF16 mean})/\text{AF16 mean}$ and this was used for mapping. Secondly, we analyzed each of the experimental blocks independently (Figure 2B).

In combination, these approaches identify three QTLs on chromosomes IV, V and the X, that affect the number of dauer larvae

present at food exhaustion (co-localizing QTLs present in both Figure 2A and B) (Table 1). The analysis is also suggestive of additional QTLs on chromosomes II and V (Figure 2A and B; Table 1). A similar analysis of the population size at food exhaustion did not detect any QTLs.

Discussion

For a comparative analysis of dauer larvae development in growing populations, a panel of *C. briggsae* RILs were analyzed. These analyses indicate that the general properties of growing populations of *C. briggsae* are similar to those described for *C. elegans*^{5,6}. This means these methodologies will be suitable for more direct comparisons between the species. The RIL analysis identifies a number of QTLs

Figure 2. Quantitative trait mapping identifies regions affecting the number of dauer larvae at food exhaustion. A) CIM of the number of dauer larvae (solid line), and the number of dauer larvae scaled to the numbers in the AF16 (dashed line) or HK104 (dotted line) controls from that experimental block. **B)** CIM of the number of dauer larvae observed in each experimental block separately. Horizontal lines indicate thresholds.

Table 1. QTLs affecting the number of dauer larvae at food exhaustion.

Position is the 1 LOD interval for the QTL. R^2 denotes the proportion of the inter-RIL variance explained by the QTL, and effect indicates how the QTL alters dauer larvae numbers, with a positive value indicating that the AF16 allele increases the number of dauer larvae compared to the HK104 allele.

Chromosome	QTL	Position (Mbp)	R^2	Effect	Support
II	1	13.7–14.5	8.3	-	AF16-scaled
IV	2	14.9–17.5	12.6	-	AF16-scaled
		15.1–16.4	12.6	-	HK104-scaled
		15.1–15.6	14.4	-	block 2
		14.9–16.4	37.5	-	block 3
V	3	2.5–5.6	8.0	+	AF16-scaled
		3.0–5.6	8.4	+	HK104-scaled
		2.8–14.3	20.0	+	block 2
V	4	16.1–16.8	17.4	-	block 3
X	5	3.0–6.2	8.8	+	AF16-scaled
		0–11.6	15.5	+	HK104-scaled
		0–7.6	17.2	+	block 1
		4.7–6.2	26.3	+	block 3

(Figure 2; Table 1) although these are not resolved to narrow genomic regions and therefore contain many hundreds of genes. They do however provide a starting point for attempts to identify causal loci.

Data availability

F1000Research: Dataset 1. The number of dauer larvae and the population size at food exhaustion for replicate populations of the RILs and the respective AF16 and HK104 controls from three experimental blocks, [10.5256/f1000research.7546.d109088](https://doi.org/10.5256/f1000research.7546.d109088)¹²

F1000Research: Dataset 2. Marker distances (from Ross *et al.* 2011) for informative markers in the RILs analysed, [10.5256/f1000research.7546.d109089](https://doi.org/10.5256/f1000research.7546.d109089)¹³

F1000Research: Dataset 3. RIL genotypes (from Ross *et al.* 2011) and analysed trait values for the RILs, [10.5256/f1000research.7546.d109090](https://doi.org/10.5256/f1000research.7546.d109090)¹⁴

Author contributions

JG and SH conceived the study and designed the experiments. JG carried out the research. SH and JG analyzed the data, wrote the paper and have agreed to the final content.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

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Christian Braendle

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This research note presents a very brief summary of a QTL analysis based on a phenotyping assay of dauer formation (at food exhaustion) in *C. briggsae* AI-RILs.

The main issue here is the low reproducibility of the phenotyping assay across replicates and blocks. Given this problem, it appears rather surprising that some significant QTL were picked up. Further assays would be required to validate these, and this might be worthwhile doing (given that reproducible assays can be developed).

If I understood correctly, adjusted or unadjusted numbers of dauers were used as trait values – why not rather use the proportion of dauers (or number of dauers adjusted by population size) for mapping? [higher densities are expected to increase dauer formation]

What is the relationship between proportion of dauers and population size? Can the variability in dauer formation be explained by variability in population size? Does mapping of population size give the same QTLs?

