1 # Mutation independently affects reproductive traits and dauer

2 # larvae development in mutation accumulation lines of

3 # *Caenorhabditis elegans*

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22   **Abstract**

23   Developmental decisions are important in organismal fitness. For the nematode *Caenorhabditis*

24   *elegans*, which is naturally found in the ephemeral food patches formed by rotting plant material,

25   correctly committing to dauer or non-dauer larval development is key to genotype survival. To

26   investigate the link between reproductive traits, which will determine how populations grow, and

27   dauer larvae formation, we have analysed these traits in mutation accumulation (MA) lines of *C.*

28   *elegans*. We find that reproductive traits of individual worms – the total number of progeny and

29   the timing of progeny production – are highly correlated with the population size observed in

30   growing populations. In contrast, we find no relationship between reproduction traits and the

31   number of dauer larvae observed in growing populations. We also do not observe a mutational

32   bias in dauer larvae formation. These results indicate that the control of dauer larvae formation

33   is distinct from the control of reproduction and that differences in dauer larvae formation can

34   evolve rapidly.

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36   **Introduction**

37   In the wild, animals will encounter a wide variety of environmental conditions. This means that

38   no single phenotype can be optimal at all times. This can provide a selective advantage to

39   genotypes that are capable of modifying their phenotype in response to the environment

40   experienced (Pigliucci 2005). Given the importance of phenotypic plasticity, it is critical to

41   understand how such response are controlled and how they vary between genotypes. A model

42   important in understanding the genetic control of phenotypic plasticity is the developmental

43   switch between dauer and non-dauer larval development in nematodes. Dauer larvae are

44   specialised alternate third larval stage animals that are found in the Rhabditae and are related

45   to the infective larvae of many parasitic nematodes (Sudhaus 2010).

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47   Within *Caenorhabditis elegans*, a species that is a coloniser of ephemeral bacterial blooms in

48   rotting vegetation (Frézal and Félix 2015), appropriate dauer larvae development is critical to

49   successful migration between ephemeral food resources. Dauer larvae form under conditions

50   that are not suitable for reproduction, specifically low food and high population density (Hu

51   2007). Individual worms assess population density based on the relative amounts of a complex

52   mix of ascarosides and other related molecules (von Reuss et al 2012). Most work on dauer

53   larvae development relies on exposing synchronised cohorts of larvae to defined amount of

54   exogenous pheromone. However, under natural conditions dauer larvae will form within growing

55   populations and hence the population dynamics will be important. Further, the production of

56   ascarosides – in terms of both amounts and types of molecule produced – varies depending on

57   the conditions experienced by the worms (von Reuss et al 2012). In a growing population of

58   worms, the dynamic production of ascarosides will therefore potentially allow individuals to

59   perceive, and hence respond to, a very nuanced picture of their environment.

60

61   The decision to develop as a dauer larvae is variable between isolates of *C. elegans* (Viney et

62   al. 2003; Green et al. 2013; Diaz and Viney 2015). This variation between isolates is a

63   consequence of differences between isolates in both the composition of the pheromone they

64   produce and their response to pheromone within the environment (Choe et al. 2012; Diaz et al.

65   2014). The importance of pheromone perception in this is supported by comparisons of dauer

66   larvae formation within growing populations and dauer larvae formation of synchronised cohorts

67   of larvae exposed to defined amount of exogenous pheromone (Green et al. 2015). In this

68   comparison, most quantitative trait loci (QTLs) affecting dauer larvae formation are detected in

69   both conditions, suggesting that the same variants are affecting the decision (Green et al.

70   2015).

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72   Here we use mutation accumulation (MA) lines to further investigate the relationship between

73   reproductive traits, which will determine rates of population growth, and dauer larvae formation

74   in growing populations. MA lines are maintained at very low population sizes – for *C. elegans*,

75   worms are maintained at a population size of one worm per generation – such that the effects of

76   natural selection are minimised. Here we report the variation between MA lines produced from

77   the N2 isolate in lifetime reproductive success (LRS), the intrinsic rate of increase (*r*), and the

78   population size and the number of dauer larvae at food exhaustion in growing populations.

