Hills, A., Green, J. and Harvey, S.C. (2017) Mutation independently affects reproductive traits and dauer larvae development in mutation accumulation lines of Caenorhabditis elegans. Development genes and evolution, 227 (6). pp. 411-414. ISSN 0949-944X.

http://dx.doi.org/10.1007/s00427-017-0596-1

Mutation independently affects reproductive traits and dauer larvae development in mutation accumulation lines of *Caenorhabditis elegans*

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Acknowledgments

Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). MA lines were provided by Charles Baer, University of Florida, who we also thank for advice with regard to the analyses undertaken. This work was supported by Canterbury Christ Church University.

Abstract

Developmental decisions are important in organismal fitness. For the nematode *Caenorhabditis elegans*, which is naturally found in the ephemeral food patches formed by rotting plant material, correctly committing to dauer or non-dauer larval development is key to genotype survival. To investigate the link between reproductive traits, which will determine how populations grow, and dauer larvae formation, we have analysed these traits in mutation accumulation (MA) lines of *C. elegans*. We find that reproductive traits of individual worms – the total number of progeny and the timing of progeny production – are highly correlated with the population size observed in growing populations. In contrast, we find no relationship between reproduction traits and the number of dauer larvae observed in growing populations. We also do not observe a mutational bias in dauer larvae formation. These results indicate that the control of dauer larvae formation is distinct from the control of reproduction and that differences in dauer larvae formation can evolve rapidly.

Introduction

In the wild, animals will encounter a wide variety of environmental conditions. This means that no single phenotype can be optimal at all times. This can provide a selective advantage to genotypes that are capable of modifying their phenotype in response to the environment experienced (Pigliucci 2005). Given the importance of phenotypic plasticity, it is critical to understand how such response are controlled and how they vary between genotypes. A model important in understanding the genetic control of phenotypic plasticity is the developmental switch between dauer and non-dauer larval development in nematodes. Dauer larvae are specialised alternate third larval stage animals that are found in the Rhabditae and are related to the infective larvae of many parasitic nematodes (Sudhaus 2010).

Within *Caenorhabditis elegans*, a species that is a coloniser of ephemeral bacterial blooms in rotting vegetation (Frézal and Félix 2015), appropriate dauer larvae development is critical to successful migration between ephemeral food resources. Dauer larvae form under conditions that are not suitable for reproduction, specifically low food and high population density (Hu 2007). Individual worms assess population density based on the relative amounts of a complex mix of ascarosides and other related molecules (von Reuss et al 2012). Most work on dauer larvae development relies on exposing synchronised cohorts of larvae to defined amount of exogenous pheromone. However, under natural conditions dauer larvae will form within growing populations and hence the population dynamics will be important. Further, the production of ascarosides – in terms of both amounts and types of molecule produced – varies depending on the conditions experienced by the worms (von Reuss et al 2012). In a growing population of worms, the dynamic production of ascarosides will therefore potentially allow individuals to perceive, and hence respond to, a very nuanced picture of their environment.

The decision to develop as a dauer larvae is variable between isolates of *C. elegans* (Viney et al. 2003; Green et al. 2013; Diaz and Viney 2015). This variation between isolates is a consequence of differences between isolates in both the composition of the pheromone they produce and their response to pheromone within the environment (Choe et al. 2012; Diaz et al. 2014). The importance of pheromone perception in this is supported by comparisons of dauer larvae formation within growing populations and dauer larvae formation of synchronised cohorts of larvae exposed to defined amount of exogenous pheromone (Green et al. 2015). In this comparison, most quantitative trait loci (QTLs) affecting dauer larvae formation are detected in both conditions, suggesting that the same variants are affecting the decision (Green et al. 2015).

