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Environmental and genetic influences on dauer larvae development in growing populations of Caenorhabditis species

by

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ABSTRACT

Phenotypic variation manifests from either simple (monogenic) or complex (multigenic) traits. Variation due to genetic and environmental influences is important because the ability to produce a range of phenotypes is essential for adaptive evolution. Complex traits are important not only for evolution, but because many diseases are complex traits. The genetic architecture of complex traits can be very multifaceted, with a large number of causal genes each having a small effect on the overall heritability of the trait, and as such our understanding of the genetic architecture and control of complex traits is limited. Complex traits are studied through quantitative trait loci mapping and genome-wide association studies. Since there are a great range of resources available for the nematode *Caenorhabditis elegans*, this is an appropriate system in which to study the genetic architecture of complex traits. Dauer larvae development represents a suitable complex trait as many of the genes involved and their genetic pathways have been identified. This trait is also important for the clear links between the dauer larvae of free-living species and the infective stages of many parasitic nematodes, and is therefore important as a model complex trait. Dauer larvae are routinely studied under unnatural conditions, with a cohort of aged-matched worms exposed to concentrated pheromone from many worms, conditions that are not obviously ecologically or evolutionally relevant. It is therefore important to understand the dynamics of growing populations in the laboratory both specifically to understand *C. elegans*, and generally to understand the genetics of complex traits.

Methods have been established for the analysis of population growth assays, and experiments to validate this style of assay have been carried out for the analysis of dauer larvae development in a growing population. In this, extensive variation in dauer larvae development between natural wild isolates and the canonical isolate N2 has been shown,

variation which has previously not been demonstrated in standard dauer larvae assays. The genetic basis of this variation was also investigated using two Recombinant Inbred Line (RILs) panels made from two distinct parental genotypes of *C. elegans*, Isogenic Lines (ILs) of *C. elegans* and also a *C. briggsae* RIL panel. These analyses revealed that the genetic architecture of dauer larvae development in growing populations is highly complex, with a large number of QTLs affecting this trait. Also, comparison of the results from the different mapping approaches (RILs vs. ILs) revealed variation in their power to detect QTLs, as the ILs were capable of identifying far more QTLs than the RILs. Three candidate genes which have an effect on dauer larvae development in growing populations were identified and analysed. These candidates are *npr-1*, *srg-36* and *srg-37*, each showing a negative effect on dauer larvae development in a growing population and an allelic effect of variation at *npr-1*. Together, these results demonstrate that extensive variation in dauer larvae development can be analysed in growing populations, that the underlying genetics can be mapped and that candidate genes can be identified for the underlying regions.

CHAPTER ONE: Introduction

Complex traits

All organisms show phenotypic variation; this can be at multiple biological scales including morphology, physiology and behaviour. This variation can manifest as disease. Some of this variation is comparatively simple, with phenotypes controlled by a single gene (monogenic or Mendelian traits), such as obesity in mice (Coleman and Eicher, 1990; Leibel *et al.,* 1997) and male sterility in *Drosophila melanogaster* (Wakimoto *et al*., 2004). In contrast, other variation is complex with phenotypes controlled by multiple genes (multigenic), such as with three genes affecting geotaxis in *D. melanogaster* (Toma *et al.,* 2002), and many diseases, for example, the three known genes leading to early-onset Alzheimer's Disease (Blacker *et al.,* 2003). Both simple and complex traits are not, however, only under genetic control, they also show gene-environment interactions with environmental specific effects, for example, many behaviours and some aspects of morphology (Anholt and Mackay, 2004; Forbes *et al.,* 2010).

The manifestation of complex traits is therefore the result of the relationship between an organism's genetic makeup and the environment in which they live. Variation due to the environment can be seen in reaction norm studies. Reaction norm studies allow the visualisation of phenotypic plasticity, where organisms of the same genotype produce phenotypic variation due to varying environmental conditions, for example, age and size at maturity (Stearns and Koella, 1986) and changes in life-history traits, morphology and behaviour induced by predation (Dodson, 1989). Variation in reaction norms is important, as the ability to promote or inhibit adaptive change, depending on the nature of the phenotype-environment interactions, is an important aspect of evolutionary adaptation (Lande and Shannon, 1996). The environmental specificity of complex traits is strongly supported in studies of human disease, for example in smoking and lung cancer, where

smokers are more susceptible to lung cancer than non-smokers through the inhalation of the carcinogens present in cigarette smoke (Watson and Conte, 1954; Cornfield *et al*., 2009). The link between sunlight and skin cancer has also been studied, showing the p38 signalling pathway plays a vital role in solar ultraviolet skin carcinogenesis (Liu *et al.,* 2013). This demonstrates that variation in environmental conditions can have negative effects on an organism's health and can interact in a complex way with genetic variation. Complex traits are important for their effects on disease but are also important for adaptive evolution, such as in the wing shape of *D. melanogaster* where 35 quantitative trait loci (QTLs) for this trait were identified (Zimmerman *et al.,* 2000) and the adaptation of *Arabidopsis thaliana* to a wide range of climates (Hoffmann, 2005). However, because genetic architecture is sometimes very complex, with each causal gene of a complex trait only making a small contribution to the overall heritability of the complex trait (Fisher, 1918), our understanding of complex traits is limited. It is therefore important to understand the genetic architecture and control of complex traits; as complex traits are important for evolutionary change, show variability in their phenotypes are under genetic and environmental control and can also manifest as disease.

The genetics of complex traits

Genome-wide association studies (GWAS), which find associations between polymorphic alleles and phenotypes of interest, are an effective way in which the genetic basis of complex traits is studied. In humans, GWAS studies have discovered hundreds of marker loci and single nucleotide polymorphisms (SNPs), associated with diseases such as Crohn's disease (Barrett *et al.,* 2008), type-1 diabetes (Barrett *et al.,* 2009), obesity (Greenawalt *et al.*, 2011) and Parkinson's disease (Rhodes *et al.,* 2010). Many trait-associated SNPs

identified in studies such as these are thought to impact the relevant phenotypes through effects on gene expression as they are found to be in non-coding regions (Shastry, 2009), for example, many of the SNPs identified in studies of tumorigenesis in human cancers (Esteller, 2011). Gene expression has also been examined using SNPs which allow for the mapping of quantitative levels of gene expression as expression QTLs (eQTLs) (Cookson *et al.,* 2009). Studies of gene expression in blood and brain tissue (Hernandez *et al.,* 2012), follicular lymphoma (Conde *et al.,* 2013) and eQTLs in human heart (Koopmann *et al.,* 2014) show that combining SNP data from GWAS with expression profiling data can be used to further characterise the functions of trait-associated SNPs (Conde et al., 2013; Koopmann et al., 2014).

The majority of GWAS have been done to identify loci affecting human disease, using human populations (Hindorff *et al.,* 2009; Hindorff *et al.,* GWAS catalog; Visscher *et al.,* 2011), however a more limited number of studies have been undertaken in model organisms. Data gained in these studies has led to insights on the genetic basis of disease, and from there to disease models. For example, in Alzheimer's Disease, candidate genes were examined using model organisms including a yeast model of Aβ toxicity (Treusch *et al.,* 2011), a *Drosophila* model of Aβ and Tau (Shulman *et al.,* 2011), and a *Caenorhabditis elegans* model for drug development (Lublin and Link 2013). Post-GWAS testing, like these Alzheimer's models, have promoted extensive pharmaceutical research and have led to the development of medications for disease.

QTL mapping can also be used to identify candidate genes for complex traits. QTL mapping utilises molecular markers (for example SNPs) linked to at least two classified genotypes (Mackay, 2006; Mackay *et al.,* 2009), where individuals of different genotypes at marker loci will have different trait values (Sax, 1923; Lander and Botstein, 1989). These crosses are

called Recombinant Inbred Lines (RILs). RILs are a collection of strains used to map QTLs. They are created by crossing two related genotypes selected for their differing phenotypes, these parental strains must produce fertile offspring, forming the F1 generation. The F1 progeny are crossed to generate the F2 generation. F2 progeny are crossed again for a number of generations until chosen lines are inbred, either through selfing or sibling pair mating to create genetically stable recombinations (Pollard, 2012) (Fig 1.1). Nearly Isogenic Lines/Introgression Lines (NILs/ILs) are another type of cross used to map QTLs. They are created in a similar way to the RILs, though each time a cross is made it is mated to one of the original parental lines (Fig 1.2). The final NILs are selected for homozygosity, which can be done through PCR of simple sequence repeats (Miao *et al.,* 2005).

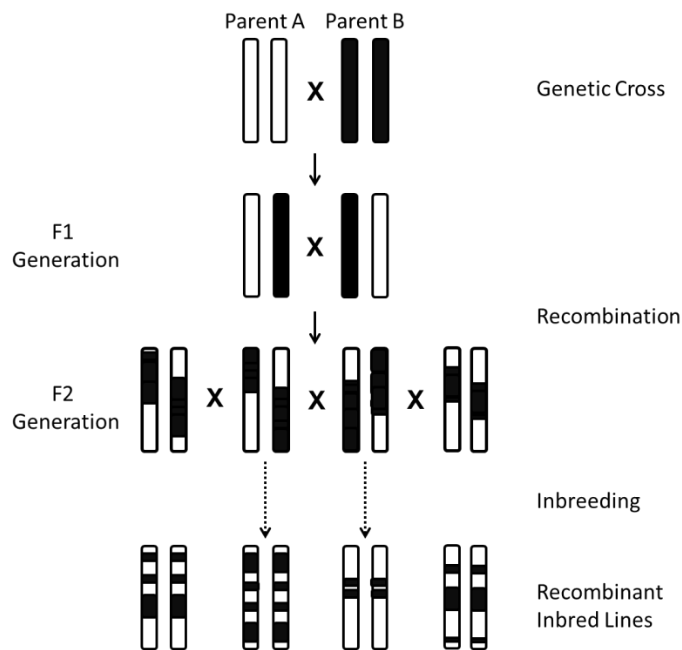

Figure 1.1: Recombinant inbred lines. Parents are crossed to create an F1 generation. F1s are then inter-crossed to create the F2 generation. The F2s are crossed for a number of generations and then left to inbreed to produce a panel of genetically stable RILs. Individuals are represented by a set of diploid chromosomes. Each parent genotype is represented by either white or black.

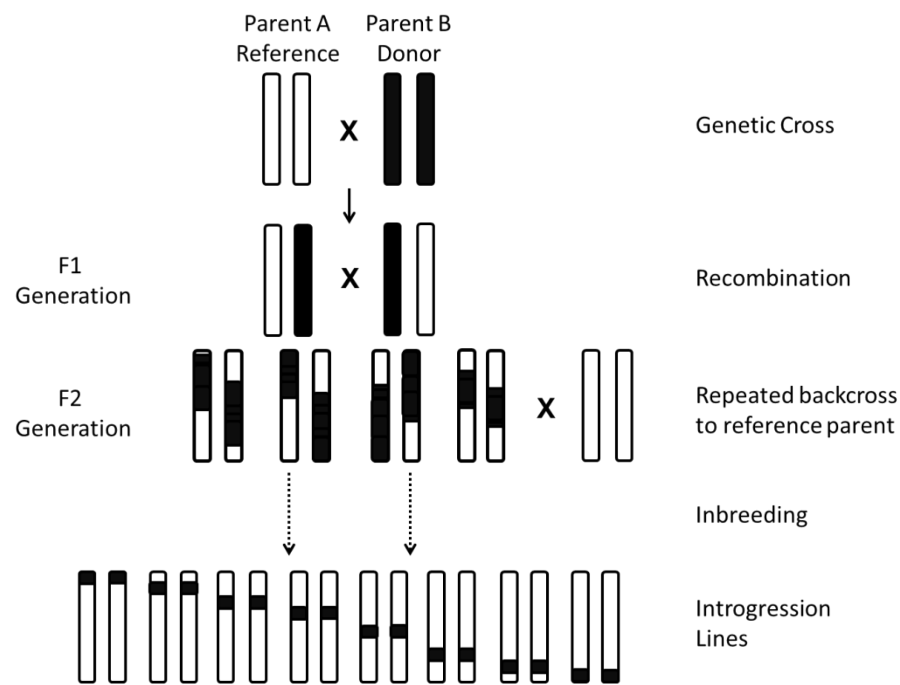

Figure 1.2: Introgression lines. Parents are crossed to create the F1 generation. F1 progeny are crossed, where recombination occurs, to produce an F2 generation. F2s are then crossed with the reference parent for several generations and then allowed to inbreed, at which point homozygous individuals are selected. Individuals are represented by a set of diploid chromosomes. Each parent genotype is represented by either white or black.

A common trait analysed in QTL studies is lifespan, which is an important trait as understanding the biology of ageing is crucial to understanding age related diseases. While knowledge in this field is growing, the causes of ageing are still not fully understood (Gems and Partridge, 2013). QTL mapping has been used to study natural variation of lifespan using *D. melanogaster* (Nuzhdin *et al.,* 2005; Lai *et al.,* 2007), *C. elegans* (Ayyadevara *et al.,* 2003; Vertino *et al.,* 2011) and *Mus musculus* (Lang *et al.,* 2010; Leduc *et al.,* 2011).

Several genetic mutations and pathways have been found to increase lifespan, such as the Insulin/insulin-like growth factor 1 (IGF-1), forkhead box O transcription factor (FOXO) and Target of rapamycin pathways (Gems and Partridge, 2013). For example, in the insulin/IGF-1

pathway, lifespan is affected through a response of the IGF-1 receptors activating a downstream signalling pathway containing proteins which affect lifespan in both mice and humans (Kenyon, 2010). Just as there are conserved homologues between *D. melanogaster*, *C. elegans* and *M. musculus*, there are also human homologues that could have the potential to increase lifespan, such as the association of the FOXO3A gene and increased human lifespan (Willcox *et al.,* 2008). QTL mapping has also been used to identify potential disease related genes. Such studies on disease related genes have identified, for example, 73 candidate genes for hypertension in humans (Hubner *et al*., 2005) and 51 candidate genes for abnormal corpus callosum in humans (Poot *et al*., 2011).

Other evolutionarily relevant complex traits have also been studied. For example, in work extending back to the 1980's on *D. melanogaster*, on whole genome variation on fitness (Mackay, 1985), copulation latency (Moehring and Mackay, 2004; Mackay *et al.,* 2005), starvation resistance (Harbison *et al.,* 2004; Harbison *et al.,* 2005), lifespan (Vieira *et al.,* 2000; Leips and Mackay, 2000), chill coma recovery (Morgan and Mackay, 2006) and locomotor reactivity (Jordan and Mackay, 2006; Jordan *et al.,* 2007; Yamamoto *et al.,* 2007). These studies have identified significant features of the genetic architecture of *D. melanogaster*, including the identification of extensive pleiotropy between the traits, for example, genes affecting defence response to bacteria are common between starvation resistance and fitness. It was also shown that around half of expressed transcripts have a sex-bias in *D. melanogaster* (Ayroles *et al.,* 2009).

RILs are widely available for many model organisms, and with more advanced lines coming into circulation, for example, the *Arabidopsis* multi-parent advanced generation inter-cross lines (Kover *et al*., 2009), the *Drosophila* Synthetic Population Resource (King *et al*., 2012) and the recombinant inbred advanced intercross lines (RIAILs) of *C. elegans (Rockman and*

Kruglyak, 2009). Using such lines it is now possible to identify QTL to single polymorphisms (e.g. McGrath *et al*., 2009). However, due to the critical role of cross structure in generating sufficient numbers of recombination events and the number of lines needed for the high resolution, i.e. the number of markers, and statistical power, only a few of these advanced panels are available. *GWAS and QTL mapping studies are usually a pre-curser to the identification of the causative nucleotides of trait variation. However, both QWAS and QTL studies are valuable in their own right, for example, in understanding the relationship between environmental and genetic determinants, which do not necessarily require gene identification.*

Forward genetics approaches, principally undertaken via induced mutagenesis, can also be used to identify genes affecting particular traits. Ethyl methanesulfonate (EMS) is the standard method of inducing mutations in C. elegans and Drosophila (Brenner, 1974; Emery, 2007) and N-ethyl-N-nitrosourea (ENU) is the standard for inducing mutations in mice (Justice et al., 1999). EMS is an efficient mutagen for generating point mutations, causing GC – AT transitions as well as small deletions and chromosomal rearrangements (Sega, 1984; Anderson, 1995). ENU is also an efficient mutagen which produces transversions as well as transitions (Anderson, 1995). The standard protocol for EMS and ENU mutagenesis on C. elegans is to incubate fourth larval stage worms (See Fig. 1.3) or young adults for four hours in EMS, followed by thorough washing when finally mutagenised individuals are screened for a trait of interest (Brenner, 1974; De Stasio and Dorman, 2001). For instance, mutagenesis has been successfully used to identify genes that affect lifespan in mice (Holzenberger et al., 2002), D. melanogaster (Rogina et al., 2000) and C. elegans (Lin et al., 1997). This method of gene identification is considered easier than QTL and GWAS because of its ability to induce single-gene mutations in defined genetic backgrounds (Belknap et al., 2001; Nadeau and

Frankle, 2000). It is however not clear the extent to which mutagenesis defines the same genes and types of variants as that identified by GWAS or by QTL analysis.

A recurring problem with GWAS and QTL studies is that the SNPs and QTLs identified as statistically significant only explain a small proportion of the variance, and the variance of a trait can be explained by either the genetics (the heritability), or the environmental component (Hindorff *et al.,* 2009; Manolio *et al.,* 2009). A general biological issue with QTL mapping and GWAS analysis is also that changes in environmental conditions have an influence on the genetic control of life-history traits (Hoffmann and Willi, 2008), therefore many QTLs could be hidden if not analysed in a range of environments. Variation in the environment, such as changes in temperature and nutrient availability, will induce differential expression of genes (Li *et al*., 2006), allowing organisms to adapt phenotypes to maximise their fitness (Hansen *et al.*, 2012). For example, a higher than normal temperature results in faster growth rates, shorter development times, and smaller adult size in insects and other ectotherms (Angilletta Jr. *et al*., 2004; Zuo *et al*., 2012) in order to cope with this environmental pressure.

A technical issue with QTL mapping and GWAS is the experimental reproducibility of the QTLs and SNPs detected, as environmental changes can affect variation of phenotypes. Gene expression changes due to short intervals of large temperature change (heat shock) have been widely studied in a number of organisms; the free-living yeast *Schizosaccharomyces pombe* (Xue *et al*., 2004), the pathogenic yeast *Cryptococcus neoformans* (Kraus *et al*., 2004), the bacteria *Escherichia coli* (Guisbert *et al*., 2004), the fruit fly *D. melanogaster* (Sørensen *et al*., 2005) and the nematode *C. elegans* (GuhaThakurta *et al*., 2002). However, organisms raised under natural conditions are often exposed to longer periods of less extreme temperature changes. The ability to respond to these changes differs among genotypes. This

is clearly demonstrated by studies that have investigated gene expression plasticity in *C. elegans* (Li *et al.,* 2006). Li *et al.,* investigated this by growing populations of RILs, made from the parental lines CB4856 and N2, at two different temperatures of 16°C and 24°C (Li *et al*., 2006). They demonstrated that temperature differences lead to drastic differences in gene expression, and that this response was largely regulated by trans-genes (Li *et al*., 2006). In addition, differences between the genetic correlations between life-history traits in populations of *C. elegans* RILs grown at 12°C and 24°C have also been analysed (Gutteling *et al*., 2007a). In both of these studies the genetic architecture was analysed using QTL mapping.

Gene expression plasticity has also been studied in two yeast strains grown under two different conditions (glucose and ethanol as carbon source) (Smith and Kruglyak, 2008), where the linkage of 1555 gxeQTL (Gene-environment QTL) were tested, revealing 17% of the gxeQTL showing an effect in only the glucose environment and 21% showing an effect in only the ethanol environment (Smith and Kruglyak, 2008). Environmental influences on phenotypic variation have been shown in different species', however variation is also seen between genetically identical individuals (Rea *et al*., 2005). Rea *et al.,* showed that isogenic individuals of *C. elegans* when heat shocked (exposing worms to a high temperature above their ideal temperature for a short time, 1 or 2 hours), resulted in wide variation in lifespan, from around 3 – 16 days, and thermotolerance, from 4 – 9.5 hours (Rea *et al*., 2005). The experiments of Rea *et al* indicate that trait variation and the interaction of genetic components with environmental influences is fundamental to understanding how an organism adapts, and therefore how evolution continues.

Complex traits and fitness

Complex traits evolve in order to increase an organism's fitness, to improve its chances of survival (Monteiro and Podlaha, 2009). For example, pigmentation as camouflage in butterflies (Monteiro and Prudic, 2010), mate selection in *Achroia grisella* (Limousin *et al.,* 2012) and dispersal in flying insects (Zimmerman *et al.,* 2000) or dauer larvae in some species of nematode (Riddle *et al.,* 1981). Environmental conditions, such as temperature, can change rapidly and unpredictably and can affect the reproduction and growth of many organisms (Jump and Peñuelas, 2005; Arendt, 2010). Living at different temperatures can result in trade-offs in performance , for example, between enzyme stability and function, where enzymes selected for stability at high temperatures are less functional at lower temperatures and vice versa (Fields, 2001; Arnold *et al.,* 2001). In some cases, trade-offs which have been positively selected can manifest as disease associated alleles (Hindorff *et al.,* 2009). In humans, for example, there is evidence for prostate cancer susceptibility (Summers and Crespi, 2008) and heart disease risk (Rockman *et al.,* 2004) being positively selected. A reason for positive selection of disease causing alleles is due to the increase in fitness they confer via their effects on other traits or in certain environments. A simplistic example of this is demonstrated in sickle cell anaemia, a debilitating disease which leads to a considerably shortened lifespan (Stuart and Nagel, 2004), which might be expected to have been removed from the gene pool by natural selection. However, in certain geographical areas this disease, and the alleles that produce it, are highly prevalent (Piel *et al.,* 2010) because it confers a selective advantage in protection against malaria parasites (Aidoo *et al.,* 2002; Piel *et al.,* 2010; Duraisingh and Lodish, 2012).

Complex traits and dispersal

Dispersal is a fundamental feature of populations, having important consequences on population dynamics; demographics, genetic drift, extinction rates, and on individual fitness; reproductive success, colonisation ability and defence strategies (Ronce and Oliveri, 1997; Bowler and Benton, 2005). Given that many species rely on dispersal, in one form or another, the fitness of dispersal morphs is of key importance for species survival (Clutton-Brock and Lukas, 2011; Edelsparre *et al.,* 2014) not just in the methods for dispersal, but in the success of the dispersal morph at colonising new habitats. To increase the chances colonisation success, the timing of dispersal, the number of dispersal morphs and their ability to effectively use the available resources need to be considered (Noblin *et al.,* 2012). Dispersal is particularly important for species which rely on ephemeral habitats (Travis and Dytham, 1999), as dispersal is an absolute requirement, due to the local extinctions taking place when a habitat's resources are depleted (Dytham and Travis, 2006).

There are three categories of dispersal; emigration, inter-patch movement and immigration (Bowler and Benton, 2005). A number of studies have suggested various theories as to the evolution of dispersal and these include; reducing competition between kin (Ronce and Promislow, 2010), reduced inbreeding (Lebigre *et al.,* 2010), limiting resource competition (Gowaty, 1993), reaction to overcrowding (Clotuche *et al.,* 2010) and reaction to environmental stochasticity (Bach *et al.,* 2007). Organisms need to make decisions on the habitat they are in before dispersing in the hope of finding a resource rich habitat, considering:

1. the population density; the amount of competition and how many members of kin there are;
2. the amount of available resources (i.e. the patch size);

3. the distance to the next resource patch; predators, energy usage, and the ratio of males to females (Bowler and Benton, 2005).

There is extensive variation in the mechanisms for dispersal across species, ranging from flight (e.g flying insects), flagella (e.g. some unicellular organisms and bacteria) and legs (e.g mammals) to organisms that depend on wind and gravity, such as reproducing plants. There is also intraspecific variation in some species. For example, predominantly male-biased dispersal is often observed in mammals, while female-biased dispersal is more common in birds (Greenwood, 1980; Pusey, 1987; Lawson Handley and Perrin, 2007). Another form of within species variation is in the morphological variation of distinct dispersal morphs, as demonstrated by Fjerdingstad *et al.,* (2007). Fjerdingstad *et al.* showed that the ciliated protist, *Tetrahymena thermophile*, was able to transform their cell shape to produce morphs varying in swim-speed. Similarly, Innocent *et al.* (2010) showed two distinct, long-winged and short-winged, female dispersal morphs in the parasitic wasp *Melittobia australica*. Interestingly, other life-history traits varied between the two morphs; short-winged females with lower rates of dispersal emerged with more fully developed eggs and lay a higher proportion in their first clutch (Innocent *et al.,* 2010).

Models of environmentally dependant complex traits

A fundamental issue in biology is determining how phenotypes are produced by genotypes and how environmental variation acts on these genotype-to-phenotype maps. That organisms respond to their environment is clear, as is the adaptive nature of a subset of these environment interactions, with organisms modifying their behaviour, physiology or phenotype to maximise fitness (Pigliucci, 2005; Rodriguez *et al*., 2012) referred to as

phenotypic plasticity. Establishing that environmentally induced variation in a trait is actually adaptive is not however straightforward. Phenotypic plasticity is the ability in which a genotype can produce a variety of phenotypes, morphologies and behaviours in response to different environmental conditions (West-Eberhard, 1989). Understanding how phenotypic plasticity evolves and how it is maintained by selection is therefore also required if we are to understand life-history evolution (Pigliucci, 2005). To achieve such an understanding of phenotypic plasticity, it is necessary to understand not only the genetic pathways that control a given polyphenism, but also how these specific pathways are affected by variation in other traits. Understanding the genetic architecture of phenotypic plasticity, specifically that which controls developmental switches, is important in determining how the control of phenotypic plasticity is related to the control of the trait itself and where variation between genotypes acts on these developmental switch pathways. Developmental switching is an important phenotype for many organisms, for example, the switch between free-living and parasitic morphs in parasitic nematodes (e.g. Ashton *et al.,* 1998) and for most species there is either a good understanding of the genetics of a trait, or of the ecological relevance of that trait. A case in point is the development of dauer larvae, an environmental resistant dispersal stage, in nematodes. Here there is an excellent understanding of the genetics of dauer larvae formation in some nematode species, particularly the model nematode *Caenorhabditis elegans*, but understanding of the ecological significance is limited. However, *C. elegans* is a good model for the analysis of an environmentally dependant complex trait because of the tools and resources available to study *C. elegans*. Further, there is evidence that suggests that the plasticity of dauer larvae development in *C. elegans* is related to variation in other life history traits such as the population growth rate (Harvey *et al*., 2008).

Introduction to *Caenorhabditis elegans*

The model organism, *C. elegans* is a free-living, bacteria eating, nematode. One of the key traits making it such a useful model organism is its relatively rapid life-cycle (See Fig 1.3). In a favourable environment, eggs hatch into the L1 stage, then develop through 3 further larval molts before the reproducing adult stage is reached. When environmental factors such as temperature, population density and food abundance are unfavourable, the development of an alternative larval stage is influenced, known as dauer larvae (Cassada and Russell, 1975; Klass and Hirsh, 1976; Riddle, 1977). The average lifespan of wild-type *C. elegans* isolates is between two and three weeks, although variation in lifespan is observed between different strains of N2 (the wild-type strain used in most studies of *C. elegans*). This implies that lifespan can evolve rapidly, as variation in the lifespan of individuals derived from a common ancestor was shown to be between 12 and 17 days (Gems and Riddle, 2000). As would be expected, many environmental factors have been found to affect lifespan in *C. elegans*, for example, temperature and food concentration (Klass, 1977) and oxygen levels (Honda *et al*., 1993).

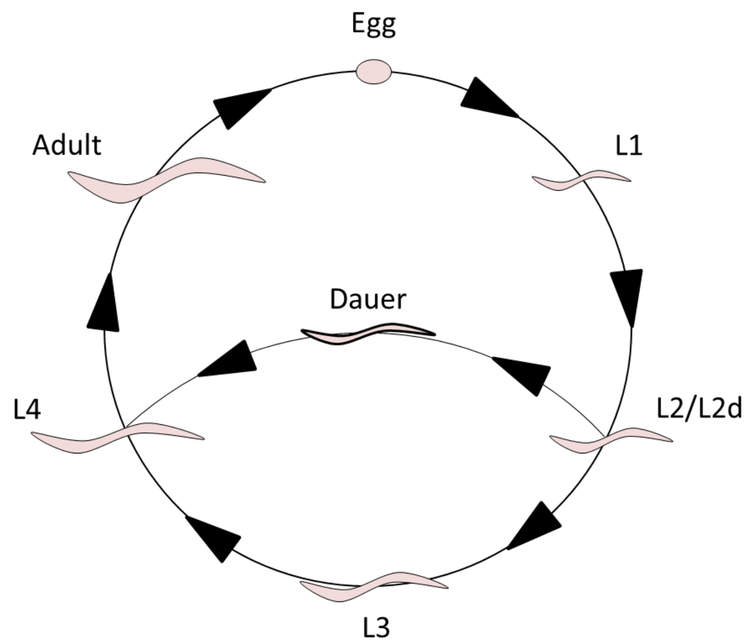

Figure 1.3: The life-cycle of *C. elegans*. *C. elegans* has a 14 hour embryogenesis period which is followed by 36 hours of development from larval stage 1 to adult, at 25°C (Byerly *et al.,* 1976; Riddle *et al.,* 1997). In periods of environmental stress, an alternative 3rd stage larval morph, dauer larvae, is chosen.