79

80   **Methods**

81   *Worms*

82   N2 was obtained from the *Caenorhabditis* Genetics Centre. The 19 MA lines assayed here are a

83   subset of lines derived from N2 and obtained from Charles Baer (University of Florida). At

84   assay, lines had undergone 331 generations of MA – 250 prior to their arrival at Canterbury and

85   a further 81 generations after arrival – using methods as described by Baer et al (2005).

86   Ancestral "pseudolines" (see Vassilieva and Lynch 1999) were not created or analysed here.

87

88   *Assays*

89   All assays were performed at 20°C and were initiated with fourth larval stage worms (L4s)

90   grown from synchronised, arrested, L1s. Assays for LRS were undertaken on NGM plates with

91   *Escherichia coli* OP50 strain as food (Stiernagle 2006), with individuals moved to new plates

92   daily throughout the reproductive period and progeny counted after 2-3 days further incubation

93   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

94   represents the age specific survivorship to day *x* and m_x represents the fecundity on day *x*

95   (Vassilieva and Lynch 1999). Assays for the population size and the dauer larvae number at

96   food exhaustion were performed as described by Green and Harvey (2012) and Green et al

97   (2013), with populations initiated on sloppy agar plates with a 4 gl^{-1} agar concentration using

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98   100µL of a 10% w/v concentration of OP50 from overnight culture. At food exhaustion, identified

99   by the worms dispersing from the exhausted area, population size and dauer larvae number

100   were determined. Within each experiment, plates were blind- coded and treatments (lines) were

101   randomised. Plates that became contaminated or on which the population had failed to grow

102   were discarded.

103

104   *Statistics*

105   Data from the MA lines were analysed using a random-effects GLM, with line fitted as a random

106   factor, and the among-line component of variance used as the relevant measure of genetic

107   variation to calculate the mutational variance V_m (determined as among-line variance/[2 x

108   number of generations]). To compare between traits, we determined mutational heritability,

109   determined as V_M/V_E, and mutational variability, determined as V_m/[trait mean]^2 (Houle et al.

110   1996), for the number of dauer larvae, the population size and LRS. Differences between MA

111   lines and N2 were determined by weighted pairwise Student t-tests for each trait. The

112   relationship between traits was also investigated by Pearson correlation. Analyses were

113   undertaken in Minitab 17 (Minitab Inc.).

114

115   **Results and Discussion**

116   As expected, given previous work on MA lines, significant variation between the 19 MA lines

117   were observed for all traits (Figure 1) (LRS: $F_{18,237}$ = 23.35, p < 0.001; r: $F_{18,231}$ = 38.23, p <

118   0.001; population size: $F_{18,67}$ = 6.07, p < 0.001; number of dauer larvae: $F_{18,67}$ = 3.51, p < 0.001).

119   Estimates of mutational variability (CV^2) were similar for the population size and for LRS

120   (0.00011 and 0.00014, respectively), and are in line with previous estimates for fitness-related

121   traits. Again, similar estimates of mutational heritability (V_M/V_E) were seen for these traits

122   (0.0017 and 0.0025, respectively). In contrast, the mutational variability for the number of dauer

123   larvae was very high (0.00114). These estimates are however predicated on the assumption

124   that there is no residual among-line variance in the ancestor. Given that no ancestral

125   pseudolines were analysed here, these values may therefore be overestimates. The estimates

126   of mutational heritability and mutational variability for the number of dauer larvae also need to

127   be treated with caution as the residuals are not normal. Traits closely associated with fitness are

128   likely to exhibit strong mutational biases, as they are typically expected to be under strong

129   directional selection (Keightley and Eyre-Walker 1999; Lynch et al. 1999) and thus the majority

130   of mutations will move the trait directionally away from the original value. Here, LRS, r and

131   population size at food exhaustion all decrease in the MA lines in comparison to N2 (for all MA

132   lines these traits are all either significantly lower than N2 or do not differ from N2). This matches

133   what has been seen in previous studies of fecundity in *C. elegans* MA lines (e.g. Vassilieva and

134   Lynch 1999; Baer et al. 2005) and shows that this is also found when the size of growing

135   populations is considered.