Here we use mutation accumulation (MA) lines to further investigate the relationship between reproductive traits, which will determine rates of population growth, and dauer larvae formation in growing populations. MA lines are maintained at very low population sizes – for *C. elegans*, worms are maintained at a population size of one worm per generation – such that the effects of natural selection are minimised. Here we report the variation between MA lines produced from the N2 isolate in lifetime reproductive success (LRS), the intrinsic rate of increase (*r*), and the population size and the number of dauer larvae at food exhaustion in growing populations.

Methods

Worms

N2 was obtained from the *Caenorhabditis* Genetics Centre. The 19 MA lines assayed here are a subset of lines derived from N2 and obtained from Charles Baer (University of Florida). At assay, lines had undergone 331 generations of MA – 250 prior to their arrival at Canterbury and a further 81 generations after arrival – using methods as described by Baer et al (2005). Ancestral "pseudolines" (see Vassilieva and Lynch 1999) were not created or analysed here.

Assays

All assays were performed at 20°C and were initiated with fourth larval stage worms (L4s) grown from synchronised, arrested, L1s. Assays for LRS were undertaken on NGM plates with *Escherichia coli* OP50 strain as food (Stiernagle 2006), with individuals moved to new plates daily throughout the reproductive period and progeny counted after 2-3 days further incubation at 20°C. LRS data was used to estimate *r* for each line, by iteration from $\Sigma e^{-rx} l_x m_x = 1$, where l_x represents the age specific survivorship to day x and m_x represents the fecundity on day x (Vassilieva and Lynch 1999). Assays for the population size and the dauer larvae number at food exhaustion were performed as described by Green and Harvey (2012) and Green et al (2013), with populations initiated on sloppy agar plates with a 4 gl^{-1} agar concentration using

100μL of a 10% w/v concentration of OP50 from overnight culture. At food exhaustion, identified by the worms dispersing from the exhausted area, population size and dauer larvae number were determined. Within each experiment, plates were blind- coded and treatments (lines) were randomised. Plates that became contaminated or on which the population had failed to grow were discarded.

Statistics

Data from the MA lines were analysed using a random-effects GLM, with line fitted as a random factor, and the among-line component of variance used as the relevant measure of genetic variation to calculate the mutational variance V_m (determined as among-line variance/[2 x number of generations]). To compare between traits, we determined mutational heritability, determined as V_M/V_E, and mutational variability, determined as V_m/[trait mean)^2 (Houle et al. 1996), for the number of dauer larvae, the population size and LRS. Differences between MA lines and N2 were determined by weighted pairwise Student t-tests for each trait. The relationship between traits was also investigated by Pearson correlation. Analyses were undertaken in Minitab 17 (Minitab Inc.).

Results and Discussion

As expected, given previous work on MA lines, significant variation between the 19 MA lines were observed for all traits (Figure 1) (LRS: $F_{18,237} = 23.35$, $p < 0.001$; r: $F_{18,231} = 38.23$, $p < 0.001$; population size: $F_{18,67} = 6.07$, $p < 0.001$; number of dauer larvae: $F_{18,67} = 3.51$, $p < 0.001$). Estimates of mutational variability (CV^2) were similar for the population size and for LRS (0.00011 and 0.00014, respectively), and are in line with previous estimates for fitness-related traits. Again, similar estimates of mutational heritability (V_M/V_E) were seen for these traits (0.0017 and 0.0025, respectively). In contrast, the mutational variability for the number of dauer larvae was very high (0.00114). These estimates are however predicated on the assumption

that there is no residual among-line variance in the ancestor. Given that no ancestral pseudolines were analysed here, these values may therefore be overestimates. The estimates of mutational heritability and mutational variability for the number of dauer larvae also need to be treated with caution as the residuals are not normal. Traits closely associated with fitness are likely to exhibit strong mutational biases, as they are typically expected to be under strong directional selection (Keightley and Eyre-Walker 1999; Lynch et al. 1999) and thus the majority of mutations will move the trait directionally away from the original value. Here, LRS, *r* and population size at food exhaustion all decrease in the MA lines in comparison to N2 (for all MA lines these traits are all either significantly lower than N2 or do not differ from N2). This matches what has been seen in previous studies of fecundity in *C. elegans* MA lines (e.g. Vassilieva and Lynch 1999; Baer et al. 2005) and shows that this is also found when the size of growing populations is considered.