C. elegans has two sexes, hermaphrodites (XX) and males (XO), and can reproduce by both self-fertilisation and cross-fertilisation (Hodgkin, 1987). Progeny produced from self-fertilisation are predominantly hermaphrodites with around 0.2% males occurring from nondisjunction of X chromosomes, while cross-fertilised broods contain an equal number of hermaphrodites and males (Hodgkin and Doniach, 1997). Sex determination was among the first traits to be characterised by genetic analysis (Hodgkin and Brenner, 1977; for a review see Haag, 2005), after the initial genetic studies by Sydney Brenner (Brenner 1974; Sulston and Brenner, 1974), and the process is now well understood. Each hermaphrodite produces around 300 sperm to be used for self-fertilisation, and as they can produce more oocytes than sperm, close to 100% of the sperm are used producing around 300 selfed progeny (Ward and Carrel, 1979). A hermaphrodite can also be mated with a male to produce up to

2500 more cross progeny (Hodgkin and Doniach, 1997). For both mated and unmated hermaphrodites, most progeny are produced during the first five days of the reproducing period.

C. elegans dauer larvae development

C. elegans' dispersal morph is known as the dauer larvae, an alternate third larval stage (Fig 1.3). Before the discovery of their natural habitat, where all stages of development can be isolated, most nematodes isolated from the wild are found as dauer larvae. Dauer larvae are developmentally arrested, develop at high conspecific population densities when food availability is low and are specialised for long-term survival and dispersal (Cassada & Russell, 1975; Golden & Riddle, 1984a, b; Hu, 2007). Isolating only dauer larvae suggested conditions suitable for reproduction were rare and that development of dauer larvae is important in the *C. elegans* lifecycle. However, *C. elegans* is commonly studied under very unnatural conditions and little is known about the properties of growing populations. For instance, most analyses of growth, reproduction and aging in *C. elegans* are performed under non-competitive conditions with an *ad libitum* food source (*e.g.*, Dorman *et al.*, 1995; Hodgkin & Doniach, 1997; Harvey *et al.*, 2008). Similarly, dauer larvae formation is normally analysed in cohorts of age-matched individuals developing at high pheromone concentrations and a limited amount of food (*e.g.*, Golden & Riddle, 1984b; Viney *et al.*, 2003; Harvey *et al.*, 2008) and has not been analysed in growing populations. Crucially, the limited number of studies that have looked at *C. elegans* under more natural conditions often reveal novel effects or explain apparent contradictions between theory and analyses undertaken using standard laboratory conditions. For example, fitness costs of *age-1* loss of function, a mutation that dramatically increases lifespan, were only revealed under competitive conditions (Walker *et*

al., 2000; Jenkins *et al.*, 2004). Also, the otherwise paradoxical sperm-limited fecundity seen in *C. elegans* was not observed when nematodes were grown in more natural resource limited conditions (Goranson *et al.*, 2005). Such observations suggest that potentially important variation may only be observable when *C. elegans* is cultured in more natural conditions.

Dauer larvae are formed when conditions are unsuitable for growth and reproduction, triggered by a high level of dauer pheromone coupled with low levels of food. Dauer pheromone, a complex mix of structurally related ascarosides (Jeong *et al.,* 2005; Butcher *et al.,* 2007, 2008, 2009a and 2009b; Pungaliya *et al.,* 2009; Park *et al*., 2012), is used by *C. elegans* to assess population density, with these ascarosides also acting to regulate aggregation, mate recognition and dispersal (Srinivasan *et al*., 2008; Pungaliya *et al.,* 2009; Harvey, 2009; Izrayelit *et al.,* 2012; Jang *et al.,* 2012). There are three major pathways involved in dauer larvae formation: 1. the transforming growth factor β (TGF-beta), 2. the insulin/insulin-like growth factor (IGF) and 3. the guanylyl cyclase pathways. Sensory information from unfavourable environmental cues, such as ascarosides components of the dauer pheromone and nutrient-sensing, are transmitted through each of them (Juilfs *et al.,* 1997; Sommer and Ogowa, 2011).

The TGF-beta and the IGF pathways control dauer larvae formation through the regulation signalling cascade pathways using the DAF encoded proteins; DAF-2 (Kimura *et al.,* 1997), DAF-23 (Gottlieb and Ruvkun, 1994) and DAF-16 (Shaw *et al.,* 2007; Ruaud *et al*., 2011). Under suitable growth conditions, the TGF-beta and IGF pathways are suppressed which allows normal reproduction to occur, and are uninhibited when conditions become unfavourable for growth (Ren *et al.,* 1996; Sommer and Ogowa, 2011). The guanylyl cyclase pathway, an olfactory signal transduction pathway, regulates dauer formation through the

inhibition of *daf-11*, which encodes cilium-localized guanylyl cyclase, by ODR-1 (L'Etolile and Bargmann, 2000; Jensen *et al*., 2010a), identifying the role of cilia activity in dauer larvae development (Albert and Riddle, 1983).

A number of genes have been identified, through mutagenesis studies, which affect dauer larvae development in standard dauer larvae formation assays (see Appendix Table 1 for a list of these genes). This demonstrates the extensive knowledge of genes and gene pathways affecting dauer larvae development, though in a very unnatural way. This in turn suggests that a more natural investigation into dauer larvae development, i.e. in a growing population, could lead to the discovery of ecologically relevant dauer genes, and could also validate the already discovered genes.

There are clear morphological links between the dauer larvae of free-living species and the infective stages of many parasitic nematodes. For example, both are non-feeding morphs, both are surrounded by a strong cuticle, both are resistant to environmental factors and both exhibit different behaviours to the organisms other larval stages (Lee, 2002). There are also genetic links between the dauer larvae of free-living species and the infective stages of many parasitic nematodes. Examples are the genes *daf-7,* which controls entry into infective or free-living stage of the parasite *Strongyloides papillosus* (Ogawa *et al.*, 2009), and the *daf-16* ortholog, *fktk-1,* in the parasite *Strongyloides stercoralis* (Castelletto *et al*., 2009). However, there are still major differences too, for example, parasitic nematode orthologs of the TGF-beta pathway (a key regulatory pathway of dauer larvae formation in *C. elegans*) have no role in the formation of the infective stage in parasitic nematode species (Viney *et al*., 2005). This indicates that while these two processes are similar they are not entirely analogous traits, as parasitic nematodes have evolved to use the common signalling pathways in different ways (Viney *et al.,* 2005).

Ecology of *C. elegans*

Counter to the wealth of knowledge into the genetics of *C. elegans*, very little is known of its ecology (Félix and Braendle, 2010). Up until very recently, it was widely believed to be a soil-dwelling nematode (Barrière and Fèlix, 2005; Caswell-Chen *et al.,* 2005). Indeed, soil and compost is where *C. elegans* populations were sought (Barrière and Fèlix, 2005). More recently, specific sampling of nematodes on rotting fruit and vegetable matter has led to the discovery of 16 new species of *Caenorhabditis*, strongly suggesting *Caenorhabditis* are not soil nematodes, but are "fruit worms" (Kiontke *et al*., 2011; Félix *et al.,* 2013). Consistent with this, large proliferating populations of up to 10,000 individuals, of *C. elegans* and *C. briggsae* have been found on rotting plant material (Félix and Duveau, 2012).

N2, the isolate of choice for developmental and genetic studies, displays very different behaviours under laboratory conditions than all other wild isolates. These behaviours include bordering, burrowing and clumping. The standard N2 strain was cultured for over 10 years before being frozen in 1969 (Hodgkin and Doniach, 1997) and is used in the majority of studies using *C. elegans*. Due to the early culturing of N2, and the certain laboratory adaptations it would have gained, it is unclear if experimental observations are due to laboratory adaptations or are incorrectly inferring natural variation. A point in case for laboratory adaptation is the *npr-1* gene, which encodes for a G protein-coupled neuropeptide receptor. The G proteins are the largest family of eukaryotic signal transduction proteins that communicate across the membrane (Cherezov *et al.,* 2007). NPR-1 is used in oxygen sensation, a process in which N2 has a unique combination of alleles compared to wild strains, the difference being a single amino acid, at number 215, being either phenylalanine or valine (de Bono and Bargmann, 1998). This difference in the amino

acid sequence of the NPR-1 protein has been attributed to domestication to laboratory conditions (Weber *et al.,* 2010).

Extensive variation in dauer larvae formation has been shown between laboratory adapted and natural isolates of *C. elegans* (Diaz *et al.,* 2014), however, this variation has been investigated under conditions which are very unnatural for the wild species. Indeed, with the discovery of the natural habitat of *C. elegans* it has been shown that large propagating populations, consisting of all larval stages (Félix and Duveau, 2012), can endure in a harsh and fluctuating environment with populations likely to have been initiated with a small number of dauer larvae. With the extensive potential for genetic and environmental variation to influence the formation of dauer larvae, the variation seen in natural and laboratory adapted populations is contentious, with careful consideration needed to any comparisons made. Because of the unnatural way of investigating the trait, coupled with our limited understanding of the ecology of *C. elegans*, it is important to understand the dynamics of growing populations in the laboratory and how dauer larvae develop in such populations. Also, the analysis of the genetics of complex traits has a more general significance as many complex traits can be linked with the manifestation of diseases.

Analysis of growing populations of *C. elegans*

The principle cues for dauer larvae development, pheromone level and food availability, will be different for a growing population than for the standard dauer larvae assay. The population growth rates, pheromone production and consumption of bacterial food will change as a consequence. Given this difference between the two assays, the ecological relevance of the extensive variation between *C. elegans* isolates in their sensitivity to dauer

inducing conditions as assessed by analysing age-matched individuals at high pheromone concentrations and limiting amounts of food is unclear (Viney *et al*., 2003; Harvey *et al*., 2008). The predominantly selfing mode of reproduction in *C. elegans* and the observation of extensive large haplotype blocks in natural isolates (Anderson *et al.,* 2012) complicate the analysis of this variation. QTL mapping however is a possible way in which variation of the causal genetic regions can be identified.

Comparative analysis of *C. elegans* and *C. briggsae* has the potential to increase the understanding of the genetic basis of dauer larvae development. Both of these species are free-living, self-fertilizing hermaphrodites with facultative males (Hillier *et al.,* 2007), have the same number of chromosomes and have similar genome sizes (Stein *et al*., 2003), occupy the same ecological niche (F**élix and Duveau, 2012**) but diverged around 100 million years ago.

Aims of this thesis

The aims of this thesis are to investigate dauer larvae development in a growing population. This will establish a new method of investigating dauer larvae, a method which will more closely relate to the natural habitat of *C. elegans*, in that a population will be allowed to grow from a single or small number of founding nematodes. These population assays will differ from the standard dauer larvae assay by changing determinates for dauer larvae formation; food availability, pheromone and allowing natural behaviours. The number of dauer larvae in the population will be established and compared to the total number of nematodes in the population, as opposed to the number of dauer larvae formed within a set number of nematodes. Specifically, the aims are:

1. to establish methods for the analysis of dauer larvae development in growing populations, and to carry out experiments to validate this style of assay for the analysis of dauer larvae development (Chapter Two).
2. to investigate the genetic basis of dauer larvae development in a growing population through QTL mapping, using Recombinant Inbred Lines (RILs) and Isogenic Lines (ILs) (Chapters Three and Four).
3. to investigate comparative differences in the genetic basis of dauer larvae development in growing populations, using crosses between different of *C. elegans* genotypes, and a related species, *C. briggsae* (Chapter Four).
4. to validate the QTLs by investigating their reproducibility and the effect environmental conditions have on their reproducibility. To investigate candidate genes using mutant isolates and RNA interference (RNAi) (Chapter Five).

CHAPTER TWO: Development of *Caenorhabditis elegans* dauer larvae in growing populations

Work reported in this chapter has been published as:

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SUMMARY

Natural populations of *Caenorhabditis elegans* exhibit rapid population growth within resource-rich patches of decaying organic material and subsequent dispersal, primarily as developmentally-arrested dauer larvae, between patches. The properties of growing populations of *C. elegans* are, however, poorly understood. Here methods that allow the analysis of growing populations of *C. elegans* have been developed. Using these methods show that food availability, dauer pheromone (a measure of conspecific population density) and temperature affect dauer larvae development in growing populations as would be predicted from analyses of single synchronised cohorts of worms. These analyses demonstrate that as food patch size increases, dauer larvae are formed prior to patch exhaustion and that the number of dauer larvae present increases after the patch is exhausted, *i.e.* worms that had not completed development as dauer larvae when the food was exhausted continue development in the absence of bacterial food. Analysis of post-dauer development of dauer larvae formed under different conditions indicates that the subsequent reproductive fitness of dauer larvae that complete development after the exhaustion of the bacterial food patch is reduced in comparison with dauer larvae that develop prior to patch exhaustion. Also shown are the differences between the population size, the number of dauer larvae and lifetime fecundity between populations grown in 2 and 3 dimensional environments. Analysis of mutant isolates with abnormal growth and physiology are shown to have subsequent effects on dauer larvae development. Overall, this work demonstrates that population level analyses of *C. elegans* are feasible, support previous studies of the environmental factors affecting dauer larvae development and suggest an adaptive benefit for variation between isolates in the sensitivity of dauer larvae development.

INTRODUCTION

Patchily distributed ephemeral habitats, *e.g.*, rotting fruit, fresh compost and other nutrient- and bacteria-rich substrates are important for many species (Hanski and Beverton, 1994) such as, earthworms (Edwards, 1983), nematodes (Félix and Duveau, 2012) and fungi (Rayner and Boddy, 1999). The species associated with such habitats are important providers of ecosystem services, e.g. soil aeration and decomposition, and help to shape many aspects of the wider ecosystems (Bengtsson, 1998; Lavelle *et al.*, 2006). Species reliant on ephemeral resources often have high local extinction rates, and commonly follow metapopulation dynamics (Hanski, 1998). Given this, genotype fitness will depend on how quickly and efficiently resources are used within a patch, the timing of dispersal stage production, the number of dispersal morphs produced and the subsequent success of these dispersal morphs at colonising new patches. Changes in environmental factors, such as temperature and both food abundance and quality, will affect the dynamics of these populations, altering both within-patch actions and dispersal success (Bowler and Benton, 2009). Genetic differences will also affect population dynamics and the potential exists for local adaptation in both life history traits (Moiroux *et al.*, 2010) and behaviour (Cousyn *et al*., 2001). Therefore, it is important to determine how traits affecting both population growth and dispersal are controlled and how such traits are related.

Laboratory-based studies of model organisms represent one way in which such questions can be addressed. For instance, mesocosm studies of the soil mite *Sancassania berlesei* have shown the importance of both density and of maternal effects in population growth (Bowler and Benton, 2005, 2008) and of inter-patch distance in population dynamics (Bowler and Benton, 2009). Other model systems have, for example, demonstrated the rapid evolution of infection strategy in *Steinernema feltiae* in response to changes in host (*i.e.*, patch)

availability, identifying trade-offs between infection rate and other life-history traits (Crossan *et al.*, 2006) and the effects of infection with a bacterial parasite on dispersal of the ciliate *Paramecium caudatum* (Fellous *et al.*, 2010). The free-living nematode *Caenorhabditis elegans* is potentially another good laboratory-based model for the analysis of dispersal and of adaptation to ephemeral habitats, but relatively little is known of the ecology and population dynamics of the species (Barrière and Félix, 2005, 2007, although see Félix and Duveau, 2012 for recent findings). Specifically, there has been no previous investigation of dauer larvae development in growing populations.

Here I have developed and validated methods to allow the analysis of dauer larvae formation in growing populations. The first stage in developing and validating the methods was to investigate dauer larvae development in growing populations under different environmental conditions; different temperatures and amounts of food, to see if the numbers corresponded with standard dauer larvae assays. The role of the dauer pheromone in a growing population was also explored, as this is a key component of the standard dauer larvae assays.

The second stage of method development and validation was to produce a working environment for the worms where they could exhibit their natural behaviour and at the same time be analysed at the end of the assay period. An agar medium, referred to as the 3D environment, was created and compared to the standard agar medium, referred to as the 2D environment. Growing population assays were performed in the 2D and 3D environments to compare population size and the number of dauer larvae. Lifetime fecundity, population growth rate and the start of dauer larvae formation in a growing population was also analysed in the 2D and 3D environments.

After the initial validation of the growing population assay, a further three ecologically relevant questions regarding dauer larvae development in a growing population were investigated. Firstly, how does the population size and number of dauer larvae in a population vary when started with different numbers of individuals? Secondly, can L1 and L2 stage worms complete dauer larvae formation when all food has been exhausted? Finally, is there a fitness difference between dauer larvae formed in the presence of and without food?

The last part of the methodology and validation section was to examine growing populations of mutant lines. These genetic mutations, some of which are known to effect dauer larvae formation, were investigated to see if, (1) those which are known to have an effect on dauer larvae formation in standard dauer assays show the same effect in a growing population and (2) to see if genetic defects in gene pathways necessary for the sensation of and interaction with their environment may affect other phenotypes.

In combination, these analyses show that growing populations of *C. elegans* can be analysed in a way suitable for large scale mapping studies. Results also validate the dauer larvae development in growing populations assay, showing that dauer larvae formation is affected as would be predicted by previous studies of dauer larvae development using single cohort assays.

METHODS

NEMATODES

N2, the canonical *C. elegans* wild-type, CB4856, the Hawaiian strain and mutants, DR476: *daf-22 (m130)II,* CX2205: *odr-3(n2150)V*, DA1113: *eat-2*(*ad1113)II, DA465: eat-2(ad465)II, DR27: daf-16(m27)I,* PR680: *che-1(p680)I and TJ1052: age-1*(*hx546)II* were obtained from the *Caenorhabditis* Genetics Centre. DR476, an isolate produced by mutagenesis of N2 worms, that is defective in dauer pheromone production (Golden and Riddle, 1982) was used to test the role of dauer pheromone in growing populations. The mutant isolates were used to test the role of various disruptions to *C. elegans* development and physiology in the formation of dauer larvae in growing populations.

Isolates were maintained using standard methods on NGM plates (Stiernagle, 2006), with the OP50 strain of *Escherichia coli* as a food source. For all assays, nematodes were synchronised by isolating eggs from gravid hermaphrodites by hypochlorite treatment (Stiernagle, 2006), and placing them at 20°C on NGM plates without food. The resulting hatched larvae arrested development at the first-stage larvae (L1). Arrested L1s were then transferred to plates with food and allowed to develop to fourth-stage larvae (L4s), before use in assays.

For all experiments, plates were blind-coded and treatments were randomised, with plates that became contaminated, or failed to grow were excluded from analysis. For the assays using 2D environments, plates on which worms burrowed into the agar were also excluded from analysis as such plates did not allow the recovery of worms. All worm numbers represent living worms.

POPULATION SIZE AND DAUER LARVAE FORMATION IN GROWING POPULATIONS

Food concentration and temperature

To investigate the role of food availability and temperature on dauer larvae formation and population growth, growing populations were initiated with one N2 L4 hermaphrodite on 55 mm diameter dauer agar plates (Viney *et al.*, 2003). At 20°C, populations were initiated on plates with 100 μl of 5, 10 or 20% (w/v) *Escherichia coli* (See Appendix Fig 1 for standardisation). At 15 and 25°C, populations were initiated with 100 μl of a 20% (w/v) suspension of *E. coli*. To prepare bacterial food, overnight cultures, grown in LB broth, were centrifuged and the supernatant discarded, with the bacterial pellet re-suspended in water at the required percentage w/v concentration. All food within an experiment was from the same batch of prepared bacteria. However, differences between batches of bacteria mean that worm numbers are not directly comparable between experiments (see Chapter Five for further investigation of the effects of variation due to variation between bacteria). Plates were monitored daily until patch exhaustion, defined by the depletion of all food, a stage recognised by the dispersal of all worms from the exhausted area (Hodgkin and Barnes, 1991). At this point, all worms were washed from the plates and suspended in a total volume of 10 ml. Population size was determined by counting the number of worms in 1 ml of this solution, with a further ten-fold dilution used to count large populations. The number of dauer larvae was determined by incubating the remaining 9 ml of suspended worms for 30-40 min in a 1% (w/v) solution of sodium dodecyl sulphate, a treatment that kills all non-dauer stages of *C. elegans* (Stiernagle, 2006). The suspension was then centrifuged for 2 min at 1000 *g*, the supernatant removed and worms re-suspended in water. This wash step was repeated and then worms were transferred to plates with food. Dauer larvae were then allowed to recover overnight prior to counting.

Pheromone

To investigate the role of the dauer pheromone, growing populations were initiated with either two N2 L4 hermaphrodites or with one N2 and one DR476 L4 hermaphrodites. At food exhaustion, population size and the number of dauer larvae were determined as above. DR476, a *daf-22* mutant, is incapable of producing the dauer pheromone as DAF-22 is required for pheromone biosynthesis (Butcher *et al.,* 2009) but the mutant is able to sense it (Riddle and Golden, 1985).

2D VERSUS 3D ENVIRONMENTS

To investigate differences in the structure of their environment, population assays were also performed as described above except that agar concentration was varied, with normal, dauer agar, plates containing 20g/l (2D environment) and sloppy agar plates containing 4g/l (3D environment). Using the 3D environment corrected an initial issue with the population assays where worms tended to burrow into the agar when the population size was very large, making recovery and accurate counting impossible. Sloppy agar is solid enough to hold a pellet of bacterial food, allows worms to burrow freely and allows recovery of all worms for assay. To investigate the effects of this 3D environment on *C. elegans* life history, population growth was analysed by counting the population size and dauer larvae each day in plates. This was done using the above methods, however, the contents of the plates were washed into a centrifuge tube and suspended to a total volume of 10 ml, 1 ml of this was taken for the population count and 1 ml was taken to determine the number of dauer larvae. The population count sample was further suspended to a total volume of 10 ml, with 1 ml used to determine to the total number of worms. The dauer larvae sample was suspended in 1% (w/v) solution of sodium dodecyl sulphate to a total volume of 5ml. Finally,

lifetime fecundity determined by moving the adult worm to fresh plates every 24 hours for 5-6 days until reproduction had stopped, with the offspring on the old plates left to grow to L2s and counted, and reproductive timing, i.e. the onset of reproduction, were investigated for N2 and CB4856 in each of the environments (Byerly *et al.,* 1997; Hodgkin and Barnes, 1991).

Populations started with differing numbers of worms

To determine the effect on population size and the number of dauer larvae of starting populations with differing numbers of worms, population assays were performed using 3D plates and started with 1, 2, 5, 10 or 20 N2 L4s with 100 μl of a 20% (w/v) concentration of OP50 and incubated at 20°C until food was exhausted, at which point the population size and the number of dauer larvae were determined as described.

POPULATION SIZE AND DAUER LARVAE FORMATION AFTER FOOD EXHAUSTION

A single N2 L4 was placed onto the plates with 100 μl of a 5% (w/v) concentration of OP50 and incubated at 20°C (no dauer larvae are formed prior to patch exhaustion at this food level). Population size and the number of dauer larvae were determined as described above for ten replicate plates at food exhaustion and then daily for each of the next 8 days.

DAUER LARVAE RECOVERY, LIFETIME FECUNDITY AND REPRODUCTIVE SCHEDULE

Dauer larvae were isolated, as described above, from plates with either 5% (dauer larvae formed after food exhaustion) or 20% (w/v) (dauer larvae formed before food exhaustion) food patches. This allowed the isolation of dauer larvae that formed both before and after

patch exhaustion at the same time. These dauer larvae were then individually transferred to plates with food and incubated at 20°C. The onset of reproduction, the time when the first egg is laid, was then determined by observing the plates hourly for a 9 h period starting 41 h after worms were transferred onto food. Progeny production in these worms was also determined as described above. For these data, the start of the onset of egg laying and the daily cumulative fecundity were analysed by Mann-Whitney *U*-test.

DAUER LARVAE FORMATION IN MUTANT WORMS

Various mutant isolates were used to investigate the role of genetic defects on dauer larvae formation in growing populations. To test the role of sensory neurons, strains CX2205 and PR680, which have mutations in the *odr-3* and *che-1*, respectively, were investigated. *odr-3* encodes a G protein alpha subunit and *che-1* encodes a C2H2-type zinc finger, mutations in these genes cause severe olfactory defects (Roayaie *et al.,* 1998) and defective chemotaxis of water-soluble attractants (Lewis and Hodgkin, 1977), respectively. To test the role of feeding, strains DA1113 and DA465*,* both of which contain mutations of the *eat-2 g*ene were investigated. EAT-2 is a ligand-gated ion channel subunit that regulates pharyngeal pumping (Avery, 1993) and is also required for normal life-span (Lakowski and Hakimi, 1998) and defecation (Thomas, 1990). The effect of age alteration was tested using the strain TJ1052, which has a mutation in *age-1*, a gene that encodes a homologue of mammalian phosphatidylinositol-3-OH kinase (PI3Ks) catalytic subunits and is required for normal development and normal senescence (Morris *et al.,* 1996), and salt-sensing (Tomioka *et al.,* 2006). Finally, an isolate which has abnormal dauer formation, DR27, due to a mutation in the *daf-16* gene, was tested. DAF-16 is the only forkhead box O (FOXO) homologue in *C.*

elegans, and it acts in the insulin/IGF-1 signalling pathway (Ogg et al., 1997), regulating lifespan and dauer larvae development (Gems and Riddle, 2000; Lin *et al.,* 2001).

ANALYSIS

Population sizes were analysed by one-way analysis of variance (One-way ANOVA), with *post hoc* testing by Fisher's least significant difference test, as data were normally distributed and the variances between groups were equal. Dauer larvae numbers were not normally distributed and were analysed by Kruskal-Wallis test. As suggested by Dytham (2003), Mann-Whitney *U* tests were used for pairwise testing as the Kruskal-Wallis test lacks a *post hoc* test. All analyses were carried out using Minitab® Statistical Software (Minitab, Coventry, UK).

RESULTS

DAUER LARVAE FORMATION IN GROWING POPULATIONS

Food concentration and temperature

Analysis of population size at food exhaustion indicates that, as expected, larger populations develop at higher resource levels ($F_{2,29}$ = 30.58, p < 0.001) (Fig 2.1A). The population size at food exhaustion also varied in response to the temperature ($F_{2,47}$ = 14.67, p < 0.001) (Fig 2.1A).

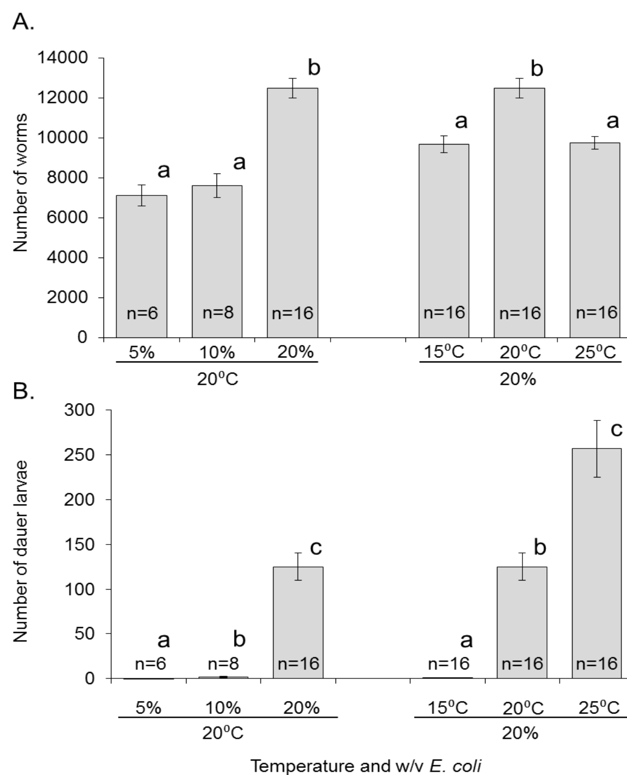

Figure 2.1: *C. elegans* dauer larvae development in growing populations depends on food availability and temperature. The mean number (±1 SE) of (A) worms and (B) dauer larvae present at patch exhaustion for populations started with one N2 fourth-stage larva (L4) and 100 µl of 20% (w/v) *E. coli* incubated at different temperatures, and 100 µl of different concentrations of food incubated at 20°C. n denotes the sample sizes for each group. Treatments marked with the same letter do not differ significantly; those marked with different letters are significantly different (p < 0.05; Fisher's Least Significant Difference).

These data also show that dauer larvae formation varies with resource availability (H = 23.11, df = 2, p < 0.001) (Fig 2.1B), with no dauer larvae developing prior to food exhaustion on the 5% (w/v) plates. Dauer larvae formation was also affected by temperature (H = 18.61, df = 2, p < 0.001) (Fig 2.1B), with a much greater proportion of the population developing as dauer larvae at 25°C. These indicate that a larger population size does not necessarily result in the highest number of dauer larvae, as suggested by the food availability assays.

Pheromone

Analysis of the pheromone manipulated populations indicates that similar sized populations developed in populations derived of N2 + N2 and of N2 + DR476 (two worms initiating the population) ($F_{1,19}$ = 0.32, p = 0.58) (Fig 2.2A). These suggest both that changes in pheromone levels are not sufficient to affect population growth rates and that DR476 does not have a reduced growth rate in comparison to N2. By contrast, analysis of dauer larvae formation in these populations indicates that dauer larvae formation does depend on pheromone level in the environment (H = 7.92, df = 1, p = 0.005) (Fig 2.2B).