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137   There is however no mutational bias seen in the number of dauer larvae produced at food

138   exhaustion in the MA lines (Figure 1). Here, fewer dauer larvae are observed in some MA lines

139   than are seen in N2, whilst other MA lines have a greater number of dauer larvae. Retesting of

140   two MA lines and N2 controls replicates this result (Figure 2) and indicates that the difference in

141   dauer larvae number is not a consequence of an increased population size – line 15 having the

142   smallest number of worms and the largest number of dauer larvae at food exhaustion (Figure 2).

143   This suggests that dauer larvae development may be being selected for some optimal and

144   intermediate value.

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146   Comparison between traits in the MA lines indicates that the various reproductive traits are

147   highly correlated (Table 1). That is, lines that produce a lower number of progeny are also likely

148   to do so more slowly and hence to also have a low r. This comparison also indicates that the

149   MA lines with a low LRS and a low r are likely to exhaust food at a lower population size than

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150   lines with a high LRS and high r, suggesting that they are less efficient at utilizing the available

151   food. In contrast to the observed links between the various reproductive traits, the number of

152   dauer larvae observed at food exhaustion was not correlated with any of the other traits (Table

153   1). A positive correlation between the numbers of worms and of dauer larvae might have implied

154   that a response to the size of the population was driving dauer larvae numbers, *i.e.* that

155   differences in the number of dauer larvae were a trivial result of changes in the population size

156   (see also Figure 2). That this is not seen suggests that the differences between MA lines in

157   dauer larvae numbers are the result of either changes in how they are perceiving their

158   environment or in how such information is assessed in the dauer/non-dauer decision. This

159   supports the view that the majority of the differences between *C. elegans* lines in dauer larvae

160   formation within growing populations will be a consequence of changes in dauer-specific traits

161   (Green et al. 2015).

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163   The development of dauer larvae under natural ecological conditions is not well understood, but

164   there is significant variation in the response of strains to pheromone under laboratory conditions

165   (Viney et al. 2003; Diaz et al 2014). It has also been shown that the number of dauer larvae

166   produced in growing populations varies between isolates (Green et al. 2013). The results from

167   our comparisons of the MA lines indicates that dauer larvae formation can change quickly and

168   that this is largely independent of changes in other traits. Given the limited number of mutations

169   that will have been sampled here, this implies that it is likely that *de novo* mutation alone would

170   be sufficient to allow the rapid evolution of both increased and decreased dauer larvae

171   formation. Dauer larvae formation might therefore represent a good trait in which to look for

172   local adaptation in *C. elegans*.

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

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176   **References**

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219   modular library of small-molecule signals in C. elegans. *Journal of the American Chemical*

220   *Society*, 134(3), pp.1817-1824.

221 Table 1. Relationship between lifetime reproductive success (LRS), the intrinsic rate of increase

222 (*r*), and the number of worms and of dauer larvae at food exhaustion in growing populations for

223 mutation accumulation lines of *C. elegans*. Shown are the Pearson correlation and the

224 associated *p* values.