Figure 1 shows how strong replicate and block effects are, just by looking at the parental lines, AF16 and HK104. Does an ANOVA analysis of these data (or the RIL data) actually reveal a significant genotype effect (so far, only block effects are given)? What are the heritabilities?

How does this QTL analysis in *briggsae* compare to the QTL analysis done in *elegans* (N2xCB4856) – number of QTLs, regions?

In summary: it would be useful to add some more info and data analysis to this note.

Additional comments

- perhaps mention that the lines used are advanced-intercross recombinant inbred lines (AI-RILs)
- the authors mention 451 markers but I had the impression many more were generated in

Ross *et al.* 2011

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 20 January 2016

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Eric Haag

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In this report, Green and Harvey address the genetic architecture governing variation in one of the best-studied examples of developmental plasticity in animals, the dauer larva *Caenorhabditis* nematodes.

Dauer larvae are dispersive forms of nearly all terrestrial nematodes, and are typically induced by the onset of food limitation and/or pheromone cues that are correlated with it. Given that the correctness of the decision to form a dauer (and thus abandon the direct path to reproductive adulthood) is expected to be highly contingent upon the exact ecological context in which it occurs, this trait should be both subject to strong selection and be potentially variable. This may be especially true for *C. briggsae*, which has a greater degree of geographic population structure than does its more famous congener, *C. elegans*.

The authors take advantage of a set recombinant inbred lines between genetically distinct strains (lines that my own lab played a role in characterizing in 2011; see the Ross *et al.* reference) to examine whether there is indeed heritable variation for dauer formation, and if so whether there are any loci of variation with effect size big enough to show significant association with dauer formation. I have no doubts that this project is addressing a significant issue in biology, and the authors make some headway.

General enthusiasm aside, as Erik Andersen also notes in his review the main concern here is the noisiness of the assay that is mapped. In understanding how the dauer formation assays is done, I think I may see why it is so noisy. The authors are founding each plate with a single L4 larva and a fixed amount of food. This is easy to reproduce, so I see the appeal. However, to then reach starvation on the plate, we presumably are looking at the grandchildren of the founder worm. As an exponential process, tiny differences in the generation time or rate of egg laying in the first few hours would produce big variability in the total number of worms, and also in the increase of the signals that are stimulatory for dauer production.

One could try to model the nature of this variation, but I don't think this is author's goal. An alternative, though admittedly requiring a whole new set of experiments, would be to seed plates with 20 or 200 L4 larvae, and score dauer formation in the first generation. This ought to be far less variable, yet should also be assessing the same basic phenotype, which could be seen as the threshold signal required to induce dauer development. Rather than repeating the entire scan, perhaps the authors could take their most discordant RILs and repeat this assay on them? It may produce a stronger signal, or at least confirm the initial screen.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 18 January 2016

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Erik C. Andersen

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Using techniques published previously, the authors measure the number of dauer larvae present at food patch exhaustion for a collection of *Caenorhabditis briggsae* recombinant lines. The dauer phenotype results are very noisy (as observed previously), but after data transformation quantitative trait loci (QTL) are detected using composite interval mapping. These results suggest that some regions of the *C. briggsae* genome confer differences in dauer formation, but more analysis needs to be performed to know more definitively. I would soften the abstract language (e.g. "strong support") to reflect the uncertainty in the results.

Given the variability of the phenotype measurements and the small number of recombinant lines, I am surprised about the QTL results. I would have expected no QTL to be detected, as observed in the untransformed data. It would be nice to see some more analyses, including the following:

1. What is the broad-sense heritability of this trait for these strains? It would be good to know how much of the trait variance you might expect to map to genomic regions in this study.
2. What are the results of a non-parametric linkage mapping? Composite interval mapping has many caveats. Are these QTL robust to mapping technique?
3. The significance threshold seems low given the allele frequency skews present in this mapping population. Please explain more about how that threshold was determined and how the skews were controlled.
4. Can you plot the RIL phenotypes before and after transformation so we can assess how well

the transformation affected the high variability of the assay?

If the detected QTL represent expected amounts of the genetic variance, are robust to mapping technique, and are significant with respect to the known allele frequency skews in this mapping population, then I believe this article offers preliminary evidence that some genomic regions confer differences in dauer larvae formation. These results suggest genomic regions to test for causality in the trait of interest, including regions that could lead to conserved interspecific variation between *C. elegans* and *C. briggsae*.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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