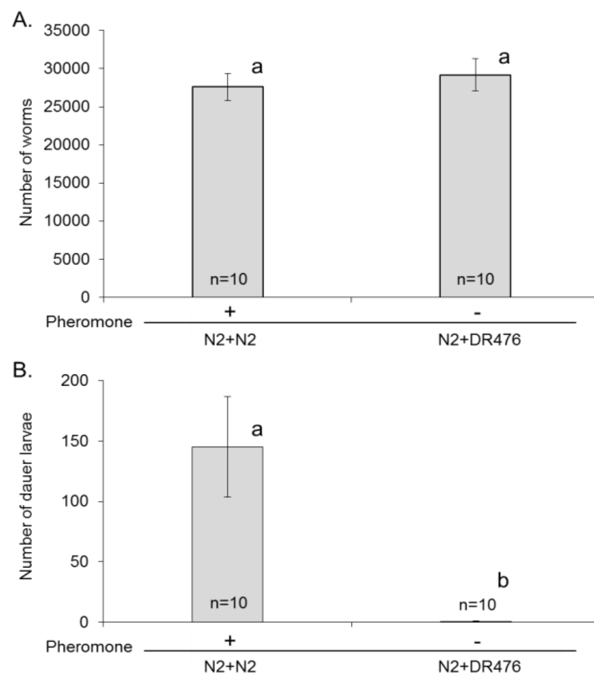

Figure 2.2: *C. elegans* dauer larvae development in growing populations depends on dauer pheromone. The mean number (±1 SE) of (A) worms and (B) dauer larvae present at patch exhaustion for populations started with one N2 L4 and one DR476 L4 with 100 µl of 20% (w/v) *E. coli*, incubated at 20°C. n denotes the sample sizes for each group. The pheromone + and – is for reference only. Treatments marked with the same letter do not significantly differ; those marked with different letters do significantly differ ($p < 0.05$; Fisher's Least Significant Difference).

2D VERSUS 3D ENVIRONMENT

Analysis at food exhaustion shows that population size and the number of dauer larvae formed are different ($F_{1,26}$=20.73, $p < 0.001$) (H = 12.04, df = 1, $p < 0.001$), respectively (Fig 2.3). To investigate the cause of these differences, analysis of the rate at of population growth in a 2D and 3D environment was done.

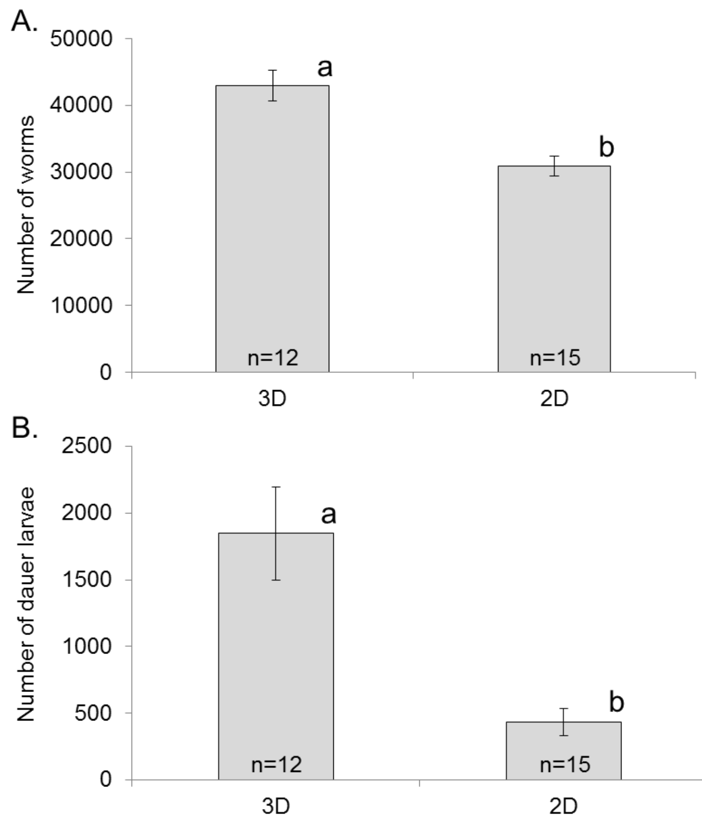

Figure 2.3: Populations of *C. elegans* grown in a 2D and 3D environment vary in the population size and number of dauer larvae produced. The mean (±1 SE) population size (A) and number of dauer larvae (B) present at patch exhaustion for populations started with one N2 L4 with 100 µl of 20% (w/v) *E. coli*, incubated at 20°C grown in 2D and 3D environment. n denotes the sample sizes for each group. Treatments marked with the same letter do not significantly differ; those marked with different letters do significantly differ ($p < 0.05$; Mann-Whitney U test).

Analyses of the rate of population growth indicate that in the first few days of population growth the population sizes don't significantly differ ($W = 2270.5$, $p = 0.80$), but on day 6 of growth the population size of the 3D environment is larger ($W = 34.0$, $p = 0.02$). These data also show that the number of dauer larvae measured daily does not differ significantly overall ($W = 2177.0$, $p = 0.35$), but days 5 and 6 demonstrate a significant difference (Day 5:

$W = 100$, $p < 0.001$. Day 6: $W = 28.0$, $p = 0.002$) (Fig 2.4). Figures 2.3 and 2.4 together indicate the importance of habitat structure on a growing population.

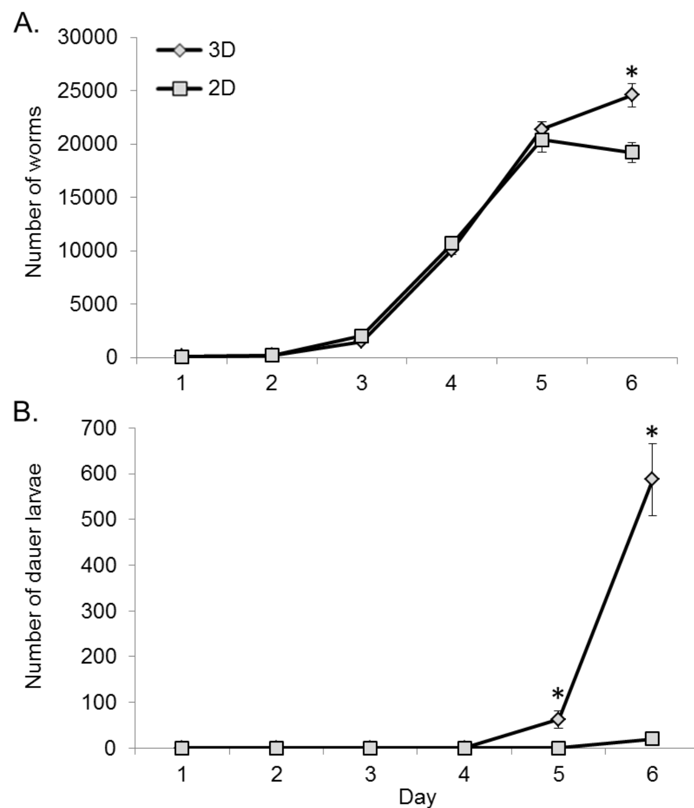

Figure 2.4: Populations of *C. elegans* grown in a 2D and 3D environment vary in growth rates. The mean (±1 SE) population size (A) and number of dauer larvae (B) present at daily intervals for populations started with one N2 L4 with 100 µl of 20% (w/v) *Escherichia coli*, incubated at 20°C grown in 2D and 3D environment. n = 8 plates per day. Asterisks (*) denotes days where the daily population size or number of dauer larvae differed between treatments ($p < 0.05$; Mann-Whitney U-test).

The mean lifetime fecundity of worms grown on the two environments, 2D and 3D, is not significantly different between the same genotype (N2: $F_{1,6} = 0.16$, $p = 0.70$. CB4856: $F_{1,8} = 0.37$, $p = 0.56$), but comparison of N2 and CB4856 on 3D shows a significant difference ($F_{1,9} = 12.32$, $p = 0.007$) which is not seen when grown on 2D ($F_{1,6} = 2.13$, $p = 0.20$) (Fig 2.5A). The cumulative daily fecundity shows that on day two for N2 there is a significant difference ($W =$

21, $p = 0.04$) while CB4856 does not vary on any days (Fig 2.5B). These suggest that environment-specific interactions are taking place and that they play an important role in how a population grows and the decision to form dauer larvae.

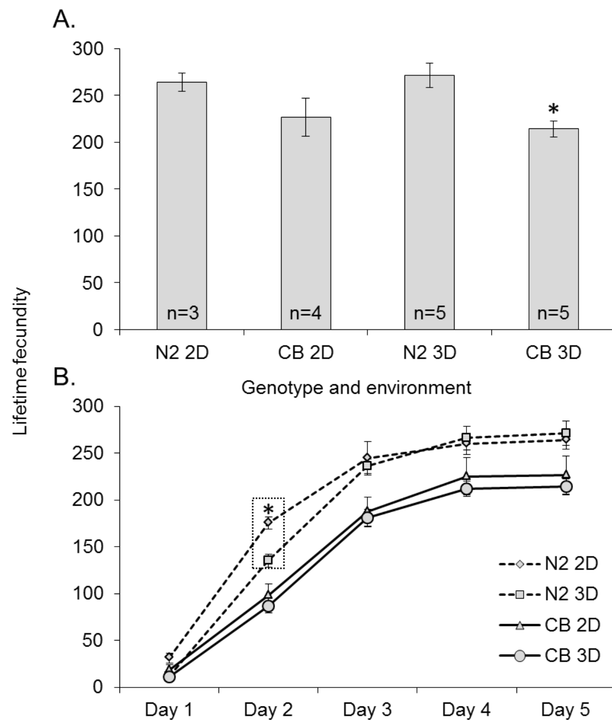

Figure 2.5: Lifetime fecundity of the same genotype does not vary between 2D and 3D environments but the reproductive schedule does. (A) The mean (±1 SE) lifetime fecundity of N2 and CB4856 genotypes. Asterisks (*) denotes where the lifetime fecundity differed between treatments ($p < 0.05$; One-way ANOVA). (B) Cumulative daily fecundity (±1 SE, which in some cases are too small to be visible). Asterisk (*) denotes days where the cumulative daily fecundity differed between treatments ($p < 0.05$; Mann-Whitney U-test). n denotes the sample sizes for each group.

Populations started with differing numbers of worms

Analysis of populations started with different numbers of worms shows that population size varies ($F_{4,84} = 19.13$, $p < 0.001$) (Fig 2.6A). The number of dauer larvae formed also varies ($H = 48.42$, $df = 4$, $p < 0.001$) (Fig 2.6B). These data indicate that a population size and structure strongly depends on how many worms initiate a population.

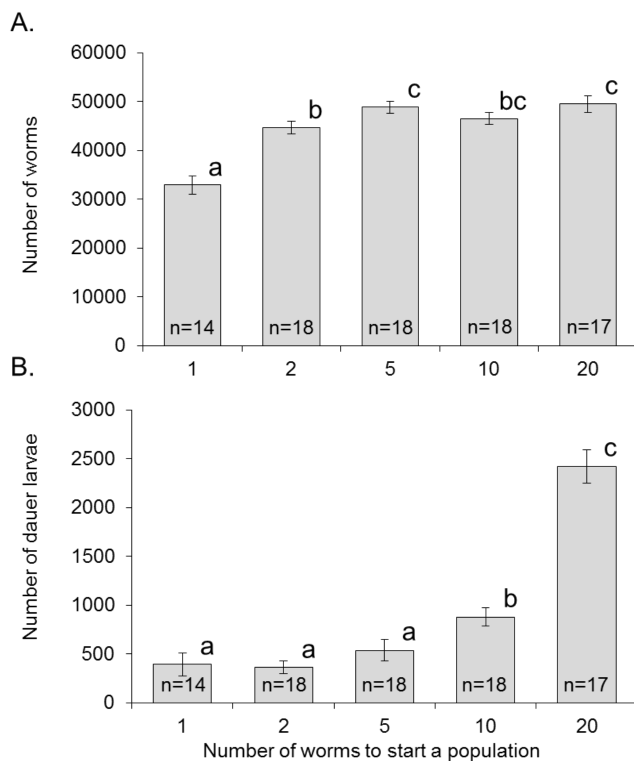

Figure 2.6: The population size and the number of dauer larvae formed are subject to the number of worms that initiate a population. (A) The mean (±1 SE) population size and (B) the mean (±1 SE) number of dauer larvae at food exhaustion for populations started with a different number of worms. n denotes the sample sizes for each group. Treatments marked with the same letter do not significantly differ; those marked with different letters do significantly differ (Population size: $p < 0.05$; Fisher's Least Significant Difference. Dauer larvae $p < 0.05$; Mann-Whitney U-test).

DAUER LARVAE FORMATION AFTER FOOD EXHAUSTION

Investigation of worm populations after food exhaustion indicates that population size continues to increase for the first few days after food patch exhaustion (Fig 2.7A), peaking on the third day after food exhaustion. During this period, the number of dauer larvae also increases slightly (Fig 2.7B). After this time (day 4 and after) the population size remain relatively constant and was predominantly composed of arrested L1s, L2s and dauer larvae, with the number of dauer larvae increasing until day 6/7. This indicates that worms which

had not completed development as dauer larvae at food exhaustion continued development in the absence of bacterial food (Fig 2.7B).

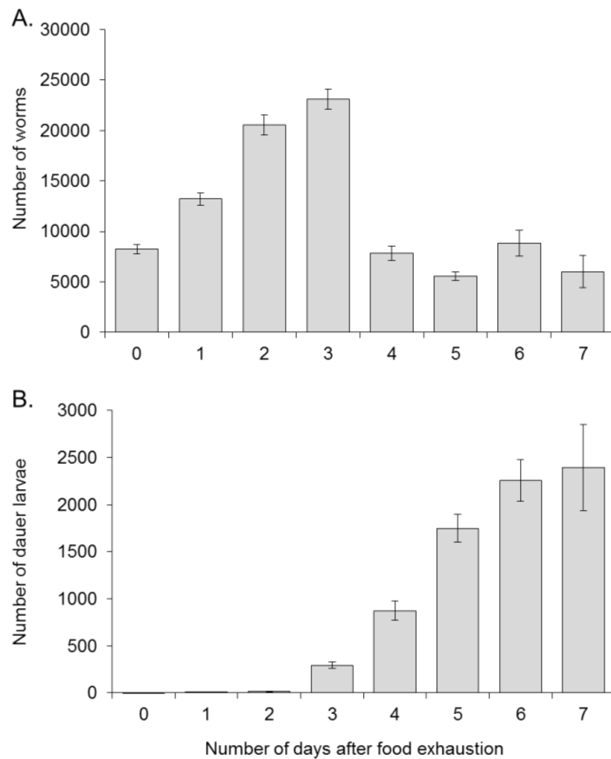

Figure 2.7: Larvae of *C. elegans* can complete development as dauer larvae after food patch exhaustion. The mean number (±1 SE) of (A) worms and (B) dauer larvae present on plates after the exhaustion of a 100 µl of 5% (w/v) *E. coli* food patch in populations at 20°C started with a single N2 L4. n = 10 plates per day.

DAUER LARVAE RECOVERY, LIFETIME FECUNDITY AND REPRODUCTIVE SCHEDULE

Comparison of subsequent reproduction between dauer larvae that had formed in the presence of food and those that had formed in the absence of food indicated that the onset of reproduction was delayed in worms that completed dauer larvae development after food exhaustion ($W = 825.25$, $df = 68$, $p < 0.001$) (Fig 2.8A). Further, comparison of the daily cumulative fecundity of these recovered dauer larvae showed that lifetime fecundity was

reduced in worms that completed dauer larvae development after food exhaustion (Day 5: $W = 1035$, $p = 0.03$. Day 6: $W = 1025$, $p = 0.03$. Day 7: $W = 1026.5$, $p = 0.03$) (Fig 2.8B).

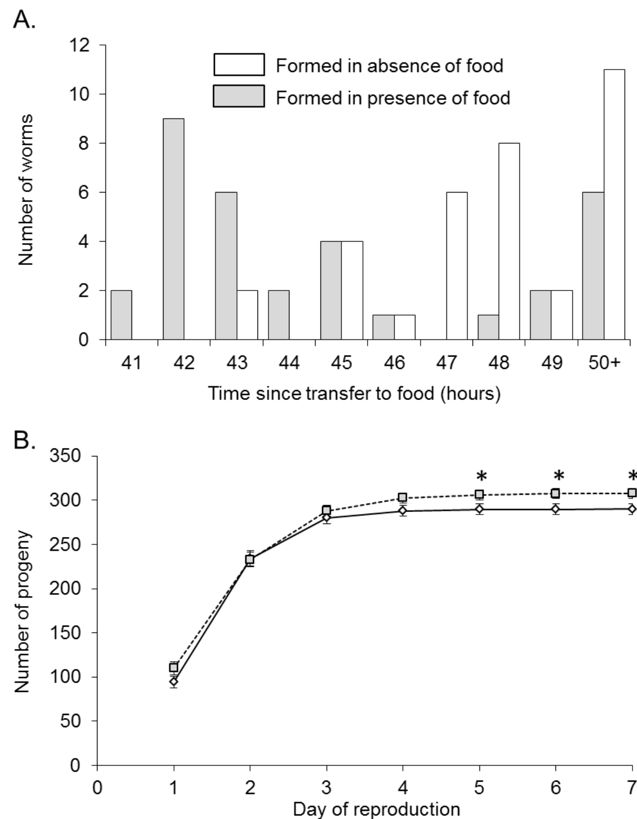

Figure 2.8: Development of *Caenorhabditis elegans* as dauer larvae after food patch exhaustion affects subsequent reproduction. (A) Time to onset of reproduction and (B) cumulative daily fecundity (±1 SE, some cases too small to be visible) of recovered dauer larvae that developed at 20°C in the presence of food (shaded bars and symbols, n = 33) and in the absence of food (open bars and symbols, n = 35). Asterisks (*) denotes days where the cumulative daily fecundity differed between treatments ($p < 0.05$; Mann-Whitney U-test).

DAUER LARVAE FORMATION IN MUTANT WORMS

The population size and number of dauer larvae formed at food exhaustion was analysed for mutant isolates. This showed the population size did not differ between genotypes, but the number of dauer larvae formed did (Population size: $F_{7,65} = 1.12$, $p = 0.37$; Dauer: $H = 36.23$, $d.f = 7$, $p < 0.001$) (Fig 2.9). DR27, the *daf-16* mutant showed very low numbers (an average

of 3-4 dauer larvae, in eight replicates) of dauer larvae formed, though it should be deficient in dauer larvae development. One of the *eat-2* mutants, DA465, showed a significantly higher number of dauer larvae formed, suggesting allelic variation between the two mutations causing different phenotypes. Interestingly, the *che-1* mutant formed fewer dauer larvae than N2, although no difference was seen in the other chemosensory mutant, CX205, which has a severe defect in chemotaxis.

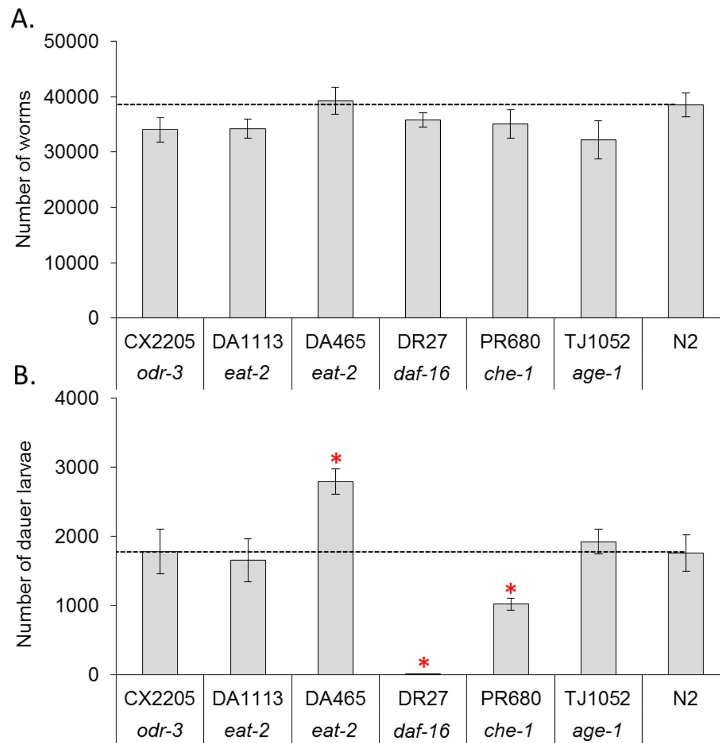

Figure 2.9: Dauer larvae formation in growing populations is effected as a consequence of gene mutations. The mean number of (±1 SE) worms (A) dauer larvae (B) present at food exhaustion. Dotted black horizontal lines show mean N2 values. Error bars indicate standard errors. Asterisks (*) denotes a difference from N2 (Population size: $p < 0.05$; Fisher's Least Significant Difference. Dauer larvae: $p < 0.05$; Mann-Whitney U-test).

DISCUSSION

Population size and dauer larvae development in *C. elegans* were examined under differing food availability and temperatures. A significantly greater population size was observed at the highest concentration of bacterial food, with smaller but similar sized populations observed at two lower food concentrations. Temperature also affected population size, with the largest populations observed at 20°C. This indicates that the efficiency of food conversion is highest at 20°C (Fig 2.1A). These analyses also indicate that dauer larvae development in growing populations is affected by food availability and temperature (Fig 2.1B). These differences in dauer larvae development are as would be predicted given what is known of the control of dauer larvae formation in *C. elegans* from the standard dauer larvae assay using synchronised cohorts of developing worms and concentrated dauer pheromone (Golden and Riddle, 1984a, b; Hu, 2007). However, these data do show that there is a critical amount of food below which dauer larvae do not develop prior to food exhaustion in growing populations. This is likely to be a consequence of the limited amounts of dauer pheromone (Golden and Riddle, 1982; Butcher *et al.*, 2007; Kaplan *et al.*, 2011), which will have been produced on the plates. This pheromone is produced throughout their life-cycle (Pomerai, 1990; Butcher *et al*., 2008) so a smaller population size will therefore have considerably lower pheromone levels. Other factors could be due to the age-structure of the population.

The importance of dauer pheromone is also demonstrated by the comparison of N2 populations with mixed populations composed of N2 and DR476. DR476, a *daf-22* mutant, is unable to produce dauer pheromone, but is able to sense its presence. The comparison of N2 and the *daf-22* mutant indicates that the population size at food exhaustion is similar (Fig 2.2A), while the mixed population shows significantly reduced numbers of dauer larvae (Fig

2.2B). Dauer pheromone is used to measure the number of worms in a particular habitat and as such, in a mixed population of N2 and DR476, half of the worms are essentially invisible. This shows that the pheromone produced by worms in growing populations is crucial for specifying dauer larvae development. These analyses indicate that the general processes that control dauer larvae development in *C. elegans* are similar in both standard assays and in growing populations i.e. high pheromone concentration in the standard dauer larvae assay leads to more dauer larvae formation, and high population size, denoting a higher pheromone concentration, leads to more dauer larvae formation in a growing population. These data also suggest that dauer pheromone *per se* is not used as a cue to alter reproduction in *C. elegans* populations at the densities observed here. These analyses of food availability, temperature and pheromone support the view that density dependent effects on reproduction are a consequence of caloric restriction rather than adaptive changes to the life history (Harvey *et al.*, 2008).

Comparison of population size and of dauer larvae formation on 2D and 3D environments indicated that there are differences in population growth rate, in the population size and the number of dauer larvae at food exhaustion. The population size and the number of dauer larvae are both significantly higher in a 3D environment (Fig 2.3). These data suggest, as would be expected given the sensitivity of the *C. elegans* life-history to its environment, that the reproductive schedule, i.e. time to reach adulthood and the onset of reproduction, is affected by the difference between the two environments (Fig 2.4). Lifetime fecundity is not different (Fig 2.5A) though early fecundity between the two environments is slightly different in N2 (Fig 2.5B), which alongside the day-to-day population growth, suggests that under these conditions, and that dauer larvae numbers are also increased, either access to food signals is reduced, or access to dauer pheromone is increased.

In their natural habitat, growing populations of *C. elegans* are likely to be initiated by more than one dauer larvae. This is likely to be the case from the prevalence of multi-species patches, such as the mixed populations of *C. elegans* and *C. briggsae* sampled in France (Félix and Duveau, 2012) and a number of species sampled from mixed populations in tropical rainforests (Félix *et al.,* 2013). This is likely to be the case as mixed populations of *C. elegans* and *C. briggsae* are routinely observed (Felix and Duveau, 2012) . Investigating starting populations with differing numbers of worms shows that the population size increases greatly from one to two founding worms with a lesser increase between two and twenty founding worms (Fig 2.6A). A difference in the number of dauer larvae formed is not seen until a population is started with ten worms (Fig 2.6B). As a new population started with a relatively large number of founder worms will grow at a greater rate, pheromone levels would increase faster and food levels would decrease much faster, more worms would subsequently develop into dauer larvae rather than reproducing adults, explaining why the population size does not carry on increasing significantly. It is interesting that the number of dauer larvae formed does not begin showing a difference until a much larger number of worms are used to start a population, suggesting that there is an important balance between the presence of food and pheromone levels.

After patch exhaustion, the population size continued to increase up until the third day (Fig 2.7A). This will be a consequence of adult worms laying eggs that were formed with resources from both the initial food patch and from the small amounts of bacteria likely to remain on the plates. The number of dauer larvae present increases after the food patch is exhausted, *i.e.*, worms that had not completed development as a dauer larvae when the food was exhausted continue development in the absence of bacterial food (Fig 2.7B). This indicates that worms are either eating the carcasses of dead worms or, in the case of worms

that have nearly completed development as dauer larvae, using internal energy reserves to complete development. It has previously been observed that starved adult hermaphrodites retain fertilised eggs within the body and that larvae hatching from these eggs can, by consuming their mothers, develop as dauer larvae (Chen and Caswell-Chen, 2004), and this is likely to be the origin of some of the dauer larvae observed in this experiment. However, given that the dauer larvae observed here will be third, and some fourth generation worms which continue into dauer larvae later, only a limited number of adult hermaphrodites (the 250-350 progeny produced by the founding worm, and a few of their first born progeny) will be present. It is therefore unlikely that this number of adults would be sufficient to explain the numbers of dauer larvae observed. The bulk of the energy required is likely therefore to have come from other third generation worms or from internal reserves obtained prior to patch exhaustion.

For *C. elegans*, the ability of dauer larvae to colonise a new resource patch efficiently and effectively is likely to be very important, and therefore the fitness of dauer larvae will be crucial. Here it has been shown that dauer larvae that form in the presence of food recover faster and have higher lifetime fecundity than those that complete development after the exhaustion of the bacterial food (Fig. 2.8). In conjunction with the observation that dauer larvae do not form before food exhaustion at low food levels, this suggests that low food patches contribute only low fitness dauer larvae to worm metapopulations. Such patches are therefore likely to be of only limited importance to worm metapopulation persistence. In contrast, for a *Daphnia magna* metapopulation, it was found that small ephemeral habitats contribute more to the production of the dispersal stage (Altermatt and Ebert, 2010), contrary to the standard main-land island model, in which migrants from a few large populations (low risk of extinction) drive smaller island populations (high risk of extinction)

(Hanski and Gilpin, 1991). This also strongly suggests that the variation observed between isolates of *C. elegans* in their sensitivity to dauer larvae-inducing conditions (Viney *et al.*, 2003; Harvey *et al.*, 2008) is likely to represent adaptation to differing quantities or qualities of food. These reproductive differences of course represent just one potential aspect of dauer larvae fitness and it would be interesting to determine if dauer larvae formed under different conditions vary in their longevity, survival and behaviour. Given that prolonged arrest as a dauer larva has serious effects on subsequent development and reproduction (Kim and Paik, 2008), a comparison of survival after a greater period of larval arrest would be particularly interesting.