There is however no mutational bias seen in the number of dauer larvae produced at food exhaustion in the MA lines (Figure 1). Here, fewer dauer larvae are observed in some MA lines than are seen in N2, whilst other MA lines have a greater number of dauer larvae. Retesting of two MA lines and N2 controls replicates this result (Figure 2) and indicates that the difference in dauer larvae number is not a consequence of an increased population size – line 15 having the smallest number of worms and the largest number of dauer larvae at food exhaustion (Figure 2). This suggests that dauer larvae development may be being selected for some optimal and intermediate value.

Comparison between traits in the MA lines indicates that the various reproductive traits are highly correlated (Table 1). That is, lines that produce a lower number of progeny are also likely to do so more slowly and hence to also have a low *r*. This comparison also indicates that the MA lines with a low LRS and a low *r* are likely to exhaust food at a lower population size than

lines with a high LRS and high *r*, suggesting that they are less efficient at utilizing the available food. In contrast to the observed links between the various reproductive traits, the number of dauer larvae observed at food exhaustion was not correlated with any of the other traits (Table 1). A positive correlation between the numbers of worms and of dauer larvae might have implied that a response to the size of the population was driving dauer larvae numbers, *i.e.* that differences in the number of dauer larvae were a trivial result of changes in the population size (see also Figure 2). That this is not seen suggests that the differences between MA lines in dauer larvae numbers are the result of either changes in how they are perceiving their environment or in how such information is assessed in the dauer/non-dauer decision. This supports the view that the majority of the differences between *C. elegans* lines in dauer larvae formation within growing populations will be a consequence of changes in dauer-specific traits (Green et al. 2015).

The development of dauer larvae under natural ecological conditions is not well understood, but there is significant variation in the response of strains to pheromone under laboratory conditions (Viney et al. 2003; Diaz et al 2014). It has also been shown that the number of dauer larvae produced in growing populations varies between isolates (Green et al. 2013). The results from our comparisons of the MA lines indicates that dauer larvae formation can change quickly and that this is largely independent of changes in other traits. Given the limited number of mutations that will have been sampled here, this implies that it is likely that *de novo* mutation alone would be sufficient to allow the rapid evolution of both increased and decreased dauer larvae formation. Dauer larvae formation might therefore represent a good trait in which to look for local adaptation in *C. elegans*.

Conflict of Interest: The authors declare that they have no conflict of interest.