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

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227

228   Figure legends

229

230   Figure 1: Dauer larvae development and reproductive traits vary between MA lines. Shown are

231   box plots of lifetime reproductive success (LRS), the intrinsic rate of increase, and the

232   population size and number of dauer larvae at food exhaustion in growing populations for N2

233   (shaded box, with median also shown by the vertical dotted line) and 19 MA lines. Lines are
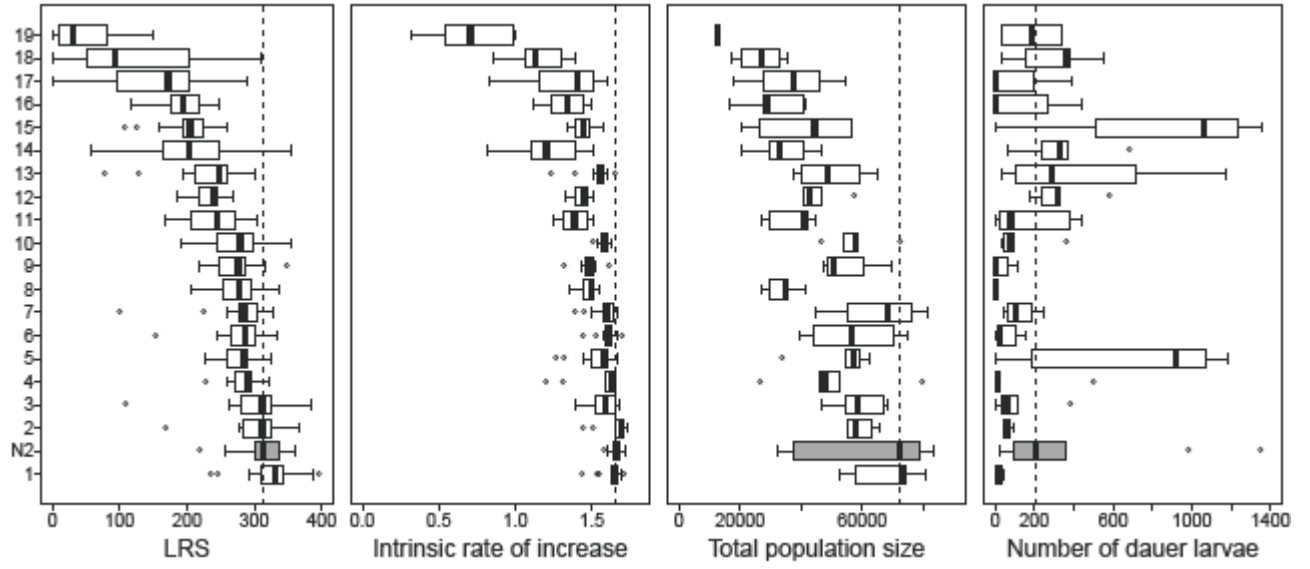
234   ordered by LRS.

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236   Figure 2: Dauer larvae development does not depend on population size in the MA lines. Shown

237   are the population size and number of dauer larvae at food exhaustion in growing populations
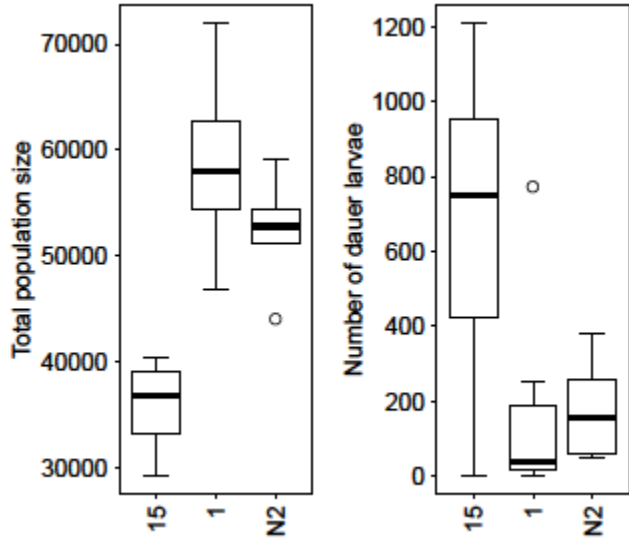
238   for N2 and 2 MA lines.

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