The population size and number of dauer larvae formed in growing populations of mutant isolates identifies variation in dauer larvae formation as a consequence of another phenotype (Fig 2.9). The sensory neurons where the *odr-3* gene is expressed have been shown to affect dauer larvae formation (Bargmann and Horvitz, 1991) and to increase dauer larvae formation in standard dauer assays (Alcedo and Kenyon, 2004). However, in a growing population, a mutation in *odr-3* showed no difference to N2 in dauer larvae formation. This could be due to the nature of pheromone increase in a growing population, with sensory neurons acting in parallel in detecting environmental stimuli; pheromone and food cues, allowing for normal chemosensation *che-1*, used for olfaction of bacterial by-products (Bargmann *et al.,* 1993; Uchida *et al.,* 2003), in a standard dauer larvae assay, formed more dauer larvae than N2 (Uchida *et al.,* 2003) due to its ability to perturb dauer-inhibiting functions (Reiner et al., 2008). A *che-1* deficient mutant in this growing population assay formed fewer dauer larvae when compared to N2, which verifies that *che-1* has a strong effect on dauer larvae formation, shown in both standard and growing population assays (Uchida *et al.,* 2003). The *daf-16* mutant is expected to show no dauer larvae formed, in

standard dauer larvae assays, as it results in dauer-formation deficient worms (Gottlieb and Ruvken, 1994). However, very low numbers of larvae were found in this growing population assay, although still significantly fewer than N2. These larvae (3 worms found on only 2 out of 8 replicates) survived the sodium dodecyl sulfate (SDS) treatment, an anionic detergent, which is the standard treatment for killing all stages but dauer larvae (Cassada and Russell, 1975; Stiernagle, 2006). Line contamination (i.e. mislabelling of plates or using the wrong isolates) is unlikely as no other lines used in the assay formed as few dauer larvae as the *daf-16* mutant isolate. Survival of worms by hatching inside a hermaphrodite is also unlikely, as the life-stage of the worms at counting (recovered L4/adult) would have broken out of its mother before that point, and it is likely there would be considerably more worms on the plate. Gottlieb and Ruvken (1994) noted that small numbers *daf-16* mutant worms, matching wild-type dauer larvae in every way except for the sealed mouth-parts, survived the SDS treatment, which could account for the very low numbers of surviving worms in the growing population assay. The two *eat-2* mutants, with abnormal pharyngeal pumping, resulted in two different phenotypes for dauer larvae formation, one showing significantly more dauer larvae than N2, whilst the other was very similar to N2 (Fig 2.9). Boyd *et al.,* (2007), showed these two mutations in *eat-2* resulted in similar feeding phenotypes, which could suggest that the polymorphisms between these two mutants also affect dauer larvae development in different ways. A lack of maternal and zygotic *age-1* promotes dauer larvae development (Morris *et al.,* 1996; Paradis *et al.,* 1999), however, in this population growth assay there was no difference from N2, suggesting that the mutant was either still able to express the gene, or the style of the assay contradicts the negative effect from the loss of function in this gene.

These data show that population level analyses of *C. elegans* are possible. Given the large number of available mutant and wild-type isolates and extensive genetic and genomic resources this therefore represents a tractable system in which to look at population dynamics, dispersal and the relationship between dispersal and other life-history traits.

CHAPTER THREE: Genetic mapping of variation in dauer larvae development in growing populations of *Caenorhabditis elegans*

Work reported in this chapter has been published as:

Green J.W.M., Snoek L.B., Kammenga J.E. and Harvey S.C. (2013). Genetic mapping of variation in dauer larvae development in growing populations of *Caenorhabditis elegans.* *Heredity* **111**: 306-313

Sequential pairwise analysis (Shao *et al*., 2010), bin mapping and IL mapping were undertaken by L.B. Snoek. These analyses are shown in Figures 3, 4 and appendix figure 2, and also contribute to the QTLs shown in Table 1.

SUMMARY

The appropriate induction of dauer larvae development within growing populations is likely to be a primary determinant of genotypic fitness, however, the underlying genetic architecture of natural genetic variation in dauer formation has not been thoroughly investigated. Quantitative trait loci (QTL) mapping represents one way in which the underlying genetics of complex traits can be investigated. Here, using methods developed for the analysis of growing populations, extensive natural genetic variation in dauer larvae development within growing populations across multiple wild isolates is identified. The genetic basis of this variation was examined using introgression lines (ILs) derived from the genetically divergent isolates N2 and CB4856. Analysis of these ILs reveals 10 QTLs affecting dauer formation. Comparison of individual ILs to N2 identifies an additional 8 QTL and sequential IL analysis reveals 6 more QTLs. These findings illustrate the complex genetic architecture of variation in dauer larvae formation in *C. elegans* and may help to understand how the control of variation in dauer larvae development has evolved. Most of the identified QTLs were validated through multiple tests of individual genotypes, and further analysis of the QTLs show that they are variable across environments.

INTRODUCTION

Determining how complex traits are genetically controlled is a requirement if we are to predict how they evolve and how they might respond to selection. This involves an understanding of how life-history traits interact and change with environmental conditions. By combining phenotypic and genotypic data, quantitative trait loci (QTL) analysis can be used to explain variation in complex traits; however, the genetic architecture of complex traits remains contentious. While it is clear that QTLs and the underlying quantitative trait nucleotides (QTNs) can be detected (Kammenga *et al*., 2007; Stern and Orgogoza, 2008; Terpstra *et al*., 2010), it is unclear to what extent current approaches are identifying ecologically and evolutionarily relevant variation (Kammenga *et al*., 2008; Mackay *et al*., 2009; Rockman, 2012).

Environmental effects contribute to the phenotypic expression of many complex traits. There are many quantitative trait studies showing differences between organisms in the same environments (Nuzhdin *et al.,* 1997; Curtsinger and Khazaeli, 2002; Rockman *et al.,* 2010), but comparatively fewer investigating differences between organisms under different environments (LeDeaux *et al.,* 2006; Boer *et al.*, 2007; Li *et al.,* 2006). QTL by environmental effects are likely to have an effect on QTLs found in many studies, with even small environmental variation effecting the expression of QTLs.

Previously, the limited number of QTL studies investigating dauer larvae development in *C. elegans* have been performed by means of the standard dauer larvae assay, using single cohorts of age-matched worms with concentrated dauer pheromone and high temperatures to promote dauer larvae development (Viney *et al.,* 2003; Harvey *et al.,* 2009). In the investigation by Viney *et al.,* 2003, 40 RILs were analysed for dauer larvae formation in response to pheromone concentration, with a total of three QTLs for the trait discovered.

Harvey *et al.,* 2009 investigated dauer larvae formation in response to different food concentrations in a set of 153 RILs, with a total of six QTLs discovered. When comparing to the number of genes which effect dauer larvae identified through mutagenesis screens (appendix table) these two QTL studies on dauer larvae formations identified comparatively fewer QTL regions than might be expected of such a complex trait.

As methods to allow the analysis of dauer larvae development in growing populations have now been validated (Chapter Two; Green and Harvey, 2012), variation in dauer larvae formation in growing populations was investigated. These conditions which, due to the dynamic nature of the food and pheromone levels in such assays, will more closely approximate the natural conditions experienced by the species, whose natural habitat has only recently been confirmed as rotting fruit and plant materials (Kiontke *et al*., 2011; Felix and Duveau, 2012). Under such conditions, populations will grow to large sizes and the principle cues for dauer larvae development, pheromone level and food availability will change as a consequence of the growth and consumption of bacterial food, and of worm population growth and pheromone production. To investigate natural variation in population size and the number of dauer larvae formed in growing populations, 20 recently isolated wild isolates were compared to N2.

To investigate the genetic architecture of natural variation in dauer larvae formation, a panel of introgression lines (ILs) derived from the genetically divergent strains CB4856 and N2 (Doroszuk *et al*., 2009) was also analysed. The genome of an IL is composed of a recipient genome contributed by one of the parental strains (Bristol N2) and a short, homozygous segment of the donor genome contributed by CB4856; introgression lines (ILs) rather than the Recombinant Inbred Lines (RILs) were used for their evenly spaced and sized specific introgressed fragments. Also, as the ILs were separated by chromosome the assays could be

done as single chromosome batches allowing variation between experimental blocks to be controlled. To identify genomic regions affecting variation between lines, a straightforward bin mapping approach was conducted, followed by comparisons to a common reference (N2), and then a sequential pairwise analysis (Shao *et al*., 2010).

MATERIALS AND METHODS

NEMATODES

N2 were obtained from the *Caenorhabditis* Genetics Centre. Wild isolates were obtained from Marie-Anne Félix (IBENS). The CB4856/N2 ILs used are described in Doroszuk *et al*., (2009) and were derived from RILs obtained from crosses between CB4856 and N2 (Li *et al*., 2006; Kammenga *et al.*, 2007 and 2008; Li *et al*., 2010; Viñuela *et al*., 2010; Elvin *et al*., 2011; Viñuela *et al*., 2012 and Rodriquez *et al*., 2012). RILs were initially constructed by mating one hermaphrodite N2 and 5 CB4856 males, with progeny then separated for line construction and inbred by allowing single worms to self-fertilise for 20 generations, to remove heterozygosity (Li *et al.,* 2006). ILs were constructed by mating RIL hermaphrodites with N2 males and backcrossing for 2-5 rounds depending on the size of the CB4856 introgression (Doroszuk *et al*., 2009). After this, each line was inbred by allowing worms to self-fertilise for 10 generations (Doroszuk *et al*., 2009). Isolates were maintained using standard methods on NGM plates (Stiernagle, 1999), with the OP50 strain of *Escherichia coli* as a food source. All assays were performed at 20°C and were initiated with fourth larval stage worms (L4s) grown from synchronised, arrested, L1s. Within each experiment plates were blind coded and treatments (genotypes) were randomised, with plates that became contaminated or on which the population had failed to grow discarded.

ASSAYS

Population assays were performed as described in Chapter Two (Green and Harvey, 2012). For the analyses of the wild isolates and of the ILs, populations were initiated with 100µl of a 20% w/v suspension of *E. coli* in water and monitored daily until food exhaustion, when the population size and the number of dauer larvae were determined. Wild isolates were

analysed in two experimental blocks with an N2 control in each block and 10 plates per isolate. Differences between the wild isolates and N2 were tested by One-way ANOVA for population size and Kruskal-Wallis for the number of dauer larvae and percentage of dauer larvae.

The CB4856/N2 ILs were analysed in six experimental blocks, one per chromosome. All ILs from that chromosome and both CB4856 and N2 controls were analysed in each block and between 10 and 15 plates initiated per isolate (this varied depending on the number of ILs per chromosome and was required to limit the total assay size).

MAPPING

For all mapping procedures, the chromosomes were treated as separate experiments. This can be done because all the ILs for one chromosome and corresponding N2 and CB4856 controls are measured within one experiment. Thresholds were determined using the statistical and graphics package R, and can be found in Appendix Table 3. The chromosome-wide significance threshold was determined by 1000 permutations by randomly distributing the phenotypic values over the ILs and N2. In each permutation run, the maximum of each chromosome-wide permutation profile was taken. These 1000 maximum values were ordered and the 950th value used as the 0.05 false discovery rate (FDR) threshold to control the expected proportion of false discoveries. This design maximises our ability to detect variation between ILs as comparisons are made between ILs within a chromosome, and between individual ILs and the N2 controls for that block. However, as ILs on the same chromosome were assayed together, linkage is conflated with growth condition variation and other potential block effects. This approach therefore limits the value of estimates of

QTL effect size and means that comparisons of effect sizes between QTLs on different chromosomes should be done with caution.

Bin mapping

Data from the ILs were analysed by bin mapping using a linear model as described by Doroszuk *et al*., (2009). For example, for marker X, the individual phenotypic scores of N2 and the ILs containing an introgression on marker X were taken and randomly re-distributed. In this way the genetic structure of the IL population was kept intact. For all analyses, outliers per genotype (outside the mean +/- 2SD) were removed per group prior to testing. No genotypes were removed completely and the maximum number of outliers per genotype was 2 (out of *c.* 15).

Single IL mapping

Genomic regions affecting variation in population size, the total number of dauer larvae and the percentage of dauer larvae at food exhaustion were also mapped by comparing each introgression line against N2 and testing for a significant difference by a two sided t-test, assuming unequal variance. The *p*-values were Bonferroni adjusted to correct for multiple testing. The ILs with Bonferroni corrected *p*-values ≤ 0.05 were determined to be significantly different from N2. QTLs were then defined by comparing the results of overlapping and bordering introgression lines as is standard for the mapping of QTLs by introgression lines. In addition, all introgression lines per chromosome where tested for significant differences. Groups were made when lines were not less statistically different ($p > 0.01$). In this way the genomic limits the various QTLs were placed. Closely linked QTLs were

tested by a linear model in which the phenotypic scores of all the ILs involved were explained by the two QTL.

Sequential IL analysis

This method was proposed by Shao *et al.,* (2010) and adjusted to the population used in this study. Introgression lines which share one break-point were selected and used in sequential (two by two) analyses to identify QTLs. A two sided t-test, assuming unequal variance, was used to determine significance, with a Bonferroni adjustment of the *p*-values used to correct for multiple testing. The pairs of ILs with genome-wide Bonferroni-corrected *p*-values ≤ 0.05 were considered to be significantly different and to indicate the presence of a QTL.

QTL CONFIRMATION

The ILs on the *X* chromosome were re-tested again to evaluate at the robustness of detected QTLs. Following this, two genome wide repeats of ILs containing QTL regions were undertaken to investigate the reproducibility of QTL detection. The ILs were chosen to include a variety of positive effect and negative effect dauer larvae QTL. These data were analysed by One-way ANOVA with Fisher's *post hoc* for population size and Kruskal-Wallis with a Mann-Whitney *post hoc* test for dauer larvae for a difference from N2.

ENVIRONMENTAL SPECIFICITY – FOOD CURVE

For the analyses of the ILs and their parental lines for environmental specificity, populations were initiated with 100µl of 10%, 15%, 20%, 25%, 30%, 35% or 40% w/v suspensions of *E. coli* in water and monitored daily until food exhaustion, when the population size and the

number of dauer larvae were determined. Population size data were analysed for genotype and food concentration effects by parametric One-way ANOVA, with Fisher's *post hoc* test for individual food concentration analysis, with dauer larvae data were analysed using the Kruskal-Wallis nonparametric test and individual concentration analysis using a Mann-Whitney *post hoc* test. Lines used were the parental N2 and CB4856, and ewIR2, ewIR72 (representing two positive effect QTLs) and ewIR89 (representing the negative effect QTL). Two isolates with positive effect QTLs are used as these prove to be consistently harder to identify.

RESULTS

COMPARISON OF WILD ISOLATES

Testing the N2 controls of these two batches showed that they were not different ($F_{1,17}$ = 4.37, p = 0.052 and $F_{1,17}$ = 0.05, p = 0.82, for population size and dauer larvae formation respectively). The two batches were therefore grouped and investigated as a combined set.

The population size and the number of dauer larvae at food exhaustion were determined for twenty wild isolates and N2. These data indicate that many of the wild isolates have a significantly lower population size than N2, ($F_{20,179}$ = 2.70, p < 0.001) (Fig 3.1A). In contrast, the observed pattern for the number of dauer larvae is reversed, with more isolates showing higher numbers than N2 (H = 83.06, df = 20, p < 0.001) (Fig 3.1B). Across the wild isolates, there was no indication that the number of dauer larvae was correlated with the population size (Pearson product-moment correlation: r = 0.08, p = 0.73), a situation that would be expected if variation in dauer larvae development was purely a consequence of variation in traits affecting population growth rates. This conclusion was supported by the observed pattern of variation in the percentage of dauer larvae, that is, the percentage of the population being dauer larvae (Fig 3.1C). These data were also used to estimate the heritability of the analysed traits. This was calculated by taking the adjusted sum of squares of the components in an ANOVA, with genotype and batch fitted as factors. The adjusted sum of squares of the genotype term was then used as the among genotype variance, which was divided by the total variance to obtain the heritability. Estimates of heritability indicate a higher heritability for the dauer larvae development traits (0.86 and 0.71 for the total number of dauer larvae and the percentage of dauer larvae, respectively) than for population size at food exhaustion (0.38). These analyses demonstrate that the differences between the wild isolates have a genetic basis. Data from Andersen *et al*., (2012) identifies

four of these wild isolates, JU1401, JU1409, JU1410 and JU1411 as belonging to a clonal set, defined as having fewer single nucleotide polymorphisms between isolates than expected given the false positive rate in sequence data from 8% of the genome. Here, our analyses identify no differences between these lines in population size, but indicate that the number of dauer larvae at food exhaustion is higher than in N2 for JU1409, JU1410 and JU1411, but not for JU1401 (Fig 3.1B).

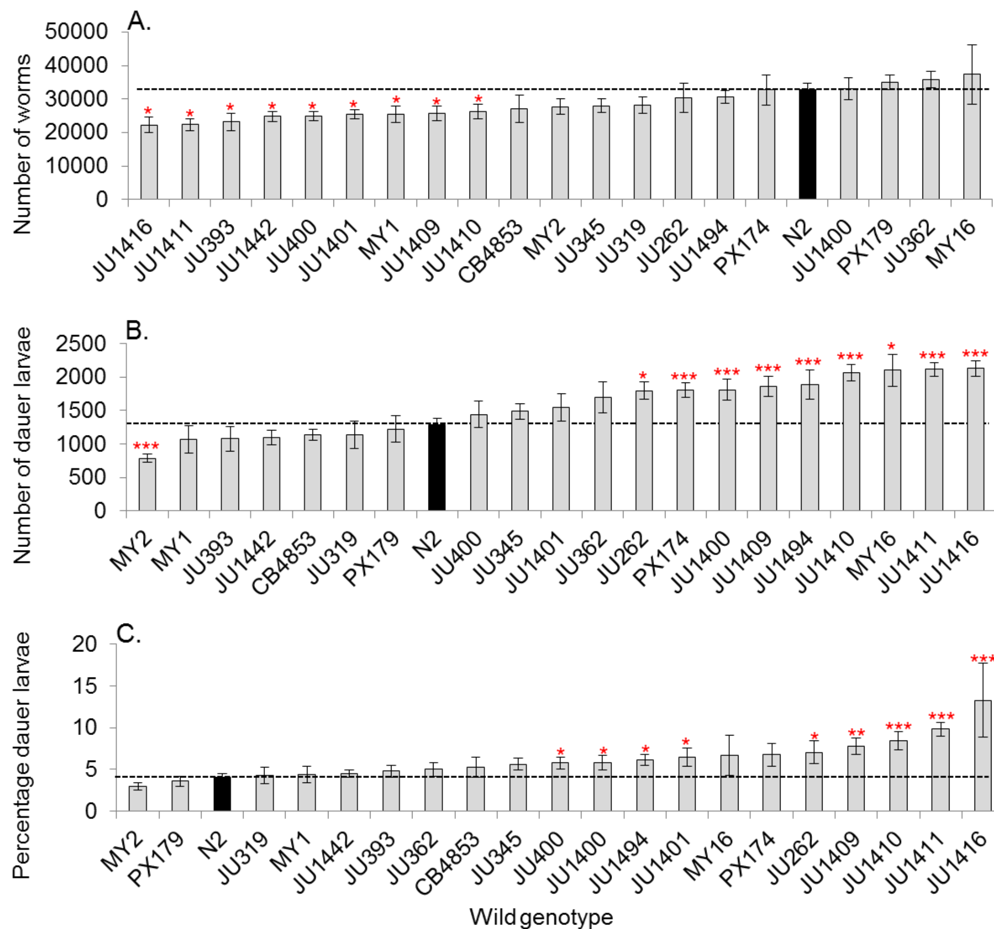

Figure 3.1: Natural variation in growing populations. The population size (A), the number of dauer larvae (B) and the percentage dauer larvae (C) at food exhaustion for N2 and 20 wild isolates. Dotted black horizontal lines show mean N2 values. Error bars indicate standard errors. Significance is shown by the asterisks on the *x*-axis (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Mann-Whitney U-test), denoting isolates that differ from N2.

A possible reason for the amount of variation seen in the *C. elegans* wild isolates (Fig 3.1) could be due to a natural adaptation within their niche environments, i.e. each isolate has an enhanced sensitivity to their own pheromone (Diaz *et al.,* 2014). Another possibility is environmental specific local adaptation, i.e. adaptation to different environmental temperatures which result in different developmental timings. These wild isolates were sampled from very different environments (Appendix Table 2), and as such, competition for ephemeral food sources may have changed some of their life-history traits; developmental timing, fecundity and lifespan. These changes in life-history traits may then result in a lower fitness when grown under laboratory conditions when compared to N2, which is well adapted to the laboratory environment. It was shown that N2 has a higher population size, but forms fewer dauer larvae than most of the wild isolates, which suggests that either the artificial laboratory conditions were not as favourable for most of the wild isolates or they have a natural adaptation for producing a higher ratio of dauer larvae to population size (Fig 3.1).

IL ANALYSIS

Population size and the number of dauer larvae present at food exhaustion were determined for 85 ILs, representing >96% of the CB4856 genome introgressed into the N2 genome (Doroszuk *et al*., 2009). Experimental blocks were examined by chromosome (Appendix Fig 3). Comparison of the ILs showed little overall variation in population size (Fig 3.2A), with 6/85 ILs having a significantly lower population size, and 3/85 ILs having a significantly larger population size than N2 (comparisons are within blocks). In contrast, comparison of the number of dauer larvae showed a large amount of variation (Fig 3.2B),

with 35/85 ILs having significantly lower and 6/85 ILs had significantly higher numbers of dauer larvae than the N2 (comparisons are within blocks).

Bin mapping

Bin mapping identified 10 dauer larvae formation QTLs on four chromosomes (Fig 3.3A), eight where the CB4856 allele decreases the number of dauer larvae and two that increase the number of dauer larvae (Table 3.1). So, even though CB4856 hardly forms any dauer larvae under these circumstances, the isolate still contains alleles that, in an N2 genetic background, have a positive effect on dauer larvae formation. Bin mapping of the population size in the ILs did not identify any QTLs in earlier analyses, while mapping of percentage dauer larvae identified six QTLs, five of which co-localise with QTLs identified in the mapping of the number of dauer larvae (Appendix Fig 1).

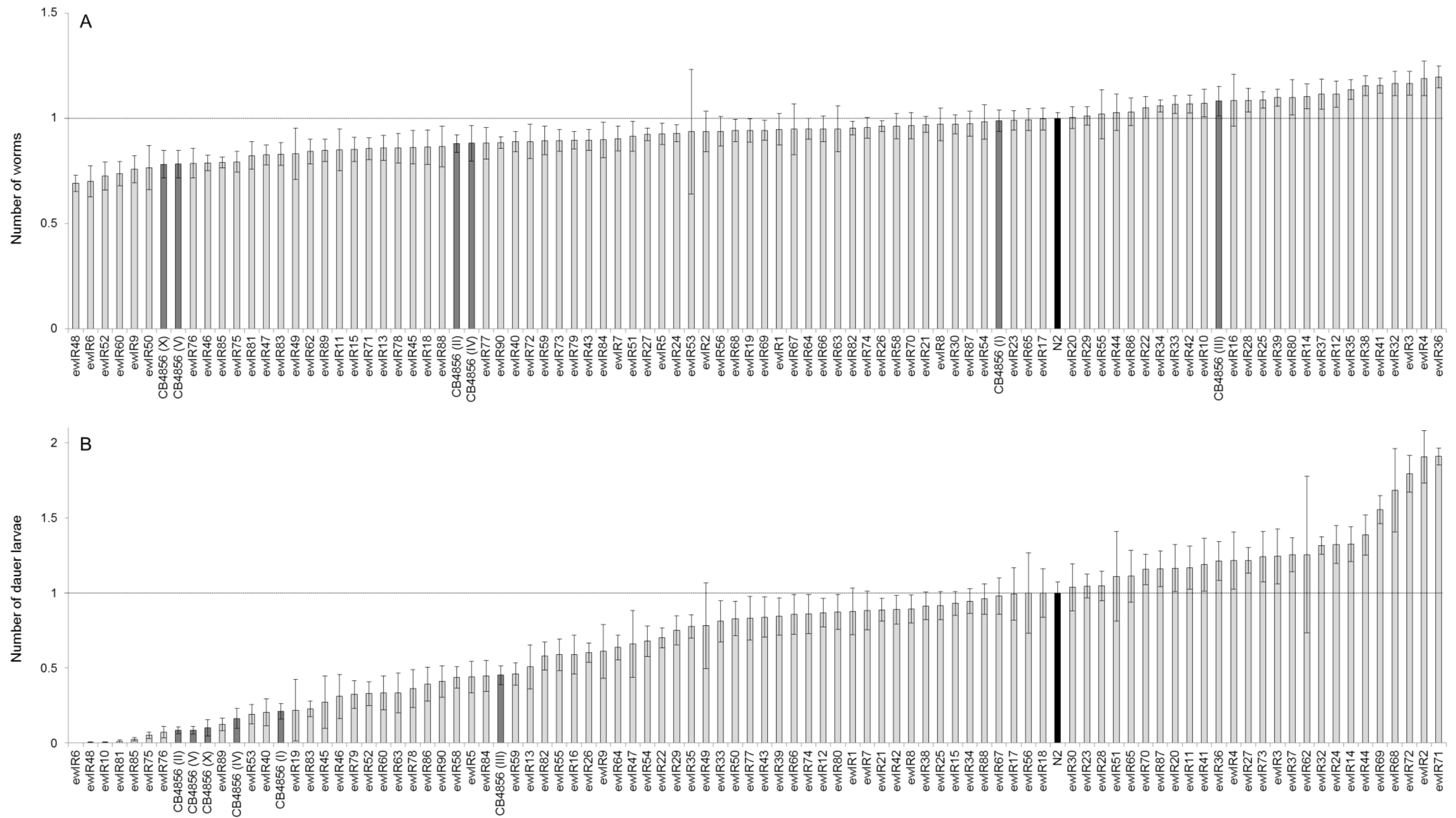

Figure 3.2: Representation the variation in population size (A) and in the number of dauer larvae (B) at food exhaustion for the ILs. Values for all ILs and for CB4856 are scaled to N2 from the block. Dotted black horizontal lines show N2 values for visual reference. Error bars indicate standard errors. Parental lines N2 and CB4856 are shown in bold. (Black and grey, respectively)

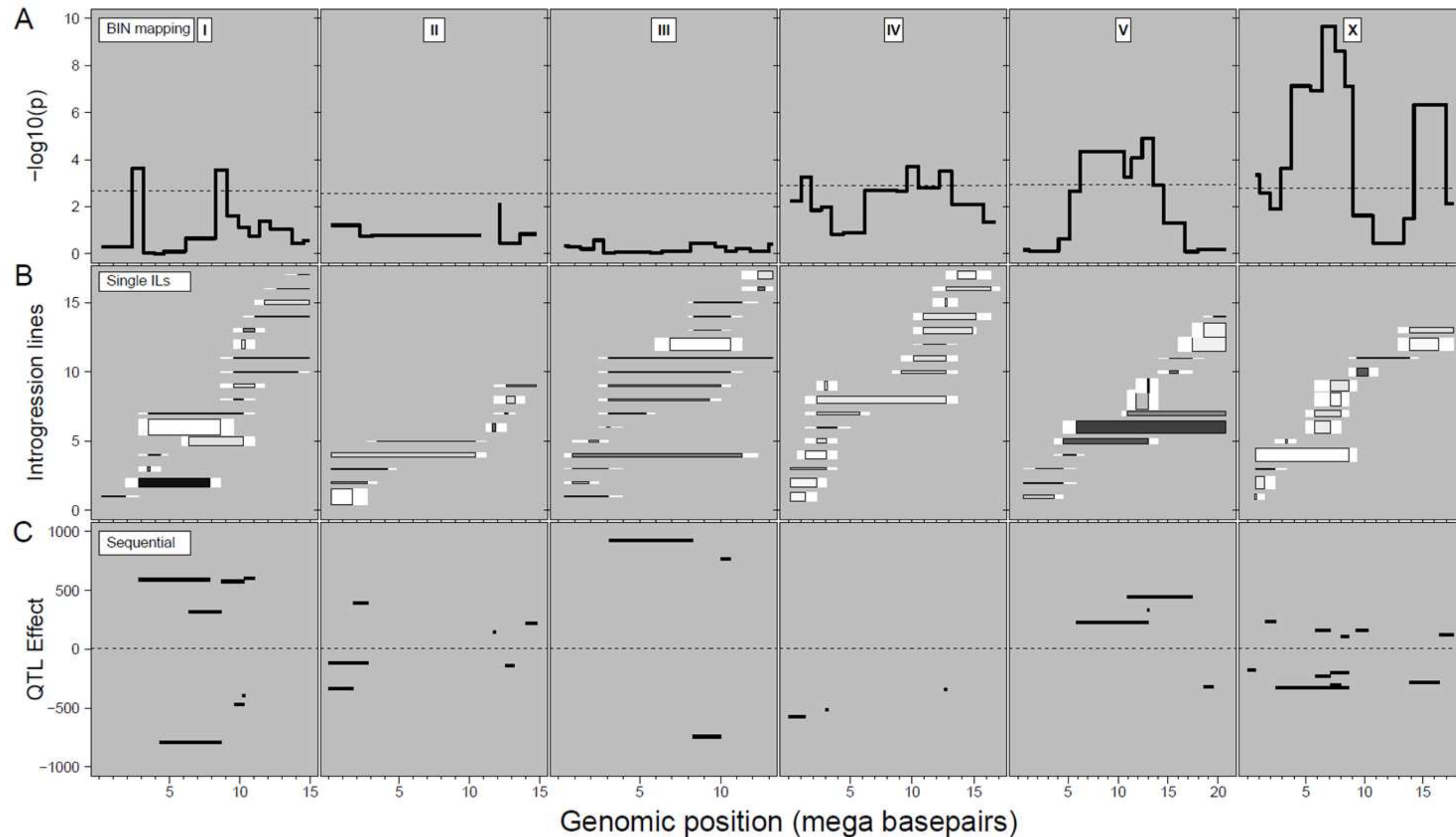

Figure 3.3: Three strategies for mapping QTLs affecting dauer larvae development.

Chromosomes are indicated by the Roman number at the top of the panels. A) Bin mapping, with chromosome specific threshold (FDR=0.05) indicated by the horizontal line and significance (-log10 (*p*)) per marker is shown in black. B) Single ILs analysis, with ILs shown as horizontal bars. The length indicates the introgression size, the shading indicates the CB4856 allelic effect (darker than background is positive and lighter than background is negative) and the width indicate the significance. C) Sequential IL analysis, with QTLs shown by the black horizontal bars. The y-axis shows the effect of the CB4856 allele.