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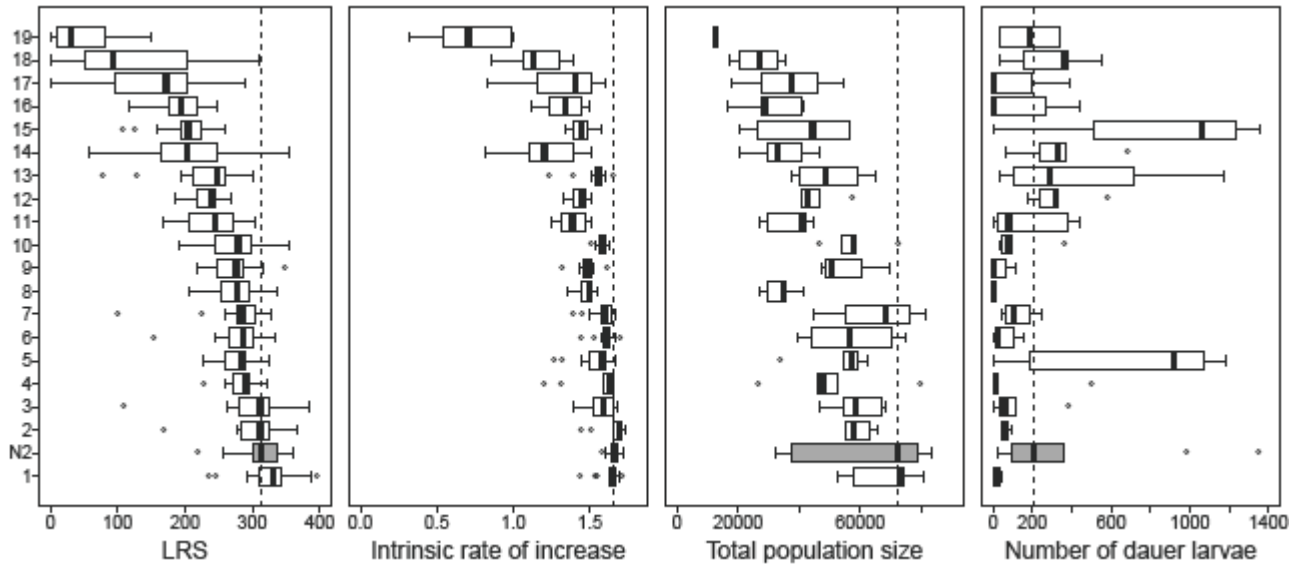
Table 1. Relationship between lifetime reproductive success (LRS), the intrinsic rate of increase (*r*), and the number of worms and of dauer larvae at food exhaustion in growing populations for mutation accumulation lines of *C. elegans*. Shown are the Pearson correlation and the associated *p* values.

	Number of dauer larvae	LRS	*R*
LRS	-0.199 0.401		
r	-0.081 0.735	0.93 <0.001	
Number of worms	-0.12 0.61	0.88 <0.001	0.90 <0.001

Figure legends

Figure 1: Dauer larvae development and reproductive traits vary between MA lines. Shown are box plots of lifetime reproductive success (LRS), the intrinsic rate of increase, and the population size and number of dauer larvae at food exhaustion in growing populations for N2 (shaded box, with median also shown by the vertical dotted line) and 19 MA lines. Lines are ordered by LRS.

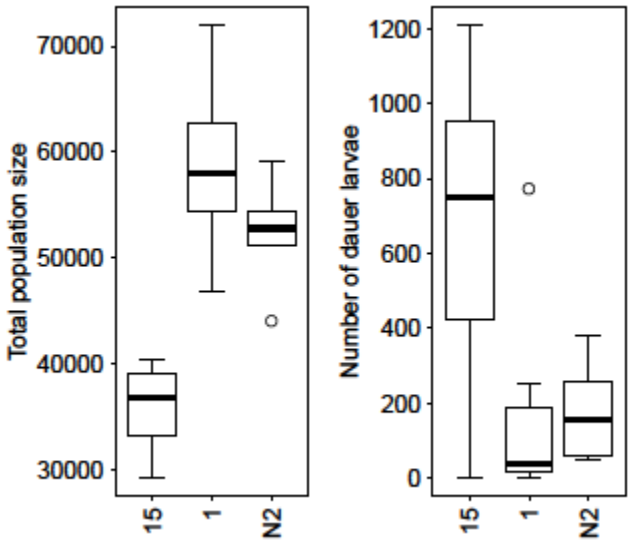
Figure 2: Dauer larvae development does not depend on population size in the MA lines. Shown are the population size and number of dauer larvae at food exhaustion in growing populations for N2 and 2 MA lines.


19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
N2
1
0 100 200 300 400
LRS
0.0 0.5 1.0 1.5
Intrinsic rate of increase
0 20000 60000
Total population size
0 200 600 1000 1400
Number of dauer larvae

243

244

245


Total population size
70000
60000
50000
40000
30000
15
1
N2
Number of dauer larvae
1200
1000
800
600
400
200
0
15
1
N2

246