Single IL mapping

Analysis of individual ILs versus N2 indicated that bin mapping underestimates the number of loci affecting variation in all traits, identifying a further eight QTLs affecting the number of dauer larvae (Fig 3.3B and Table 3.1). It is particularly noteworthy that several of the QTL detected individually switch the phenotype from one parental form to another. Similar analyses identify four QTLs affecting population size and an additional 10 QTLs affecting the percentage of dauer larvae (Table 3.1). QTLs affecting the percentage of dauer larvae predominantly co-localise with QTLs identified in the mapping of the number of dauer larvae (Table 3.1). It should be mentioned here that comparison to a common reference N2 does not attempt to predict trait architecture, but merely to localize QTL that act in the same direction as the parental difference, i.e. N2 forming high and CB4856 forming low, numbers of dauer larvae. Sequential IL analysis of the number of dauer larvae identifies, at a genome-wide significance threshold, 20 QTLs (Fig 3.3C). The bulk of these QTLs (14/20) match those identified by the other methods, but this approach does identify 6 additional QTLs (Table 3.1). The QTLs found only by this approach are all ones where the CB4856 allele increases the number of dauer larvae, as would be expected given the nature of the comparisons made in this analysis. In total, these analyses suggest the presence of a minimum of 24 QTLs affecting dauer larvae development in growing populations (Table 3.1).

Chr	QTL	Limits	Effect	Sequential IL mapping	IL mapping	Bin mapping	Other traits
I	1	1.9-3.5	+	1.9-8.7, (N2,02)	1.9-3.5, (02)	X	+ % dauer
	2	4.3-9.6	-	4.3-9.6, (04,06)	7.9-9.6, (05,06)	X	- % dauer
	3	**10.3-11.1**	**+**	**8.7-11.1, (06,07)**			
			+	**10.3-11.8, (13,14)**			
	4	9.6-11.1	-		9.6-11.1, (13)		- % dauer
II	5	0-2.8	-	0-2.8, (N2,19)	0-2.8, (19)		- % dauer
	6	**1.7-3.4**	**+**	**1.7-3.4, (19,20)**			
	7	12.6-14.0	-	12.6-14.0, (25,26)	11.8-14.0, (26)		
	8	11.2-12.6	+		11.2-12.6, (24)		- % dauer
	9	**13.2-17.5**	**+**	**13.2-17.5, (26,27)**			
III	**10**	**2.5-8.3**	**+**	**2.5-8.3, (40,36)**			
	11	8.0-10.6	-	8.0-10.6, (N2,40)	8.0-10.6, (40)		- % dauer
	12	**10.0-11.3**	**+**	**10.0-11.3, (40,41)**			
IV	13	0.8-2.3	-	0-2.3, (N2,45)	0.8-2.3, (45,46,48)	X	- % dauer
	14	2.3-3.9	-	2.3-3.9, (N2,53)	2.3-3.9, (52,53)		- % dauer
	15	9.1-10.9	-		9.1-10.9, (52)	X	
	16	11.7-13.7	-		11.7-13.7, (52,58,59,60)	X	
V	17	11.8-14.0	+	10.4-17.4, (75,70)	11.8-14.0 (69,71,72)	X	+ % dauer
			+	11.8-14.0, (N2,72)			
	18	17.4-19.5	-	17.4-19.5, (77,76)	17.4-19.5, (75,76)		- % dauer
X	19	0-1.5	-		0-1.5, (78,79,81)	X	- pop^{n} size - % dauer
	20	**1.5-3.3**	**+**	**1.5-3.3, (79,80)**			
	21	3.3-5.8	-	2.4-9.3, (80,81)	3.3-5.8, (81)	X	- pop^{n} size
	22	5.8-8.0	-	5.0-8.0, (N2,83)	5.8-8.0, (81,83,85,86)	X	- pop^{n} size - % dauer
			-	5.8-8.7, (N2,85)			
			-	5.8-9.3, (N2,86)			
	23	8.7-11.1	+	8.7-11.1, (N2,87)	8.7-11.1, (87)		
	24	13.9-17.6	-	12.9-17.6, (N2,89)	13.9-17.6, (89,90)	X	- pop^{n} size + % dauer

Table 3.1: Locations and effect of QTLs detected for the number of dauer larvae. Limits denote the maximum possible QTL region. For the Sequential and IL mapping, the limits defined by those analyses and the ILs tested are given. Other traits mapping to these regions are also noted. QTLs defined only by sequential IL mapping are shown in bold. Dotted boxes join co-localising QTLs.

QTL CONFIRMATION

Two approaches were taken in validating the QTLs detected in the genome-wide screen of the ILs. Firstly, a selection of ILs containing positive and negative QTLs for dauer larvae development, from different chromosomes, were analysed. Secondly, all the ILs with an introgression on the *X* chromosome were retested.

Analysis of ILs with candidate QTLs from across the genome showed that there was extensive variation (Fig 3.4 – 1st repeat (A), 2nd repeat (B)). The population size of both repeats are variable, with more ILs differing from N2 than in the original genome-wide analysis ((A): $F_{17,145} = 4.27$, $p < 0.001$; (B): $F_{23,231} = 5.69$, $p < 0.001$). The number of dauer larvae formed also show extensive variation ((A): $H = 93.51$, $df = 17$, $p < 0.001$; (B): $H = 104.61$, $df = 23$, $p < 0.001$), though not as much as the population size when compared to the genome-wide analysis. The majority of ILs to the left of the shaded N2 bar (signifying ILs expected to form fewer dauer larvae than N2) on A and B of figure 5 indicates that most of the negative effect QTLs are reproduced and none are observed to have significantly higher numbers of dauer larvae than N2. In contrast, none of the ILs to the right of the shaded N2 bar (signifying ILs expected to form more dauer larvae than N2) are observed to have significantly higher numbers of dauer larvae than N2, indeed the majority of these ILs formed significantly fewer dauer larvae in the second repeat (Fig 3.4B). These data indicate the difficulty in reproducing positive effect QTLs, though it also shows that the majority of the negative effect QTLs are easily reproducible (Table 3.2). This also highlights the importance of differing batches of bacteria, as the first repeat has a comparatively low population size and high dauer larvae number when compared to the second repeat, which has a much higher population size and fewer dauer larvae.

Analysis of the ILs with introgressions spanning the *X* chromosome identified that the retests for the ILs which cover QTLs 19, 21, 22 and 24, including the data from the QTL repeats in figure 3.4, supported the original genome-wide analysis; 10/12 ILs significantly lower and all the same sign (negative or positive effect QTL, +/-), 4/4 ILs all significantly lower with the same sign, 13/16 ILs significantly lower and all the same sign and 7/7 ILs significantly lower and the same sign (Table 3.2).

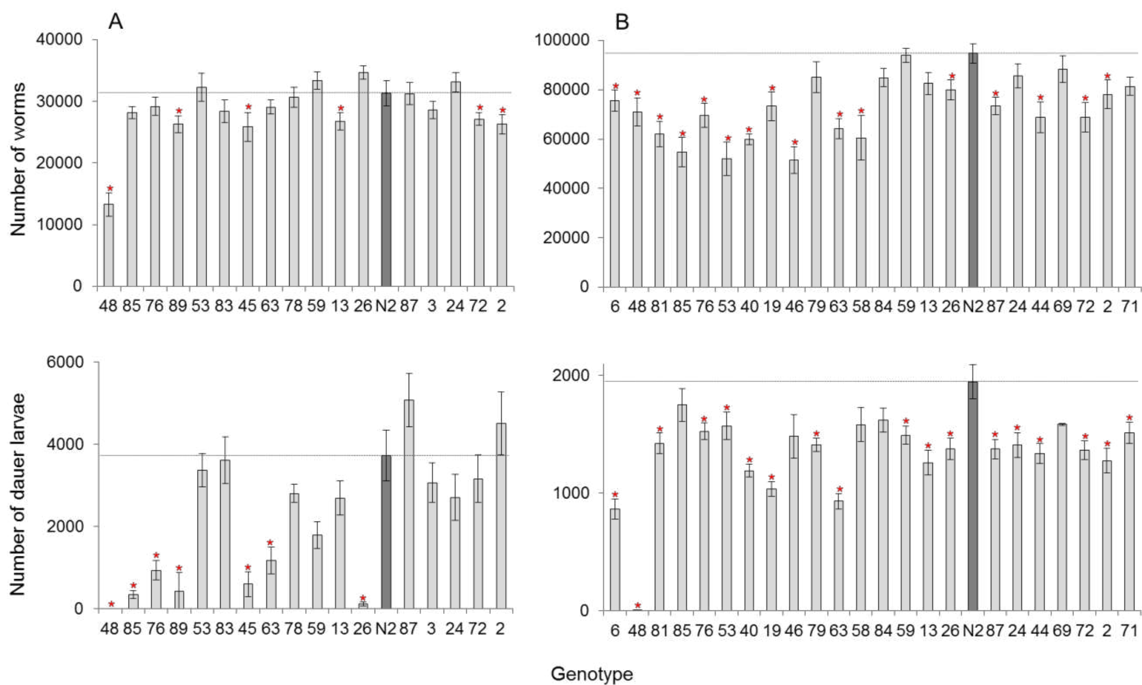

Figure 3.4: QTL region repeats. The mean number of worms and the number of dauer larvae present at food exhaustion for N2 and the ILs containing dauer larvae QTLs. First repeat block (A) and the second repeat block (B). Dotted black horizontal lines show mean N2 values. Error bars indicate standard errors and lines significantly different from N2 are shown by the asterisk (*) (Population size: $p < 0.05$; Fisher's Least Significant Difference. Dauer larvae: $p < 0.05$; Mann-Whitney U-test). Within A and B, the ILs are ordered to represent those that were lower and higher in the number of dauer larvae than N2 in the original assay (those to the left of N2 were lower, those to the right were higher).

Chromosome	QTL	QTL Effect	Line	Genome wide IL screen	X repeat	X repeat	QTL repeat 1	QTL repeat 2	Same sign	Significant in all
I	1	+	2	**1183**			780.6	**-672.4**	N	N
	2	-	5	**-731**						
		-	6	**-1305**				**-1083**	Y	Y
	4	-	13	**-644**			-1036.1	**-688**	Y	N
II	5	-	19	**-532.3**				**-912**	Y	Y
	7	-	26	**-272.0**			**-3613.9**	**-571**	Y	Y
	8	+	24	**218.6**			-1019.4	**-538**	N	N
III	11	-	40	**-1367**				**-757**	Y	Y
IV	13	-	45	**-927.5**			**-3132.1**		Y	Y
		-	46	**-879**				-466	Y	N
		-	48	**-1270**			**-3725**	**-1947**	Y	Y
	14	-	52	**-855**						
		-	53	**-1030.7**			-358.3	**-376.2**	Y	N
	15	-	52	**-855**						
	16	-	52	**-855**						
		-	58	**-718**				-366	Y	N
		-	59	**-688.3**			**-1939.3**	**-456**	Y	Y
		-	60	**-850**						
V	17	+	69	**444.9**				-363	N	N
		+	71	**727.5**				**-434**	N	Y
		+	72	**634.4**			-570	**-584**	N	N
	18	-	75	**-760**						
		-	76	**-743.6**			**-2791.7**	**-422**	Y	Y
X	19	-	78	**-478.6**	**-352.5**	-442.1	-925		Y	N
		-	79	**-508.1**	**-445**	**-1086.8**		**-539.1**	Y	Y
		-	81	**-742.2**	**-623.8**	**-1193.8**		**-525**	Y	Y
	21	-	81	**-742.2**	**-623.8**	**-1193.8**		**-525**	Y	Y
	22	-	81	**-742.2**	**-623.8**	**-1193.8**		**-525**	Y	Y
		-	83	**-581.1**	**-455**	**-882.1**	-118.8		Y	N
		-	85	**-732.1**	**-611.7**	**-1177.8**	**-3386.1**	-198	Y	N
		-	86	**-456.7**	**-402.5**	-156			Y	N
	23	+	87	120	**326.3**	-200	1343.8	**-573.6**	N	N
	24	-	89	**-658.1**	**-556.1**	**-355.6**	**-3302.8**		Y	Y
		-	90	**-442.8**	**-316.7**	**-477.8**			Y	Y

Table 3.2: Re-test of the ILs containing QTLs and of the *X* chromosome. Numbers indicate dauer larvae difference from N2, values bold are significantly different (from the same experiment). The QTL effect column shows whether it is a positive or negative QTL. The same sign column shows if the difference is in the same direction in all tests. The significant in both column shows if the difference is significant in all tests.

These data strongly support the negative effect QTLs found on the *X* chromosome as they are replicated in multiple tests of independent genotypes, strengthening the ability of this analysis for identifying negative effect QTLs. QTL 23, a positive effect QTL from the original genome-wide analysis, was not reproduced. This demonstrates the difficulty in reproducing positive effect QTLs. In total, 14/14 negative and 0/4 positive QTLs are supported from the two QTL confirmation approaches (Table 3.2).

ENVIRONMENTAL SPECIFICITY OF POSITIVE EFFECT QTLS

To investigate why the positive effect QTLs were not observed in the QTL retests, the population size and the number of dauer larvae of populations grown on differing amounts of bacterial food were analysed. These data indicate that the amount of food available has a large effect on a population's size (Fig 3.5A). These data also show that the number of dauer larvae produced is considerably more variable, depending on genotype and the amount of food available (Fig 3.5B). Analysis of the number of dauer larvae at food exhaustion for populations grown with different amounts of food showed that at every food concentration the negative effect QTL, ewIR89, was significantly lower than N2. These analyses also show that the positive QTLs were only detectable at the higher food concentrations (Fig 3.5B). These data demonstrate that negative effect QTLs are detectable under a much wider range of conditions than the positive affect QTLs for dauer larvae formation in a growing population, identifying a reason why the positive QTLs are harder to reproduce and confirm in this style of assay.

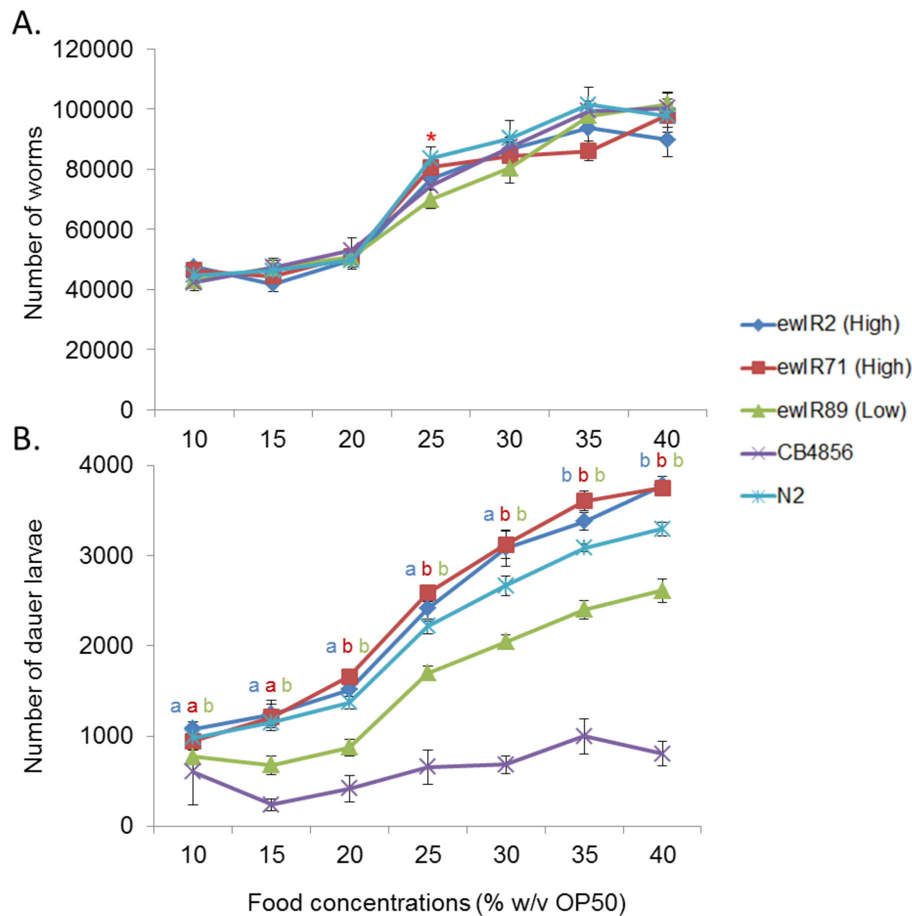

Figure 3.5: Variation due to food availability. The mean population size (A) and number of dauer larvae (B) at food exhaustion for N2 and three ILs; two positive and one negative effect QTLs. CB4856 is shown only as a reference. Error bars indicate standard errors. Asterisk shows the significant difference to genotypes at a food concentration. If the respective ILs (IL 2, 71 then 89) are not different from N2 they are marked with the letter “a”, if the ILs are different from N2 they are marked with the letter “b”. (Population size: $p < 0.05$; Fisher’s Least Significant Difference. Dauer larvae: $p < 0.05$; Mann-Whitney U-test). CB4856 data is plotted on the graph as a representation of how this genotype acts under these conditions, though the analysis is comparing the IL trait to N2 only.

DISCUSSION

Here we have identified the extensive variation between wild isolates in both dauer larvae formation and population size in growing populations of *C. elegans* (Fig 3.1). Interestingly, dauer larvae formation is significantly higher in many of the wild isolates studied than it is in N2. This contrasts with previous observations that, in standard dauer larvae development assays, N2 had higher rates of dauer larvae formation than all tested wild isolates (Viney *et al*., 2003; Harvey *et al*., 2008). This may imply that wild isolates are more sensitive to their own pheromone than they are to N2 pheromone (Viney *et al.,* 2003 and Harvey *et al.,* 2008; Kaplan *et al.,* 2012; Diaz *et al.,* 2014). Alternatively, this may reflect variation between the isolates in aspects of their biology that are not captured by standard dauer larvae development assays. For example, dauer larvae formation in growing populations would be affected by variation between isolates in population growth rates and by differences in rates of pheromone production, traits that would not affect the results of standard dauer larvae development assays. These findings also makes an interesting comparison to the findings of Mayer and Sommer (2011), which showed that 13 out of 16 tested strains of *Pristionchus pacificus* produced a pheromone that induced higher dauer larvae formation in different genotypes. Which suggests that *P. pacificus* is attempting to induce precocious dauer larvae formation in other strains (Mayer and Sommer, 2011). Analogous experiments in *C. elegans* would provide an interesting test of this idea (Kaplan *et al.,* 2012; Diaz *et al.,* 2014).

Three mapping approaches were performed to identify genomic regions affecting variation in dauer larvae development in growing populations. Bin mapping is the standard approach used in many studies (e.g. Howad *et al.,* 2005; Sargent *et al.,* 2008; Huang and Röder, 2011) and has previously been used in these ILs (Doroszuk *et al.,* 2009). Here, bin mapping identified 10 QTLs for dauer larvae development, 8 negative effect (lowering the number of

dauer larvae relative to N2) and 2 positive effect (increasing the number of dauer larvae relative to N2) (Fig 3.3A). Comparison of individual ILs to N2 revealed a total of 18 QTLs, all of which co-localise with those identified by the bin mapping and an additional 8 QTLs (Fig 3.3B). This demonstrates that the bin mapping approach is more conservative, exemplified by chromosomes *II* and *III*, where the bin mapping identified no QTLs, but the individual IL comparison identified 3 QTLs on chromosome *II* and 1 on chromosome *III*. The sequential analysis identified a total of 20 QTL, 14 of which co-localise with the previous two methods, revealing 6 new QTL. This method is therefore again less conservative than the other two, and also detects more positive effect QTLs (9/20). This is due to the nature of the comparisons made, being better suited for congenic strains, with overlapping and increasing introgression size (Shao *et al*., 2010). A number of these QTL were detected in all three methods (7/24), some were detected in at least two of the methods (9/24) and some were detected in only one of the methods (8/24). Mapping the ILs identified a total of 24 QTLs for dauer larvae development (Table 3.1). This represents an unprecedented number of QTLs for a single trait from one mapping panel in *C. elegans*. Previous QTL studies on *C. elegans* revealed 8 QTLs for lifespan (Ayyadevara *et al.,* 2001), 11 QTLs for four life-history traits (age at maturity, fertility, egg size and growth rate) at two temperatures (Gutteling *et al.,* 2007b) and 3 for dauer larvae formation (Viney *et al.,* 2003). The identification of these QTLs in the ILs provides a simplified and tractable approach to further genetic analysis of these loci.

Retesting the QTLs reveals that there is, however, difficulty in reproducing some QTLs due to variation in the numbers of dauer larvae formed at food exhaustion. Figure 3.4 shows the two repeats of ILs containing QTLs for dauer larvae development, and the variation between the two is very clear. Firstly, variation between the two repeats; one with a smaller population size and a relatively large number of dauer larvae, and the other a very large

population size and comparatively few dauer larvae formed. These data suggest a very strong environmental dependant variation. Secondly, variation in the ILs between the QTL region repeat assays and the original genome-wide analysis (Appendix Fig 3) suggest genotype dependant variation; in the first repeat, the positive effect QTLs (right side of N2) are not significantly different from N2, in the second repeat, 6/7 ILs which were originally positive effect QTLs have produced significantly lower numbers of dauer larvae than N2. The ILs containing introgressions on the *X* chromosome were also repeated to test for confirmation. Together, both QTL confirmation analyses revealed that the negative effect QTLs tested were robust and highly reproducible (14/14), however, the positive effect QTLs were not reproducible (0/4) (Table 2). Variation in and lack of reproducibility of the positive QTLs could be due to environment specific effects. Within growing populations, variation between genotypes in the number of dauer larvae could therefore be a consequence of variation in traits that affect the likelihood of dauer larvae development, the perception of the environment, or the way in which the population grows.

Previous studies have shown that environmental change can affect life-history traits in *C. elegans*, such as body mass, egg size (Gutteling *et al*., 2007a and 2007b) and reproductive timing (Hughes *et al*., 2007; Cornils *et al*., 2011). Gutteling *et al.,* (2007a) mapped variation of three life-history traits in *C. elegans* (egg size, egg number and body mass) under two different environments (12°C and 24°C), in which they identified a number of QTL, some overlapping for different traits and temperatures suggesting pleiotropic effects, and also different genetic correlations between the two temperatures. This demonstrates the amount of variation that the environment can have on a complex trait. To test an environmental effect, populations were grown with increasing amounts of available bacterial food. This demonstrated, at certain levels, the amount of food available to a population has

little effect on the population size but has a large effect on dauer larvae development (Fig 3.5), so much so that there is a significant difference seen from the lowest concentrations. The genome-wide and the QTL confirmation assays were performed at 20°C with a 20% w/v suspension of *E. coli* as a food source and at these parameters on the food curve assay, the negative effect and one positive effect QTL can be determined, while the second positive effect QTL is not visible until the highest two food concentrations. These data demonstrate the wider range of conditions at which negative QTLs for dauer larvae formation can be detected and reproduced, and demonstrate one reason why the positive effect QTLs are harder to detect. It also demonstrates that dauer larvae development is strongly effected by environmental specific change. This level of variation could be an adaptation to *survival in a rapidly and unpredictably changing environment (Hoffman, 2009; Zhou et al., 2007).*

One potentially confounding factor is that ILs can reveal the presence of Dobzhansky-Muller incompatibilities; the evolution of hybrid incompatibility. In this case, IL regions displaying Dobzhansky-Muller incompatibilities might reduce population growth rates and hence affect dauer larvae development via effects on pheromone accumulation. This would be manifested as additional QTLs where the CB4856 allele negatively affected both population size and dauer larvae development. Most QTLs do not show such effects, but QTLs displaying these characteristics are detected on the *X* chromosome (Table 3.1) and a large number of negative effect QTLs are also seen on chromosome *IV*.

There is a strong possibility that variation between isolates in dauer larvae formation is a consequence of variation in the chemoreceptors required to sense both pheromone and food signals. As ascaroside signalling is known to be complex, with some receptors showing excessive specificity and others able to respond to a range of ascarosides (Kim *et al*., 2009; McGrath *et al*., 2011; Park *et al*., 2012), variation in receptor sensitivity would allow fine-

tuning of the dauer larvae development decision. Interestingly, QTL 24 on the *X* chromosome co-localises with individual markers identified as being associated with variation in dauer larvae formation between N2 and DR1350 (Harvey *et al*., 2008) and contains *srg-36* and *srg-37*, genes known to encode receptors for ascaroside C3 (McGrath *et al*., 2011), one of the components of *C. elegans* pheromone (Butcher *et al.,* 2009; Zhang *et al.*, 2013).

Currently available isolates of *C. elegans* are characterised by a limited number of large and relatively common shared haplotypes on four chromosomes (Andersen *et al*., 2012), with population genetic modelling suggesting that this pattern of variation is a consequence of recent chromosome-scale selective sweeps (Andersen *et al*., 2012). Given the association between *C. elegans* and rotting fruit (Kiontke *et al*., 2011) it is tempting to speculate that human induced changes in the prevalence and morphology of various fruits may be related to these selective sweeps. If this is the case, then it is possible that dauer larvae development in growing populations is one of the traits under selection. The large number of QTLs identified here also contrasts with the three dauer larvae development QTLs identified by Harvey *et al*., (2008) in an analysis of RILs produced from crosses between N2 and DR1350. Given the genetic isolation of CB4856 from DR1350 (which in a population growth assay has a smaller population size and forms fewer dauer larvae than N2) and N2 it would therefore be very informative to determine if the more complex architecture revealed here between CB4856 and N2 is a consequence of the differences in methodologies (analysis of ILs and of growing populations) or reflects differences due to selective history. To investigate this, in Chapter Four, dauer larvae formation in growing populations will be analysed using two recombinant inbred line panels of *C. elegans* and also one in of *C. briggsae*.

CHAPTER FOUR: Mapping variation in dauer larvae development in growing populations in Caenorhabditis elegans and C. briggsae recombinant inbred lines

SUMMARY

Recombinant inbred lines (RILs) are commonly used for the identification and analysis of QTLs and for candidate gene identification. Here, the population size, the number of dauer larvae and percentage of dauer larvae formed by the point of food exhaustion has been analysed in three RIL panels, two constructed with different *C. elegans* parental isolates and one from *C. briggsae*. These analyses identified variation in all three RIL panels in all traits measured in a growing population. Mapping dauer larvae development identified a total of 6 distinct QTLs; one in the CB4856 x N2 RILs, one in the DR1350 x N2 RILs and four in the HK104 x AF16 RILs. These analyses allow comparison of two genetically divergent isolates of the same species and of two distinct species. The two *C. elegans* RIL panels demonstrated a difference in variation for dauer larvae formation between an isolate which is closely related to (DR1350) and a distantly related to (CB4856) N2. Comparison of dauer larvae development between the two species is difficult to fully analyse due to the lack of chromosomal synteny, however, some similarities between the QTLs are identified. In comparison to the previous analysis of dauer larvae development in a growing population, far fewer QTLs were identified here. This is important as it shows the limited power of RIL analysis compared to that of the IL analysis (in Chapter Three) and again demonstrates the complexity of dauer larvae development in growing populations.

INTRODUCTION

Quantitative trait loci (QTL) mapping is used in a wide variety of organisms to identify genomic regions affecting complex traits, which can later be analysed for the underlying genes. RILs and introgression (ILs) are commonly used for the analysis of QTLs. Significant progress has been made using these analyses to the identification of genes, for example, affecting seed size in *Arabidopsis thaliana* (Joosen *et al*., 2011; Herridge *et al*., 2011; Moore *et al*., 2013), life span in *Drosophila melanogaster* (Nuzhdin *et al*., 2005; Lai *et al*., 2007), *Caenorhabditis elegans* (Ayyadevara *et al*., 2003; Vertino *et al*., 2011) and *Mus musculus* (Lang *et al*., 2010; Leduc *et al*., 2011) and to identify potential disease related genes in humans (Hubner *et al*., 2005; Poot *et al*., 2011). However, Rockman (2012) argues that the progress made with these studies are focussing on large effect alleles, and ignoring the small effect alleles (Rockman, 2012). His example of wing size in *Drosophila*, a model complex trait, shows that large numbers of QTLs can be identified, however, no quantitative trait nucleotides (QTN), the allelic variants that underlie phenotypic variation, have yet been discovered for the trait (Rockman, 2012). Associations between phenotype and genotype can be determined by comparing the QTL regions with those of candidate genes, using marker positions and taking advantage of the bioinformatics programmes available. However, if the genomic regions containing QTLs are mapped to broad intervals the detection of candidate genes is challenging (Zou *et al*., 2005). The difference between RILs and ILs is their genetic makeup; RILs have multiple genomic regions differing between the lines, with many overlapping segments and ILs have distinct genomic regions introgressed into a homozygous background with very little overlap (Keurentjes *et al*., 2007). To increase the accuracy of QTL location and help make the identification of candidate genes easier, the number of inbred lines analysed in RIL panels can be increased, the size of the introgression

in IL panels can be reduced (Keurentjes *et al*., 2007) and the number of genetic markers increased (Beavis, 1998). ILs are usually produced after the QTL analysis of RILs to refine a genomic region for mapping analysis, for this reason they are a more powerful tool for gene identification (Szalma *et al*., 2007). A problem with the difference in how RILs and ILs function is the potential difficulty in detecting epistatic genes and identifying loci that underlie variation in polygenic traits (Kloosterman *et al*., 2010), with the latter a particular issue with IL analysis.

Previously, extensive variation in dauer larvae development in 20 wild isolates has been shown (Chapter Three), and as such requires explanation. Does it represent adaptation to different environments or does it imply that there are multiple ways to maximise fitness within the same environment? Using a panel of ILs produced from the *C. elegans* isolates CB4856 and N2, 24 QTLs for dauer larvae development in growing populations were identified (Chapter Three). To investigate the effect of mapping strategy and to test for variation in different isolate genotypes and in different species, analysis was extended to different nematode RIL panels; two *C. elegans* panels produced from the isolates CB4856 and N2 and DR1350 and N2, and one *C. briggsae* panel produced from AF16 and HK104 isolates. These analyses allow direct comparisons between different RIL panels produced from distinct parental isolates (CB4856 x N2 and DR1350 x N2) and RILs and ILs produced from the same parental isolates (CB4856 x N2 RILs and ILs) analysed for the same dauer larvae development trait. These analyses identify common QTL regions and both genotype and trait specific QTLs. Comparison with the results of analysis of variation in dauer larvae development within growing populations of *C. briggsae* RILs allows a more general picture of the control of variation of dauer larvae development in growing populations. Such an extensive analysis of a complex trait has not been done in nematodes and is useful in assessing the power of using RILs and ILs in the QTL analysis for the same traits, as well as

intra-species and inter-species comparisons of dauer larvae formation in growing populations.

MATERIALS AND METHODS

NEMATODES

N2 was obtained from the *Caenorhabditis* Genetics Centre. The CB4856 x N2 RILs were obtained from Jan Kammenga (Wageningen University) (see Li *et al*., 2006; Kammenga *et al.*, 2007 and 2008; Li *et al*., 2010; Viñuela *et al*., 2010; Elvin *et al*., 2011; Viñuela *et al*., 2012 and Rodriquez *et al*., 2012 for details). The DR1350 x N2 RILs were generated from crosses between N2 hermaphrodites and DR1350 males and reciprocal matings and allowing them to self-fertilise for at least 30 generations, as described in Harvey *et al*., (2008) and were obtained from Mark Viney (University of Bristol). The HK104 x AF16 *C. briggsae* RILs were generated from crosses between males and sperm-depleted hermaphrodites were established in both directions (AF16xHK104; HK104xAF16). From F8–F17, the lines were intentionally inbred by complete selfing using a single virgin (L4 stage) founder hermaphrodite per generation, as described in Ross *et al.,* (2011) and were obtained from Asher Cutter (University of Toronto). Isolates were maintained as previously described in Chapter Two.

ASSAYS

Population assays were performed using the 3D environment technique as described in Chapter Two. For the analyses of RILs, populations were initiated with 100µl of a 20% w/v suspension of *E. coli* in water and monitored daily until food exhaustion, when the population size and the number of dauer larvae were determined as described in Chapter Two. The CB4856 x N2 RILs were analysed in four experimental blocks, the DR1350 x N2 RILs were analysed in two experimental blocks and the HK104 x AF16 RILs were analysed in three experimental blocks. All assays were performed at 20°C and were initiated with fourth larval

stage worms (L4s) grown from synchronised, arrested, L1s. Within each experiment plates were blind coded and treatments (genotypes) were randomised, populations which had failed to grow were discarded.

ANALYSIS

Population sizes were analysed by ANOVA, with *post hoc* testing by Fisher's least significant difference test, as data were normally distributed and the variances between groups were equal. Dauer larvae numbers were not normally distributed and were analysed by Kruskal-Wallis test. As suggested by Dytham (2003), Mann-Whitney *U* tests were used for pairwise testing as the Kruskal-Wallis test lacks a *post hoc* test. All analyses were carried out using Minitab® Statistical Software (Minitab, Coventry, UK). QTL cartographer was used to perform mapping of traits in the each of the RIL panels.

QTL MAPPING

The CB4856 x N2 and the AF16 x HK104 RILs have been genotyped to 121 (Li *et al.,* 2006) and 451 markers (Ross *et al.*, 2011), respectively, with markers distributed across all 6 chromosomes (*I*, *II*, *III*, *IV*, *V* and the *X* chromosome). The DR1350 x N2 RILs have been genotyped to 39 markers (Harvey *et al.,* 2008), with these distributed across 4 chromosomes (*I*, *II*, *III* and the *X* chromosome) and covering approximately half of the genome.

Comparison of the controls (N2 and AF16) between experimental blocks indicated that the number of dauer larvae differed significantly between experimental blocks in each RIL panel; CB4856 x N2 (H = 11.29, df = 3, p = 0.01), DR1350 x N2 (H = 5.31, df = 1, p = 0.02) and AF16 x HK104 (H = 7.22, df = 2, p = 0.03). To investigate the effect of this variation on QTL mapping,

a number of mapping approaches were undertaken in the CB4856 x N2 RILs. Firstly, each experimental block was analysed separately. Secondly, data from all experimental blocks was combined and mapped using (1) the raw dauer larvae numbers, (2) the number of dauer larvae scaled to N2 within the block, (3) the number of dauer larvae scaled to the average number of dauer larvae within that block and (4) the number of dauer larvae within a block normalised so the mean and variance are equal; Mean = 0 and variance = standard deviation of block (mean + fractional SD above the mean x standard deviation). These analyses indicated that, even with the variation between blocks, using the raw numbers was the most appropriate approach and this was therefore used in the analysis of the other RIL panels. QTL mapping was performed using QTL Cartographer (Basten *et al*., 2002), using the Composite Interval Mapping (CIM) approach. The threshold was determined by 1000 permutations which the maximum values were ordered and the 950th value used as the 0.05 FDR threshold.

RESULTS

***C. elegans* CB4856 x N2 RILs**

193 lines were assayed for population size, the number of dauer larvae formed and the percentage of dauer larvae in a growing population. Analysis indicated that the population size at patch exhaustion differed between lines in all four experimental blocks ($F_{49,199}$ = 1.18, p = 0.3; $F_{47,139}$ = 2.03, p = 0.002; $F_{49,159}$ = 1.54, p < 0.001; $F_{49,188}$ = 3.43, p < 0.001: blocks 1-4 respectively)(Fig 4.1). Similarly, the number of dauer larvae at patch exhaustion also differed between lines in all four experimental blocks (H = 170.83, df = 49, p < 0.001; H = 103.51, df = 47, p < 0.001; H = 128.77, df = 49, p < 0.001; H = 152.64, df = 49, p < 0.001: blocks 1-4 respectively), as did the proportion of dauer larvae at patch exhaustion (H = 166.82 df = 49, p < 0.001; H = 94.94 df = 47, p < 0.001; H = 129.37 df = 49, p < 0.001; H = 150.42 df = 49, p < 0.001: blocks 1-4 respectively).

***C. elegans* DR1350 x N2 RILs**

99 lines were assayed for population size, the number of dauer larvae formed and the percentage of dauer larvae in a growing population. Analysis indicated that the population size at patch exhaustion differed between lines in both experimental blocks ($F_{50,236}$ = 2.06, p < 0.001; $F_{49,236}$ = 1.94, p = 0.001: blocks 1-2 respectively)(Fig 4.2). Analysis indicated that the number of dauer larvae at patch exhaustion also differed between lines in both experimental blocks (H = 65.47, d.f = 50, p = 0.07; H = 100.02, df = 49, p < 0.001: blocks 1-2 respectively). Similarly, the proportion of dauer larvae at patch exhaustion differed between lines in both experimental blocks (H = 78.25, df = 50, p = 0.007; H = 101.03, df = 49, p < 0.001: blocks 1-2 respectively).

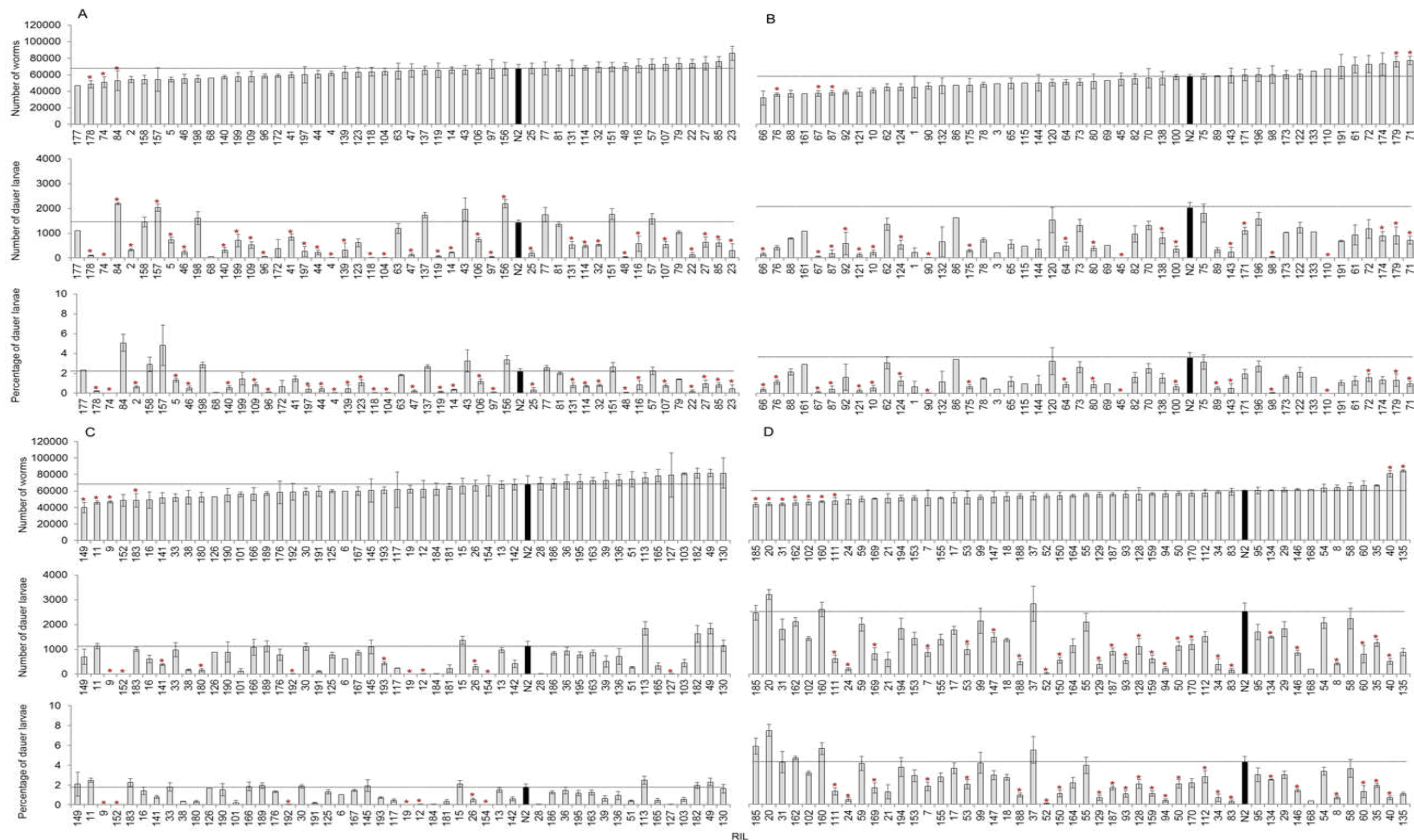

Figure 4.1: CB4856 x N2 RILs vary in population size and dauer larvae formation. A-D blocks 1-4. The mean (±1 SE) (per graph) (Top) population size, (Middle) dauer larvae and (Bottom) percentage dauer larvae present at food exhaustion for populations started with one RIL fourth-stage larva (L4) and 100 µl of 20% (w/v) *E. coli* incubated at 20 °C. Dotted black horizontal lines show mean N2 values. Error bars indicate standard errors. Asterisks (*) denote a significant difference from N2 within the block ($p < 0.05$).

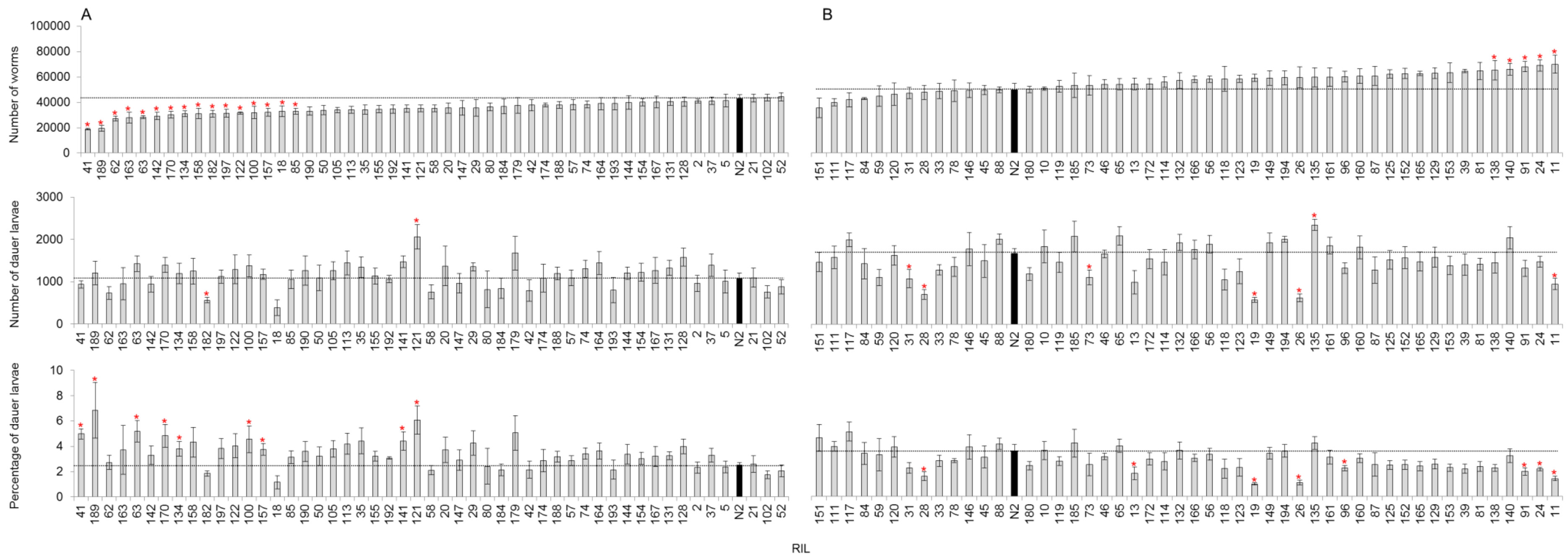

Figure 4.2: DR1350 x N2 RILs vary in population size and dauer larvae formation. A-B blocks 1-2. The mean (±1 SE) (per graph) (Top) population size, (Middle) dauer larvae and (Bottom) percentage dauer larvae present at food exhaustion for populations started with one RIL fourth-stage larva (L4) and 100 µl of 20% (w/v) *E. coli* incubated at 20°C. Dotted black horizontal lines show mean N2 values. Error bars indicate standard errors. Asterisk denotes

***C. briggsae* HK104 x AF16 RILs**

117 lines were assayed for population size, the number of dauer larvae formed and the percentage of dauer larvae in a growing population. Analysis indicated that the population size at patch exhaustion differed between lines all three experimental blocks ($F_{47,203}$ = 1.63, p = 0.014; $F_{46,217}$ = 1.77, p = 0.005; $F_{27,130}$ = 2.01, p = 0.007: blocks 1-3 respectively)(Fig 4.3). Analysis indicated that the number of dauer larvae at patch exhaustion also differed between lines in all three experimental blocks (H = 125.46, df = 47, p < 0.001; H = 155.21, df = 46, p < 0.001; H = 76.64, df = 27, p < 0.001: blocks 1-3 respectively). Similarly, the proportion of dauer larvae at patch exhaustion differed between lines in all three experimental blocks (H = 128.00, df = 47, p < 0.001; H = 140.66, df = 46, p < 0.001; H = 75.28, df = 27, p < 0.001: blocks 1-3 respectively).

The population size, number of dauer larvae and the percentage dauer larvae present at food exhaustion were determined for three RIL panels. These analyses indicate that each trait is variable between blocks; in the CB4856 x N2 panel for example, in block 1 the N2 average for the number of dauer larvae is around 1500 whereas it is around 2500 in block 4 (Fig 4.1A and D). Extensive variation in all three traits, though especially in the two dauer larvae traits is also shown, with the majority of lines forming fewer dauers than the parental, but with some lines forming higher numbers of dauer larvae; block 1 of the CB4856 x N2 panel for example shows 35 lines which are significantly different from N2, 32 forming fewer dauer larvae and 3 forming more (Fig 4.1A). This pattern is seen in both the *C. elegans* RILs and the *C. briggsae* RILs. Interestingly, the DR1350 x N2 RILs show the greatest variation in population size; in block 1 there are 17 lines showing a significantly lower population size than N2 (Fig 4.2A). Also in the DR1350 x N2 RILs, no line formed zero dauer larvae, indeed this panel shows the least amount of variation in dauer larvae development (Fig 4.2).

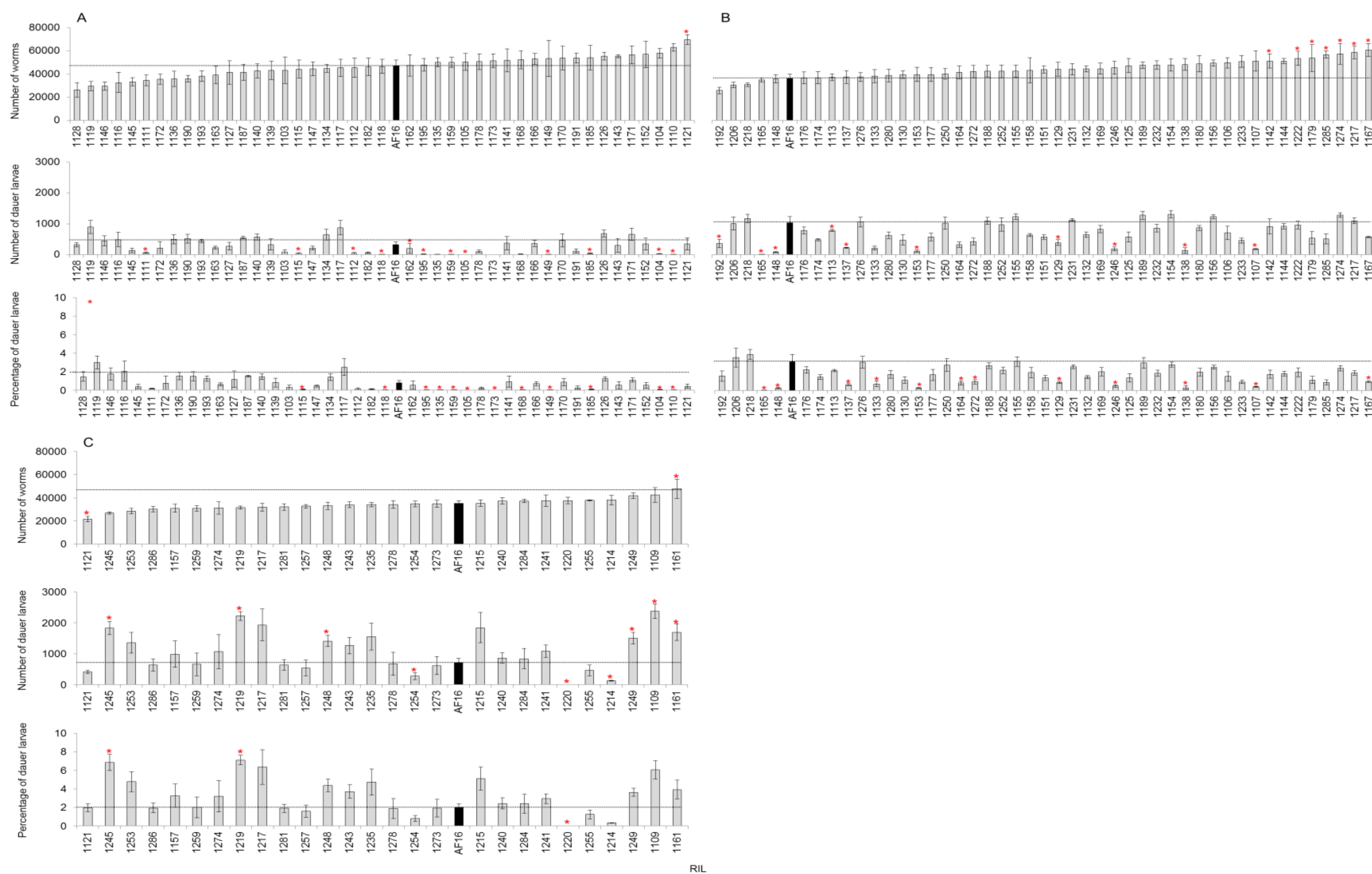

Figure 4.3: HK104 x AF16 RILs vary in population size and dauer larvae formation. A-C blocks 1-3. The mean (±1 SE) (per graph) (Top) population size, (Middle) dauer larvae and (Bottom) percentage dauer larvae present at food exhaustion for populations started with one RIL fourth-stage larva (L4) and 100 µl of 20% (w/v) *E. coli* incubated at 20 °C. Dotted black horizontal lines show mean AF16 values. Error bars indicate standard errors. Asterisk denotes a

QTL mapping

To investigate the effect of variation between blocks each block was individually mapped, using raw data for the number of dauer larvae formed in the CB4856 x N2 RILs (Fig 4.4). These analyses indicate that the overall pattern observed is similar in each block. A QTL is defined by having a value above the logarithm of odds (LOD) score.

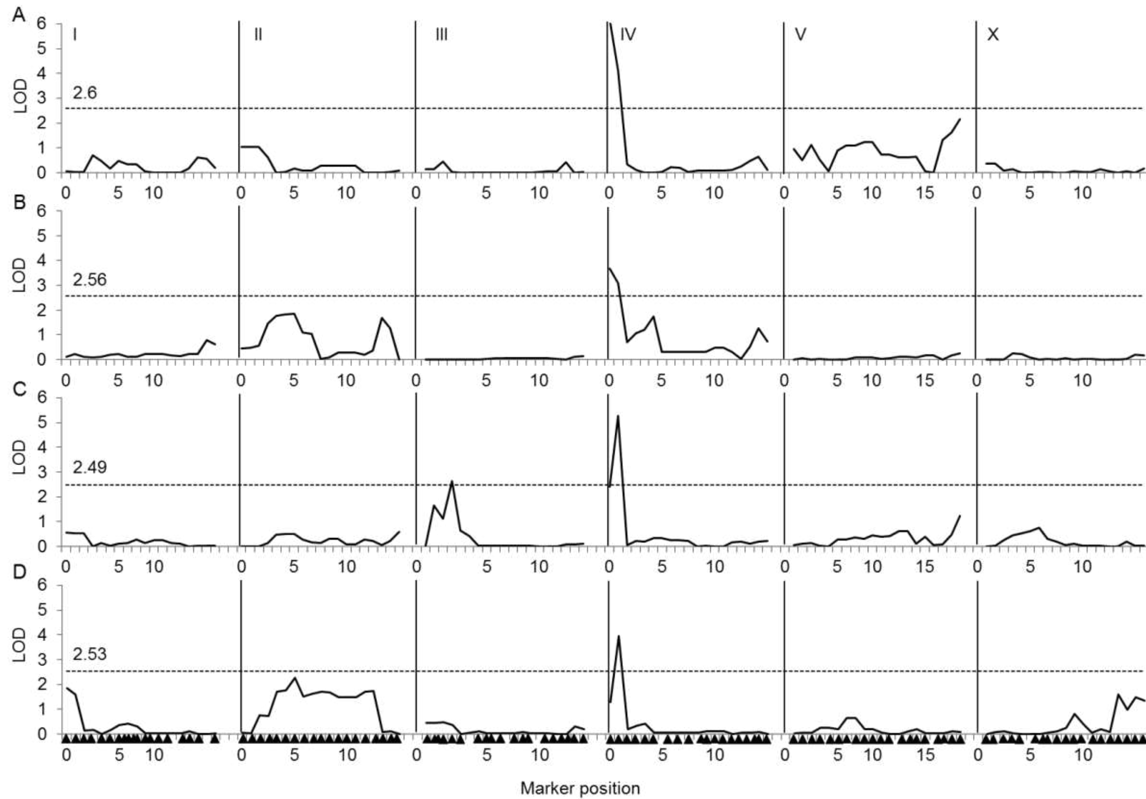

Figure 4.4: Mapping each individual block shows similarities in dauer larvae development. A-D in graphs shows blocks 1-4 for the number of dauer larvae in the CB4856 x N2 RILs. Black dotted line indicates LOD score; numbers above the line indicate the LOD value. Black triangles indicate marker position.

As there is little variation between the four blocks in the mapped data for dauer larvae, and is the main trait of interest, the data was pooled for the following mapping strategies. Following this, the number of dauer larvae in the CB4856 x N2 RIL panel were mapped using

four methods; as raw numbers, scaled to N2 average in block, scaled to the average of the block and normalised. This allowed for a comparison of the mapping methods (Fig 4.5).

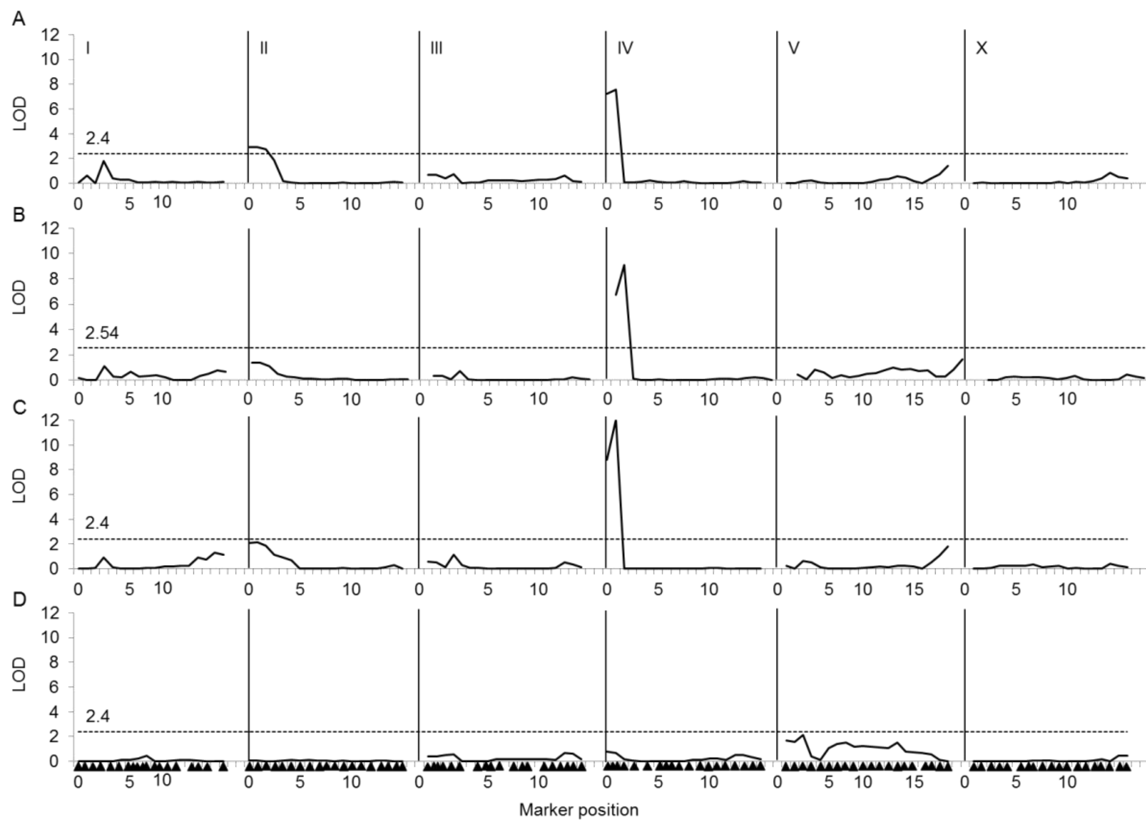

Figure 4.5: Different mapping strategies in the CB4856 x N2 RILs. A-D in graphs shows the number of dauer larvae mapped A) as raw numbers, B) Scaled to N2 within a block, C) scaled to the average of a block and D) normalised. Black dotted line indicates LOD score; numbers above the line indicate the LOD value. Black triangles indicate marker position.

These data show the mapping strategies have little variation also, giving only slightly different results, with two scaling methods, B and C, producing similar results and revealing a single QTL on chromosome *IV*. This QTL is also detected when the raw numbers are mapped. Interestingly, the strategy making the mean and variance equal lost the QTL on chromosome *IV*, which is seen on each of the other three methods.

The QTL at the beginning of chromosome *IV* visible in the dauer larvae analysis (Fig 4.5) maps in the same place as a QTL identified in a screen for genetic incompatibilities undertaken in this RIL set (HE Orbidans and SC Harvey, personal communication). This region spans the first three markers on the chromosome. To address the potential effect of this on the results, all lines that are CB4856 at those three markers were removed from the analysis. This analysis allowed the number of dauer larvae at food exhaustion to be mapped in 77 RILs (Fig 4.6).

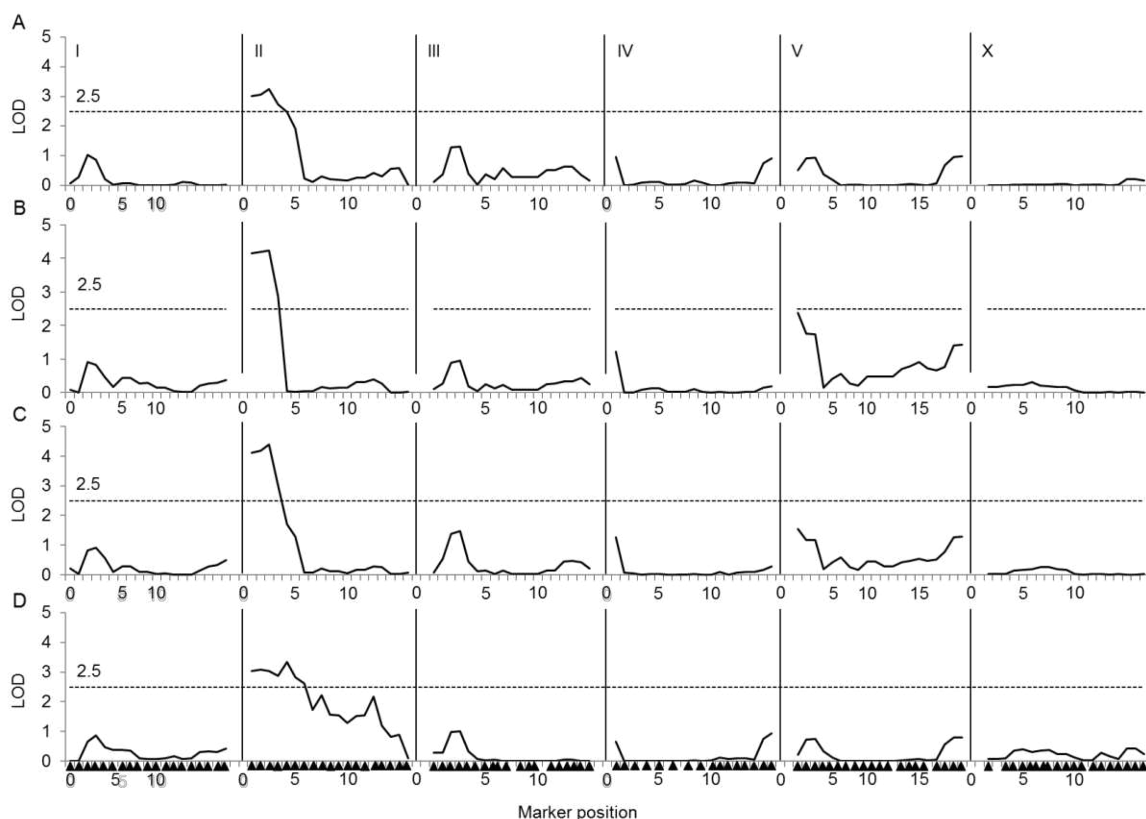

Figure 4.6: Region of incompatibility removed reveals QTLs that were not visible before. A-D in graphs shows the number of dauer larvae mapped A) as raw numbers, B) Scaled to N2 within a block, C) scaled to the average of a block and D) normalised. Black dotted line indicates LOD score; numbers above the line indicate the LOD value. Black triangles indicate marker position.

These data reveal a QTL on chromosome *II*, which was hidden by the large region of incompatibility, on all mapping strategies. This QTL was only detected using the raw data on the previous mapping which included this region of incompatibility. With the three markers removed on chromosome *IV*, there is now no variation between the mapping strategies. There is little variation in these mappings, and as this QTL was only detected using the raw data in both tests, the raw data of the other RIL panels will be used for their QTL mappings.

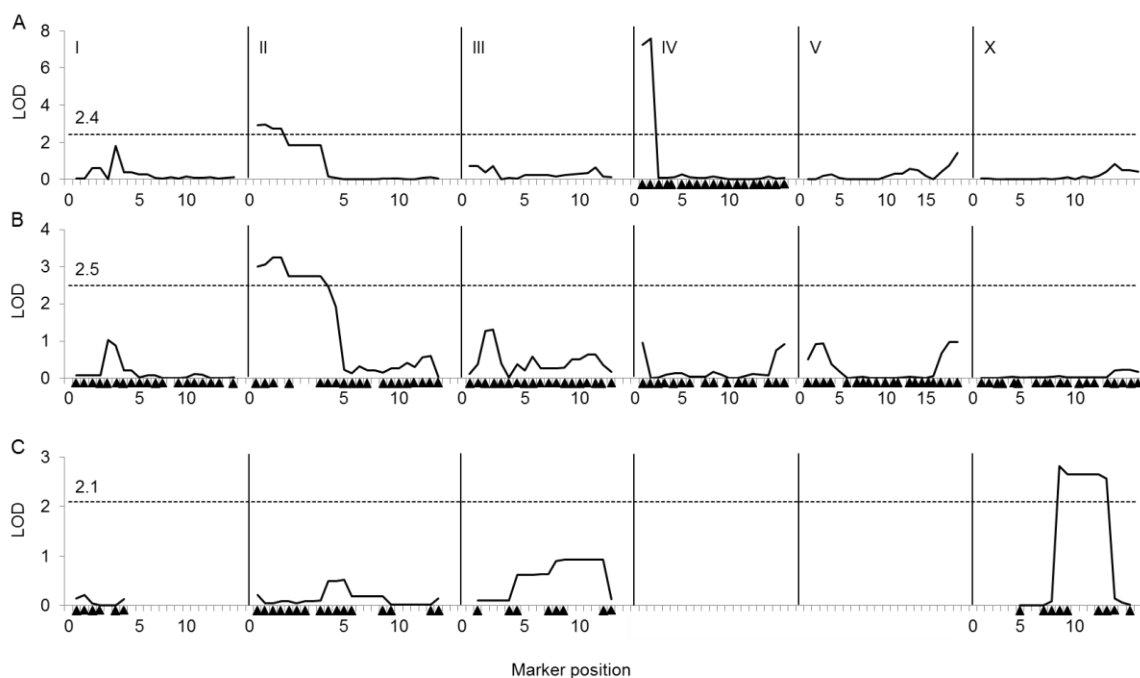

Figure 4.7: The number of dauer larvae in the two *C. elegans* RIL panels. (A) CB4856 x N2 RILs with incompatibility region included, (B) CB4856 x N2 RILs with incompatibility region removed and (C) DR1350 x N2 RILs. Black dotted line indicates LOD score; numbers above the line indicate the LOD value. Black triangles indicate marker position.

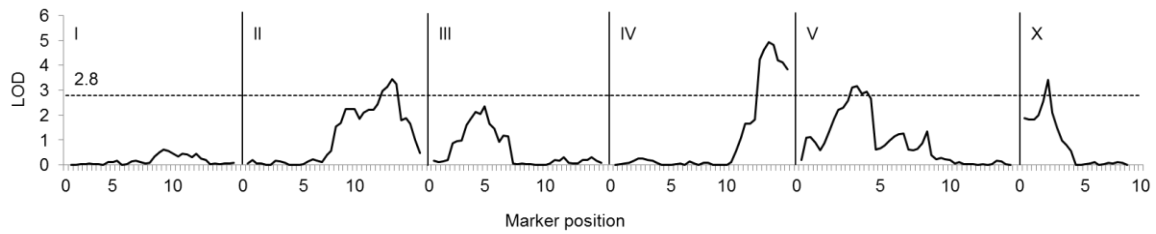

Figure 4.8: The number of dauer larvae in the *C. briggsae* RIL panel. Black dotted line indicates LOD score; numbers above the line indicate the LOD value.

These analyses show an interesting and distinct set of QTL for dauer larvae development between the two RIL panels (CB4856 x N2 (B) and DR1350 x N2 (C)), neither of which overlap with the other (Fig 4.7). Table 4.1 shows potential overlapping QTLs between the ones identified here and with the ILs used in Chapter Three.

The *C. briggsae,* HK104 x AF16, RIL panel shows the largest number of QTLs (Four) for dauer larvae development out of the three RIL panels investigated (Fig 4.8). This could be due to the number of genomic markers the RILs were genotyped to as low resolution and distance between markers lower the accuracy of comparisons and the *C. briggsae* had considerably more markers than the two *C. elegans* RILs. The proportion of phenotype variation explained by these QTLs (R^2 value in Table 4.1) was identified. The amount of variation found in these dauer larvae formation QTLs correlates with the amount of variation identified by Harvey *et al.,* (2008).

Chromosome	QTL - Chapter Three	Limits	CB4856 IL mapping	CB4856 RIL mapping	DR1550 RIL mapping	AF16 RIL mapping
I	1	1.9-3.5	X			
	2	4.3-9.6	X			
	3	10.3-11.1				
	4	9.6-11.1				
II	5	0-2.8				
	6	1.7-3.4		3.3-4 (0.15)		
	7	12.6-14.0				
	8	11.2-12.6				
	9	13.2-17.5				14.1-14.9 (0.08)
III	10	2.5-8.3				
	11	8.0-10.6				
	12	10.0-11.3				
IV	13	0.8-2.3	X	**0.5-2.7 (0.18)**		
	14	2.3-3.9				
	15	9.1-10.9	X			
	16	11.7-13.7	X			
						15.1-17.2 (0.12)
						3.7-7.7 (0.07)
V	17	11.8-14.0	X			
	18	17.4-19.5				
X	19	0-1.5	X			
	20	1.5-3.3				
	21	3.3-5.8	X			4.7-6.7 (0.09)
	22	5.8-8.0	X			
	23	8.7-11.1				
	24	13.9-17.6	X		11.9-15.3 (0.14)	

Table 4.1: Locations of QTLs detected for the number of dauer larvae in the ILs from Chapter Three and the RILs assayed in this Chapter. The proportion of the variance explained, R^2, in brackets. Bold value on chromosome *IV* shows the incompatibility QTL.

DISCUSSION

Here we have demonstrated extensive variation in both population size and the number of dauer larvae in growing populations of two *C. elegans* RIL panels and in a panel of *C. briggsae* RILs. The CB4856 x N2 RILs showed little variation in the population size, whereas most of the RILs produced significantly fewer dauer larvae than N2 (Fig 4.1 A-D). This is a similar picture to what is shown from the ILs in Chapter Three; lines which were produced from these RILs. The DR1350 x N2 RILs showed the biggest difference in the population size out of the three panels, with relatively few lines showing a difference from N2 for dauer larvae formation (Fig 4.2 A-B). From these analyses, variation in dauer larvae formation in a growing population is higher in the CB4856 x N2 RILs. This greater variation could be due to the relatedness between the parental lines which make up the RIL panel; CB4856 is highly genetically divergent from N2 with a single nucleotide polymorphism (SNP) every 840bp (Koch *et al.,* 2000) and DR1350 is less genetically divergent. This suggests the complex architecture of dauer larvae development reflects differences due to selective history.

As each panel was assayed in 2-4 blocks, the number of dauer larvae was mapped for each block using the raw numbers from the population assay in the CB4856 x N2 RILs to check for a block effect (Fig 4.4). The dauer larvae data did not show great variation suggesting that dauer larvae formation is either a more conserved trait, or that in this style of assay its underlying genetics are conserved. As the mapping showed little variation, the data was pooled and the number of dauer larvae was mapped again using the four strategies mentioned in the methods. Little variation between the four strategies was shown; a QTL on chromosome *IV*, an area of incompatibility (HE Orbidans and SC Harvey, personal communication), identified in three out of four mapping strategies, and a QTL on chromosome *II* in one of four mapping strategies (Fig 4.5). These same data were mapped

again with lines containing this region of incompatibility removed, the first three markers from on chromosome *IV*, which revealed a QTL on chromosome *II* in all four strategies (Fig 4.6). This indicates incompatibilities have the potential for masking other QTLs. The results from the four strategies were very similar. As the QTL on chromosome *II* that is identified in all four strategies after the incompatibility region removed was also seen using the raw dauer larvae numbers with the region, this was used for the mapping of the other RIL panels.

In the CB4856 x N2 RILs only one QTL for dauer larvae development was identified (as the one of chromosome *IV* is not a dauer larvae QTL) (Fig 4.7), which does not correlate with the 24 QTLs identified in the ILs identified in Chapter Three. Table 4.1 shows that this QTL potentially overlaps with a QTL found in the ILs. The difference in the number of QTL identified could be due to the nature of the differences in genomic structure between the RILs and ILs. Each RIL has more than one introgressed fragment whereas each IL has only one, and increasing the number of lines in each type will increase the mapping resolution; in RILs because recombination frequency is fixed and in ILs because minimising the introgression will mean more lines are needed. Lisec *et al.,* (2009) demonstrated a similar finding when using RILs and ILs to identify heterotic metabolite QTL in *Arabidopsis thaliana*. Using a panel of 369 RILs they identified 147 QTLs and with a panel of 41 ILs they identified 634 QTLs (Lisec *et al.,* 2009). The DR1350 x N2 RILs identified just one QTL for dauer larvae development, on the *X* chromosome (Fig 4.7). This QTL contains the genes *srg-36* and *srg-37* and co-localises with a QTL identified in the Chapter Three. This QTL also co-localises with one identified by Harvey *et al.,* (2008) for dauer larvae development in the standard dauer larvae assay using a single cohort of age-matched worms. In these two *C. elegans* RIL panels both of the QTLs here correlated with the QTLs identified in Chapter Three. The proportion of the variance explained (R^2) matches a previous study of dauer larvae development, which

supports the validity of these QTLs, where Harvey *et al.,* 2008 identified 6 QTL for dauer larvae formation in response to different food concentrations, using DR1350 x N2 RILs and the standard dauer larvae assay (Harvey *et al.,* 2008).

For a comparative analysis of dauer larvae development in growing populations, a panel of *C. briggsae* RILs using the parent isolates HK104 and AF16 were analysed. This analysis showed variation in population size and the number of dauer larvae similar to that seen in the *C. elegans* panels, with fewer numbers of dauer larvae formed in the RILs than the parent, in this case AF16 (Fig. 4.3 A-C). This panel showed more variation in dauer larvae development than the DR1350 x N2 RILs and less variation than the CB4856 x N2 RILs, which shows that HK104 is greatly genetically divergent from AF16 (Hillier *et al.,* 2007). When the raw numbers were mapped, four QTL were identified (Fig 4.8) one on chromosome *II*, one on *IV*, one on *V* and one on the *X* chromosome. This panel had considerably more markers; 451 compared to 121 in the CB4856 x N2 and 39 in the DR1350 x N2 RILs, than the other two which is a reason why more QTLs were identified, and further demonstrates the importance of increasing the number of markers and the number of lines that are assayed (Keurentjes *et al*., 2007; Beavis, 1998). Although the *C. briggsae* genome is approximately the same size as *C. elegans* (Stein *et al*., 2003; Hillier *et al.,* 2007) the positioning of certain genes are not in the same place as *C. elegans*. Due to the syntenic nature of the two species their QTLs cannot be compared directly, however, Table 4.1 shows the potential overlap of QTLs. Another reason why these two data sets cannot be directly compared is because of their ecological niches. It has been shown that while *C. elegans* out-competes *C. briggsae* at lower temperatures, at higher temperatures the roles are reversed (Félix and Duveau, 2012). Here, *C. elegans* is grown under optimal conditions (Chapter Two) for population growth whereas *C. briggsae* has a higher optimal temperature, and therefore is at a disadvantage. This *C.*

briggsae data makes a nice standalone comparative study of dauer larvae development, but cannot be directly compared to *C. elegans* until the genome of HK104 has been fully sequenced and annotated, therefore the QTLs identified here will not, at this time, be further examined.

The variation shown in Figures 4.1-3 does not correlate with the very few QTL identified in Figures 4.7-8. This is potentially due to the small numbers of genetic markers (shown in the difference between the three panels), the number of lines assayed (shown by the difference between the RILs and ILs) and shows that the power of ILs in identifying genomic regions controlling a phenotype is far greater than that of RILs. The genetic divergence of the parental lines also reduces the number of real QTLs identified due to incompatibilities, for this reason, new sets of RILs and ILs would be a valuable resource. However, robust QTLs are still identified, and are supported because they co-localise with the QTLs identified in Chapter Three. The QTLs found in Chapter Three and in the *C. elegans* panels here will be further analysed for the underlying genes effecting dauer larvae development in growing populations in Chapter Five.

CHAPTER FIVE: Candidate dauer development gene detection and analysis

npr-1 data reported in this chapter has been published as:

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Data used in Figure 5.5 was collected by an undergraduate student, Luke Rahman, and was analysed by myself.

SUMMARY

In Chapters Three and Four, a large number of QTLs were identified for variation in dauer larvae development in a growing population. Here, bioinformatic analyses of these QTLs indicates: (1) regions that contain genes previously known to be associated with dauer larvae development, (2) potential candidate genes not previously shown to have an effect on dauer larvae development, and (3) regions which indicate domestication in the N2 genotype and regions that are not present in the CB4856 genotype. Three candidate genes; *npr-1*, *srg-36* and *srg-37* are then further analysed for their effect on dauer larvae development in growing populations. Analysis of *npr-1* indicates an allelic effect which lowers the number of dauer larvae formed in a growing population without affecting the population size. Additional analysis indicates that the bordering phenotype of *npr-1* does not correlate with dauer larvae formation in wild isolates. Mutant isolates containing deletions of *srg-36* and *srg-37* show reduced dauer larvae development in growing populations, with some variation in population size, increasing the number of nematodes. RNAi analysis of *srg-36* and *srg-37* indicates that disruption of *srg-37* results in fewer dauer larvae in N2, but that disruption of *srg-36* does not. However, these analyses are inconclusive due to the life history differences observed between the mutant lines of differing RNAi sensitivity. These analyses therefore indicate that at least one domestication allele affects dauer larvae development in growing populations, suggesting that several other QTLs could be explained by similar domestication alleles, and that analysis of complex traits by RNAi in *C. elegans* is complicated by the difference between lines.

INTRODUCTION

Dauer larvae development in *C. elegans* is a highly complex polygenic trait, with dozens of known genes affecting the trait. Quantitative genetics is useful for identifying both natural variation and alleles underlying lab adaptation (Rockman *et al.,* 2010). QTL analysis using inbred lines is commonly used for dissecting complex traits (Mackay 2001; Darvasi and Pisanté-Shalom, 2002; Steinmetz *et al.,* 2002; Reddy et al., 2009; Rockman *et al.,* 2010). Progression from a defined QTL to the associated gene can be difficult as QTLs often span regions containing hundreds of genes, for example, around 500 genes underlying QTLs found in *D. melanogaster* (Mackay, 2004); 9038 genes underlying 26 QTL found in mice (Gao *et al*., 2008) and in the *C. elegans* QTLs identified in Chapter Three as few as ~300 and up to ~3000 potential candidate genes were found (Table 5.1). As many complex traits are polygenic, coupled with the large number of genes underlying a QTL region, the discovery of a single gene polymorphism is arduous, although this is now improving with the ability to increase recombination and the number of genetic markers a strain can be mapped at (Yan *et al*., 2010). McGrath *et al*., (2009) demonstrates the identification of two QTL leading to single genes using *C. elegans* Recombinant Inbred Advanced Intercross Lines (RIALS) (Rockman and Kruglyak, 2008), which have been genotyped to 1455 markers across the genome (Rockman and Kruglyak, 2009). The development of RIALS clearly demonstrates the effect increased recombination and a large number of well-spaced markers can have on gene identification.

Once a gene has been identified it can be, for example, introgressed into a wild background through mating a transgene male with wild females or hermaphrodites, with further crossing to isolate the genomic region in the wild background (Gidalevitz *et al.,* 2013). Alternatively, an inbred line can be backcrossed for many generations, producing an isogenic line which

contains the gene of one parent in another genomic background (Soller *et al.,* 1976; Econopouly *et al.,* 2011).

Another way of investigating a particular gene is through RNA interference (RNAi), a method of gene silencing using double-stranded RNA (dsRNA) (Fire *et al.*, 1998). It was first observed in *C. elegans* when dsRNA was injected into the body cavity with observable changes to phenotypes (Fire *et al.*, 1998). Following this, a library of bacterial feeding clones has been constructed. These bacterial clones express dsRNA corresponding to a single gene (Kamath *et al*., 2003) and when fed to *C. elegans*, they have shown loss of function phenotypes (Timmons and Fire, 1998). This powerful approach to gene targeting allows for fast identification of phenotype variation due to gene knockouts. The RNAi pathway is highly conserved in eukaryotes and a huge number of studies have been done in model organisms. Examples of such RNAi studies are, observing gene function in specific tissues of *D. melanogaster* (Dietzl *et al.*, 2007), identification of genes involved in root transformation in *A. thaliana* (Crane and Gelvin, 2007), targeting genes associated with Parkinson disease in *M. musculus* (Zhou *et al*., 2007b) and an RNAi screen to identify genes of the p53 pathway in human cell cultures (Berns *et al*., 2004). An RNAi screen for enhanced dauer larvae formation was performed by Jensen *et al*., (2010b). In this study, 513 RNAi clones were screened for dauer larvae formation, resulting in identification of 21 genes for this phenotype, only one of which has previously been associated with dauer larvae formation. Further analysis of genes known to affect dauer larvae formation showed increased dauer larvae formation in response to pathogenic bacteria (Jensen *et al*., 2010b).

Bioinformatic analysis of the QTLs identified in Chapter Three has revealed a number of potential candidate genes for further analysis. One of the candidates, variation in *npr-1*, a neuropeptide Y receptor homolog located under QTL 21 was further investigated for dauer

larvae development in growing populations using three mutant isolates and ewIR81; the IL which is CB4856 at this locus. This locus is of interest as the lab-derived (McGrath *et al.,* 2009) N2 allele of *npr-1,* known to be polymorphic between N2 and all wild isolates of *C. elegans*, including CB4856 (de Bono and Bargmann, 1998; Anderson and Kruglyak, 2009). *npr-1* is also of interest as it's been found to be important in a large number of phenotypes such as, thermal preference (Gaertner *et al.,* 2012), feeding behaviour (de Bono and Bargmann, 1998), response to O_2 and CO_2 (McGrath *et al.,* 2009), innate immune response (Styer *et al.,* 2008), pathogen susceptibility (Reddy *et al.,* 2009) and recently in lifetime fecundity, adult body size and susceptibility to *Staphylococcus aureus* (Anderson *et al.,* 2014). These analyses reveal the first genes to have a role in dauer larvae development in growing populations.

The other candidates, genes known to be involved in dauer larvae development, *srg-36* and *srg-37,* serpentine receptor class G*,* which are located under QTL 24 were also investigated for dauer larvae development in growing populations, as they code for the G protein-coupled receptors for ascaroside C3 (McGrath *et al.*, 2011), a part of the dauer pheromone. The *srg-* genes will be investigated using NILs containing deletions at these positions and with RNAi bacterial feeding clones.

MATERIALS AND METHODS

Nematodes and assays

C. elegans wild type N2, the *npr-1* mutants DA508: *npr-1(g320)X,* DA609: *npr-1(n1353)X* and DA650: *npr*-1(ad609)*X*, the RNAi mutants NL2099: *rrf-3(pk1426)II* and NL3321: *sid-1(pk3321)V* were obtained from the *Caenorhabditis* Genetics Centre. The *srg-36* and *37* NILs CX13249 (LSJ2 deletion) and CX13591 (CC1 deletion) (McGrath *et al*., 2012) were obtained from C.I. Bargmann at The Rockefeller University, New York, USA. RNAi feeding clones X-6L13 and X-6L23 were obtained from Source BioScience (Nottingham, United Kingdom). The wild isolates and isogenic lines used were as described in Chapter Three. Maintenance of lines and population assays were prepared as those used in Chapter Two, the 3D environment technique was used for each experiment.

Candidate gene discovery

The QTL regions found in the IL and RIL analysis in the previous Chapters were evaluated by taking the two marker positions of the outer measurements of the QTL region, the genetic marker positions of the QTLs were analysed using the WormMart tool in Wormbase (www.Wormbase.org) which highlights the genes underlying these regions. The annotated genes found in this search were examined for dauer larvae development effects, to identify candidate genes for dauer larvae development in growing populations. Genes known to be associated with laboratory domestication were investigated (Weber *et al.,* 2010) and genetic regions which are not present in the CB4856 genotype were also analysed (Maydan *et al*., 2007. The domestication and missing genes were discounted from further examination, and due of the number of known dauer larvae genes and candidate dauer larvae genes available, it was not possible to test them all, therefore two QTLs found on the *X* chromosome of the

ILs in Chapter Three and the RILs in Chapter Four were used to find candidate genes for dauer larvae development.

npr-1

Population size and number of dauer larvae at food exhaustion were investigated using the isolate DA650, which contains the g320 *npr-1* allele from RC301 backcrossed ten times into an N2 background (Gloria-Soria and Azevedo, 2008), the isolates DA609 and DA508, which contain different *npr-1* loss-of-function alleles (de Bono and Bargmann, 1998) and the IL, ewIR81, which is CB4856 for this gene. The feeding behaviour effect of bordering, where worms tended to accumulate around the edge of a bacterial lawn (de Bono and Bargmann, 1998), was analysed, using the same wild isolates used in Chapter Three, to look for associations between dauer larvae development and bordering.

srg-36* and *srg-37

Population size and number of dauer larvae at food exhaustion were investigated using RNAi clones on RNAi susceptible (NL2099) and resistant (NL3321) isolates and N2. The genes, *srg-36 and srg-37,* were also analysed using the isolates CX13249 and CX13591, NILs containing deletions of the *srg-36* and *srg-37* genomic regions (McGrath *et al.*, 2012) and the IL, ewIR89, CB4856 at this genomic region. Lifetime fecundity, as described in Chapter Two, was also analysed in the RNAi susceptible and resistant isolates and N2 on plates with OP50.

RNAi

The feeding protocol from Kamath *et al.,* (2000) was used and adapted for use in a growing population assay. Colonies of the RNAi feeding clones were picked and grown overnight in LB with 50µg/ml ampicillin. NGM plates (adjusted for the 3D environment) with added carbenicillin to 25 µg/ml of the final concentration and IPTG to 1 mM to the final concentration, were seeded with the grown bacterial cultures and left to dry and grow for 3 days, until a small lawn had formed. Four plate assays were set up, one of each feeding clone, X-6L13 and X-6L23, one with mixed feeding clones and one with OP50 as a control. (OP50 assays did not include the ampicillin, carbenicillin or IPTG). A single L4 stage nematode was then added to each plate and incubated at 20˚C, nematodes used were the two RNAi mutants, NL2099 and NL3321, and N2 as a control. On day 8 of growth (the average day for food exhaustion of 20% w/v *E. col*i) the population size and number of dauer larvae were determined as in Chapter Two.

Analysis

Population size data was analysed by One-way ANOVA with *post hoc* testing by Fisher’s least significant difference test. Dauer larvae numbers were ere analysed by Kruskal-Wallis test with Mann-Whitney U tests used for pairwise testing as the Kruskal-Wallis test lacks a post hoc test. Pearson's Correlation Coefficient was used to analyse the relationship of the bordering phenotype and dauer larvae formation. All analyses were carried out using Minitab® Statistical Software (Minitab, Coventry, UK).

RESULTS

Candidate gene discovery

Analysis of the QTLs identified in Chapter Three show that 20 out of the 24 QTL regions contain genes which are known to affect dauer larvae development. These QTLs could therefore be a consequence of variation in these genes. These analyses also show that 8 of the QTLs contain genes which are deleted in the CB4856 genotype, and 5 of the QTLs contain genes which are thought to be involved in domestication in N2 (Table 5.1). Two QTL in particular, QTL5 on chromosome *II* and QTL18 on chromosome *V*, span regions which have a large number of genes missing from the CB4856 genome; 148 and 88 respectively. Given this number of candidate genes, further analysis was restricted to the *X* chromosome. The total number of potential genes on the *X* chromosome is 7429 (Table 5.1), an impossible number of potential candidates to test in a growing population assay. Interestingly, QTL24 contains *npr-1*, a gene known to be associated with a number of phenotypes and is associated with laboratory domestication in N2 (Weber et al., 2010). Although known not to affect dauer larvae formation in standard dauer larvae assays (Viney *et al.,* 2003), a polymorphism at *npr-1* is known to affect many traits (de Bono and Bargmann, 1998; Gloria-Soria and Azevedo, 2008; Reddy *et al.,* 2009; Andersen *et al.,* 2014), therefore polymorphism at *npr-1* was investigated in a population growth assay. Also identified in a region on the *X* chromosome were the two chemosensory genes, *srg-36* and *srg-37*, within QTL24, also identified in a QTL in the DR1350 RILs from Chapter Four (Table 5.2). These genes are known to be associated with dauer larvae development, as they encode redundant G-protein-coupled receptors for ascaroside C3 (a part of the dauer pheromone) (McGrath *et al*., 2011), which makes them an interesting choice as candidates for a population growth assay. The RILs from Chapter Four did not reveal as many QTLs as the ILs; two in the CB4856 x N2, two in the DR1350 x N2 and

four in the HK104 x AF16, with only two; one from each of the *C. elegans* RILs, overlapping a QTL from Chapter Three (Table 5.2). For these reasons, candidates were investigated from the IL QTLs.

Chr	QTL	Limits	Effect	No. of genes	Known dauer larvae genes	Gene deletions (not present in CB4856)	Domestication polymorphism
I	1	1.9-3.5	+	331	***srbc-64***	Y39G10AR.5(*zeel-1*)	*F55A12.8 (nath-10)*
	2	4.3-9.6	-	2202	*aap-1*, ***daf-8***, ***dyf-1***, ***dyf-5***, *ins-8*		
	3	8.7-11.8	+	1009	*daf-16*, ***dyf-5***, ***eak-6***, ***tax-2***		
	4	9.6-11.1	-	476	*daf-16*, ***eak-6***		
II	5	0-2.8	-	913	*che-10*	C03H5.1(*clec-10*), F28A10.3, T07D3.5, T07D3.4, K02E7.9, K02E7.5, K02E7.10, K02E7.12, Y51H7BR.3, Y51H7BR.2(*fbxb-43*), Y51H7BR.1(*fbxb-42*), K05F6.5(*fbxb-44*), K05F6.4, K05F6.6(*fbxb-52*), K05F6.3(*fbxb-51*), K05F6.7(*fbxb-54*), K05F6.2(*fbxb-5*), K05F6.8, K05F6.9(*fbxb-46*), K05F6.1(*fbxb-49*), K05F6.10, C08E3.7, C08E3.8, C08E3.9, C08E3.10, C08E3.11, C08E3.12, ZC204.9(*fbxb-20*), F58E1.12, F58E1.13, F36H5.9, F36H5.11(*fbxb-12*), F36H5.3(*math-28*), F36H5.2b(*math-27*), F36H5.1(*math-26*), C08F1.4a(*math-3*), C08F1.5(*math-4*), C08F1.10, C08F1.6, C08F1.3(*fbxb-13*), C08F1.2(*str-21*), C08F1.1(*math-2*), C08F1.7(*str-22*), C08F1.8, C08F1.9, T08E11.6(*fbxb-10*), T08E11.7(*fbxa-3*), T08E11.5, T08E11.4(*math-41*), T08E11.3(*math-40*), T08E11.2(*math-39*), T08E11.8, T08E11.1, C52E2.5, C52E2.4, C52E2.6(*fbxb-97*), C52E2.7(fbxb-96), C52E2.3, C52E2.2, C52E2.1(*fbxb-95*), C52E2.8, C16C4.7, C16C4.6(*fbxb-98*), C16C4.5(*math-15*), C16C4.4(*math-14*), C16C4.15(*math-10*), C16C4.16(*math-11*), C16C4.3(*math-13*), C16C4.8(*math-16*), C16C4.9(*math-17*), C16C4.10(*math-5*), C16C4.11(*math-6*), C16C4.12(*math-7*), C16C4.13(*math-8*), C16C4.14(*math-9*), C16C4.2(*math-12*), C16C4.1, C46F9.4(*math-25*), C46F9.3(*math-24*), C46F9.2(*math-23*), C46F9.1(*math-22*), F52C6.5(*math-30*), F52C6.6(*math-31*), F52C6.7(*bath-11*), F52C6.8(*bath-4*), F52C6.9(*bath-6*), F52C6.10(*bath-7*), F52C6.11(*bath-2*), F52C6.4, F52C6.3, F52C6.2, F52C6.1(*bath-22*), F52C6.12, F52C6.13,F52C6.14, C40D2.2(*math-20*), C40D2.1(*math-19*), C40D2.4, F59H6.5, F59H6.6, F59H6.4(*math-32*), F59H6.3, F59H6.2, F59H6.8(*bath-21*), F59H6.9(*bath-1*), F59H6.10(*bath-3*),	

						F59H6.11(*bath-5*), F59H6.12, F59H6.1(*bath-19*), B0047.1(*bath-20*), B0047.2, B0047.3(*bath-24*), B0047.4(*math-1*), B0047.5(*bath-14*), F07E5.4, F07E5.2(*fbxb-35*), F07E5.5, T16A1.4, T16A1.5, T16A1.9, T16A1.1(*math-42*), K09F6.6, K09F6.9, K09F6.10, K09F6.7, K09F6.8, B0281.4, B0281.5, B0281.6, B0281.3, B0281.2, B0281.7, B0281.8, B0281.1, ZK1240.4, ZK1240.5, ZK1240.9, ZK1240.3, ZK1240.6, ZK1240.2, ZK1240.8, ZK1240.1, F43C11.11, F43C11.12, F16G10.5, F16G10.4, F16G10.3, F42G2.5	
	6	1.7-3.4	+	611			
	7	11.8-14.0	-	576	*daf-22*	F15A4.8b(chil-28), Y46G5A.7, Y46G5A.8, E01G4.5	
	8	11.2-12.6	+	461	***age-1***, *daf-22*		
	9	13.2-17.5	+	484	*asc-1, daf-5*		
III	10	2.5-8.3	+	1675	*daf-2, daf-4,* ***ncr-2***		*thoc-2, Y40D12A.1, cyk-1, K04G7.1(rnp-7), R151.2, F56C9.11 B0303.7, ZK507.1, emb-9, K04H4.2, vps-53*
	11	8.0-10.6	-	865	*asna-1,* ***hpd-1****, ncr-2,* ***tax-4***	F44E2.2b(*retr-1*)	
	12	10.0-11.3	+	323			
IV	13	0-2.3	-	484	*daf-1,* ***daf-18***	F38A1.7(*clec-168*), F38A1.14(*clec-169*), F38A1.13, R05C11.2	
	14	2.3-3.9	-	407		Y69A2AR.24, Y69A2AR.25, Y69A2AR.13, Y69A2AR.12, Y69A2AR.12, Y69A2AR.26(*nhr-242*), Y69A2AR.11, Y69A2AR.10, Y69A2AR.9, Y69A2AR.8, Y69A2AR.27, Y94H6A.10	
	15	9.1-10.9	-	707	***daf-10****,* ***daf-14****,* ***daf-15****, ins-1*		
	16	11.7-13.7	-	937	*eak-4,* ***unc-31***		
V	17	10.4-17.4	+	3042	***che-11****,* ***daf-11***	C54D10.7 (dct-3)	
	18	17.4-19.5	-	697		T27C5.7, T27C5.12, T27C5.8, T27C5.14(*srh-96*), T27C5.10, F20E11.15(*srbc-27*), F20E11.16(*srbc-28*), F20E11.2(*srsx-2*), F20E11.11(*srh-175*), F20E11.12(*srh-*	*str-200*

						154), F20E11.13(*srh-158*), F20E11.3(*srh-160*), F20E11.8(*srh-157*), F20E11.9(*srh-156*), F20E11.14(*srh-161*), F20E11.10(*srh-203*), F20E11.4(*str-200*), F20E11.1(*srz-48*), F20E11.5, F20E11.7, F20E11.6(*srw-72*), F08E10.1(*srh-235*), F08E10.8(*srh-114*), F08E10.3(*srh-123*), F08E10.2(*srbc-61*), F08E10.4(*srh-110*), F08E10.5(*srh-253*), F08E10.6(*srh-111*), F08E10.7, K03D7.9, K03D7.8, K03D7.7(*fbxa-102*), K03D7.6(*srh-118*), K03D7.5, K03D7.4(*srh-261*), C18D4.3, Y6G8.1(*srz-45*), Y6G8.2, F57G4.5, F57G4.6, F57G4.9, F57G4.7, F57G4.8, F59A1.5, F59A1.9, F59A1.8(*fbxa-129*), F59A1.12, Y94A7B.5(*srh-298*), Y94A7B.6(*srh-300*), Y94A7B.8(*srh-301*), Y94A7B.9(*srh-304*), Y94A7B.7(*srh-303*), F16H6.3, F16H6.4, F16H6.5, F16H6.6, F16H6.7, F16H6.8, F16H6.9, F16H6.10, Y37H2B.1, R10E8.7, Y51A2A.2, Y51A2A.3, Y51A2A.4, Y51A2A.11, Y51A2A.5, C08E8.3, Y69H2.10b, F11D11.1, T26H2.2(*fbxb-115*), T26H2.1 (*fbxb-1*), F21D9.6, C43D7.7, C43D7.5(*sdz-6*), C43D7.4, C25F9.6, C25F9.5, C25F9.4, C25F9.9, C25F9.2, C25F9.1(*srw-85*), C25F9.t5, M04C3.1a, M04C3.3, M04C3.2, Y43F8B.14, Y43F8B.13	
X	19	0-1.5	-	352	*che-2*, ***daf-3***, ***mrp-1***		
	20	1.5-3.3	+	565	***hid-1***		
	21	2.4-9.3	-	2585	***daf-9***, ***hbl-1***, ***hid-1***, *ins-9*, ***ist-1***, ***ncr-1***		*npr-1*
	22	5.0-9.3	-	1686	***daf-9***, ***hbl-1***, *ins-9*, ***ist-1***		
	23	8.7-11.1	+	766	*daf-12*, *ins-9*		
	24	12.9-17.6	-	1475	*akt-2*, ***daf-6***, *srg-36*, *srg-37*		

Table 5.1: QTL analysis for candidate genes. QTLs identified in Chapter Three with their genomic length, the number of genes contained and the genes of which are known to be associated with dauer larvae development, gene deletions in the CB4856 genotype (Maydan *et al*., 2007) and domesticated N2 genes (Weber *et al*., 2010), for the QTLs found. Bold genes in the 'known dauer larvae genes' column indicate having a polymorphism between N2 and CB4856.

RIL	Chr	QTL	Overlap with ILs	Limits	Effect	No. of genes
CB4856 x N2	*II*	1	QTL6	3-3.4	N	180
	IV	**2**	**QTL13**	**0.5-2.7**	**N**	**476**
DR1350 x N2	*X*	1	QTL24	11.9-15.3	N	1185
HK104 x AF16	*II*	1		14.1-14.9	N	120
	IV	2		15.1-17.2	N	51
	V	3		3.7-7.7	N	1073
	X	4		4.7-6.7	N	328

Table 5.2: QTL analysis of the RIL panels from Chapter Four. Bold data on chromosome IV is the incompatibility QTL.

As there is, as yet, no sequence data for HK104 to check SNPs data between HK104 and AF16, further analyses comparing *C. elegans* and *C. briggsae* QTLs were not performed. A lack of chromosomal synteny, the physical co-localisation of genetic loci, between the two species increases the difficulty of further analysis, for example, *daf-18* underlies the QTL on chromosome *IV* in the *C. briggsae* RILs and QTL13 in the *C. elegans* IL. While it is known that there is no polymorphism changing the coding sequence of *daf-18* between CB4856 and N2, without the sequence data for HK104 there are no means to check for a polymorphism of *daf-18* between HK104 and AF16. These data will be further investigated in time.

npr-1

Analysis of isolates containing different alleles of *npr-1* indicates that variation of *npr-1* affects dauer larvae formation in growing populations (A. Population size: $F_{3,56} = 2.32$, $p = 0.09$; Dauer: $H = 36.58$, $df = 3$, $p < 0.001$. B. Population size: $F_{4,62} = 1.19$, $p = 0.36$; Dauer: $H = 47.0$, $df = 4$, $p < 0.001$. C. Population size: $F_{4,47} = 2.01$, $p = 0.110$; Dauer: $H = 26.72$, $df = 4$, $p < 0.001$) with alleles that produce a bordering phenotype lowering the number of dauer larvae produced (Fig 5.1). Interestingly, these analyses indicate that the effect of variation in *npr-1* on dauer larvae formation is allele-specific, with a much stronger effect on dauer larvae development observed in DA609. Although a bordering effect is shown to reduce the number of dauer larvae formed with the *npr-1* mutants, the effect of bordering in wild isolates does not correlate with the number of dauer larvae seen in wild isolates (from Chapter Three) (Pearson correlation of percentage bordering and number of dauer larvae = 0.052, $p = 0.83$) (Fig 5.2) and indeed shows that unlike the fewer number of dauer larvae formed in the *npr-1* mutants, most of the wild isolates produce more dauer larvae in a growing population than N2 (Chapter Three). This analysis does however indicate that wild isolates differ in the extent to which they border (Fig 5.2). Hence, this analysis suggests that additional loci affect bordering, either directly or by interacting with *npr-1*.

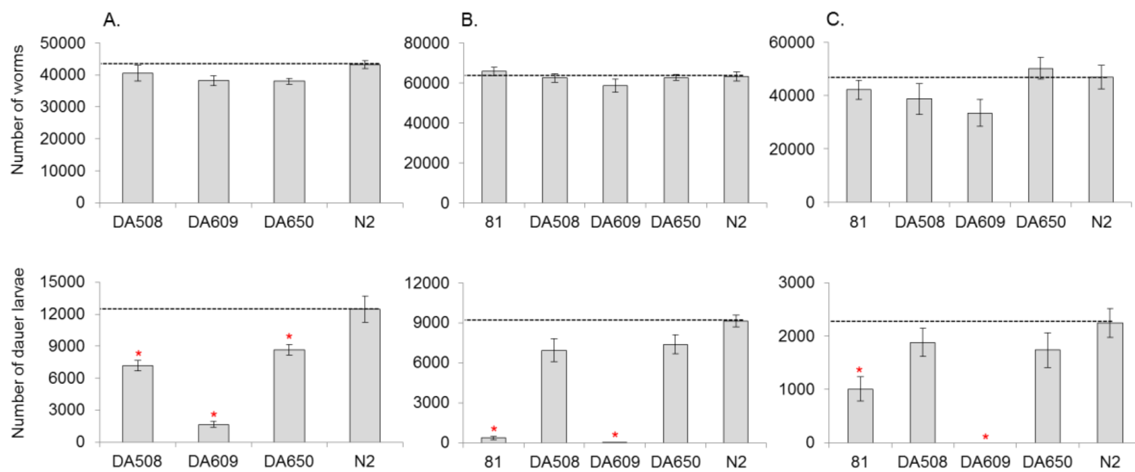


Figure 5.1: *Caenorhabditis elegans* dauer larvae development in growing populations is affected by *npr-1*. The mean number of (±1 SE) worms (Top) and dauer larvae (Bottom) present at food exhaustion for populations started with one fourth-stage larva (L4) and 100 µl of 20% (w/v) *Escherichia coli* incubated at 20°C. A, B and C represent three assay repeats, ewIR81, the IL that is CB4856 at this region was added after the first assay. Dotted black horizontal lines show mean N2 values. Error bars indicate standard errors. Significance is shown by the asterisks on the x-axis denoting isolates that differ from N2. (Population size: $p < 0.05$; Fisher's Least Significant Difference. Dauer larvae: $p < 0.05$; Mann-Whitney U-test).

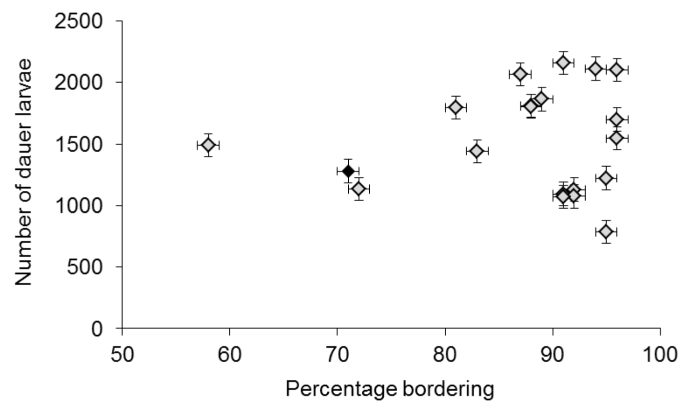


Figure 5.2: Bordering in wild isolates is not correlated with dauer larvae development in growing populations. Pearson correlation of percentage bordering and dauer larvae formation in growing populations. The solid marker indicates the value of N2. Error bars indicate standard errors.

srg-36* and *srg-37

Analysis of the RNAi susceptible and resistant lines when grown on standard OP50, shows a difference in both population size and the number of dauer larvae formed at food exhaustion (Population size: $F_{2,29} = 11.06$, $p < 0.001$; Dauer: $H = 22.58$, $df = 2$, $p < 0.001$) (Fig 5.3A and B). Analyses also shows that lifetime fecundity is effected by the two mutant isolates ($F_{2,29} = 11.69$, $p < 0.001$) (Fig 5.3C). These analyses show that the lines differ in these life-history traits. However, it is unclear if the mutations for susceptibility and resistance to RNAi cause these effects or if the lines have other mutations. This indicates that the mutations the two RNAi lines have incurred deleterious effects to their life-history traits, or have other mutations which infer the fitness loss.

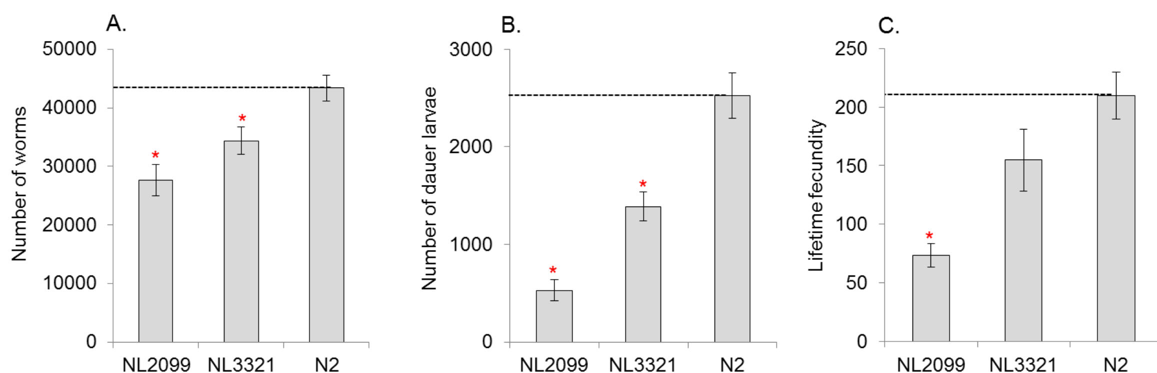


Figure 5.3: RNAi mutant lines show a difference to N2 when grown under normal assay conditions. (A) The mean number of (±1 SE) worms (B) the number of dauer larvae present at food exhaustion and (C) lifetime fecundity. Dotted black horizontal lines show mean N2 values. Error bars indicate standard errors. Significance is shown by the asterisks on the x-axis denoting isolates that differ from N2. (Population size: $p < 0.05$; Fisher's Least Significant Difference. Dauer larvae and fecundity: $p < 0.05$; Mann-Whitney U-test).

Analysis of RNAi gene knockout (Fig 5.4) indicates that growth on RNAi clone targeting *srg-36* affects the population size but not the number of dauer larvae formed within (Population

size: $F_{2,25}$ = 10.29, p < 0.001; Dauer: H = 2.25, df = 2, p = 0.28). However, growth on RNAi clone targeting *srg-37* shows an effect on both population size and the number of dauer larvae formed (Population size: $F_{2,22}$ = 22.22, p < 0.001; Dauer: H = 15.15, df = 2, p < 0.001). When both RNAi clones are used it mirrors the effect of *srg-36* (Population size: $F_{2,23}$ = 8.21, p = 0.002; Dauer: H = 2.35, df = 2, p = 0.31) where population size is effected but there is no effect on the number dauer larvae formed.

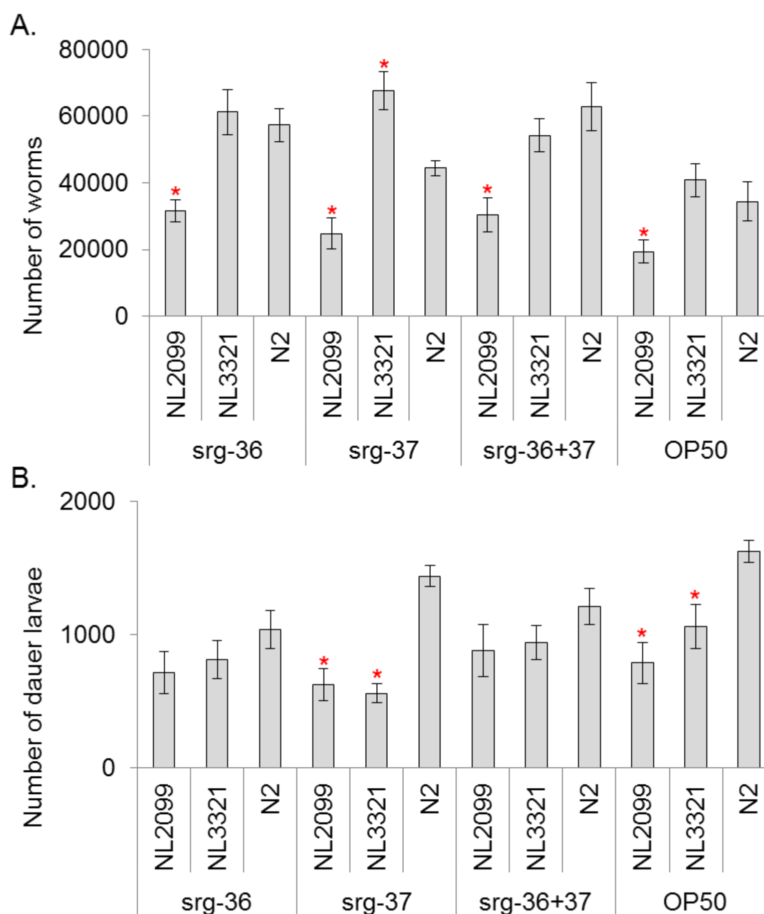


Figure 5.4: RNAi analyses of *srg-36* and *srg-37* in growing populations. The mean number of (±1 SE) worms (A) and dauer larvae (B) present at food exhaustion. Error bars indicate standard errors. Significance is shown by the asterisks on the x-axis denoting isolates that differ from N2 with the same bacterial feed. (Population size: p < 0.05; Fisher's Least Significant Difference. Dauer larvae: p < 0.05; Mann-Whitney U-test).

NILs with the genomic region for *srg-36* and *srg-37* deleted and the IL from Chapter three were analysed for dauer larvae development in growing populations. These analyses indicate that the population size of ewIR89, the IL which is CB4856 at *srg-36* and *-37*, is not different from N2. However, the number of dauer larvae and the proportion of dauer larvae are significantly lower in both repeats. The NIL, CX13249, also shows no significant difference in population size compared to N2. In both repeats, this NIL forms significantly fewer dauer larvae than N2, though in only one repeat is the proportion of dauer larvae formed significantly different from N2. The NIL, CX13591, has a significantly higher population size than N2 in one repeat and shows no difference in the other. For dauer larvae formation and the proportion of dauer larvae, the higher population size repeat showed no difference, however, the second repeat shows significantly fewer dauer larvae and a lower proportion of dauer larvae than N2 (Fig 5.5).

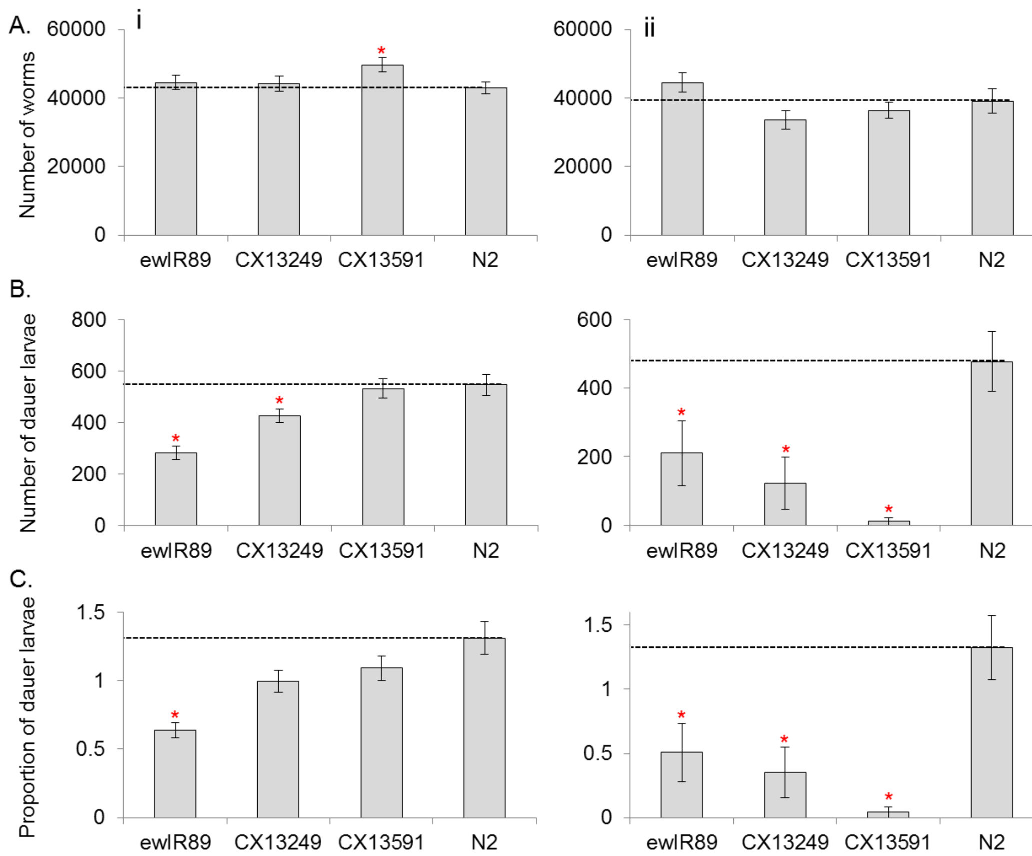


Figure 5.5: *srg-36* and *srg-37* NILs show variation in dauer larvae development in a growing population. The mean number of (±1 SE) worms (A) dauer larvae (B) and the proportion of dauer larvae (C) present at food exhaustion, i and ii represent two repeats. Dotted black horizontal lines show mean N2 values. Error bars indicate standard errors. Significance is shown by the asterisks denoting isolates that differ from N2. (Population size: $p < 0.05$; Fisher's Least Significant Difference. Dauer larvae: $p < 0.05$; Mann-Whitney U-test).

DISCUSSION

Here we have analysed the QTLs previously identified from ILs in Chapter Three and RILs in Chapter Four. The analysis of the IL QTLs reveal large numbers of genes within the regions, some of which are known to affect dauer larvae development; affecting the dauer pathways such as the *daf* family, or chemosensory genes used in assessing their environment such as the *che* family. It is also shown that a number of the QTL regions contain, in some cases, large numbers of genes which are deleted in the CB4856 genotype and so were not further analysed. Finally, these analyses identify a number of these QTLs contain genes which are associated with laboratory domestication in N2, defined through a comparison with the LSJ1 genome sequence (Weber *et al.,* 2010). N2 and LSJ1 were cultivated separately before freezing methods became available, and since then have accrued 1208 genetic differences (Weber *et al.,* 2010). Some of these genes are interesting because of their known functions; such as *srg-47* and *str-200*, both chemosensory genes, and *npr-1* for its wide array of phenotypes. From this analysis, the QTLs on the *X* chromosome were chosen for candidate genes discovery, as these QTLs contained no gene deletions in CB4856. *npr-1* was chosen as a candidate gene for further analysis because of its position within QTL21 and it's well known for a number of phenotypes but is not known to affect dauer larvae development. *srg-36* and *-37* were also chosen as candidates for further analysis, located underlying QTL24, they have recently been shown to affect dauer larvae development on standard dauer assays (McGrath *et al.,* 2011).

Analysis of the three *npr-1* mutants show an effect on dauer larvae development in growing populations of *C. elegans*, to reduce the number of dauer larvae formed without affecting the population size (Fig 5.1). These data also indicate an allelic-specific effect, with one

mutant, DA609, showing a more pronounced negative effect, and the other two mutants, DA508 and DA650, only showing significantly lower numbers in one out of the three assays. *npr-1* is responsible for the choice between social (bordering on bacterial lawn) and solitary (evenly dispersed on bacterial lawn) feeding in *C. elegans* (de Bono and Bargmann, 1998). Given that it has previously been shown there is no effect of bordering behaviour on dauer larvae development in standard dauer assays (Viney *et al*., 2003), this demonstrates an example of an indirect effect on dauer larvae development. Lower numbers of dauer larvae are observed in the *npr-1* mutants, the opposite result to what one might predict if clumping behaviour, another feeding behaviour associated with *npr-1* (de Bono and Bargmann, 1998), where large numbers of worms gather in one area on a bacterial lawn and tend to burrow into the agar medium, produces high local pheromone concentrations and low local food levels. The reduced numbers of dauer larvae formed in the *npr-1* mutants also suggests that there is significant genetic variation which would act to increase dauer larvae development in many wild isolates as the majority of these lines display the clumping phenotype and still produce more dauer larvae than N2 (Fig 5.2 and Chapter Three).

For the RNAi analysis of *srg-36* and *-37*, the susceptible and resistant lines were analysed for any differences from N2. These analyses indicated that the mutations in the lines had deleterious effects, reducing the population size, the number of dauer larvae formed in a growing population and their lifetime fecundity (Fig 5.3). This unfortunately means that the RNAi analysis of *srg-36* and *-37* is inconclusive due to the differences life-history traits of the mutant lines. RNAi analysis of these genes reveals interesting results however. Analysis of the three lines, NL2099 (*rrf-3* gene conferring hypersensitivity to RNAi), NL3321 (*sid-1* gene conferring resistance to RNAi) and N2 when fed OP50 show the deleterious effects, but this

difference is not seen when they are fed the RNAi clone for *srg-36* and a mixture of *srg-36* and *-37*. This could indicate that N2 is also affected by the RNAi clone, and the number of dauer larvae formed is being reduced. Further analysis of *srg-36* and *-37* was performed using the NILs CX13249 (N2 background and LSJ2 at this genomic region) and CX13591 (N2 background and CC1 at this genomic region), and the IL ewIR89 which contains the CB4856 region for these genes. This reveals that all lines form fewer dauer larvae with no difference in the population size in a growing population, only CX13591 has a higher population size on one of the assays and no difference in the number of dauer larvae formed. Together, these analyses indicate that there is an effect by the two *srg* genes to lower the number of dauer larvae formed in a growing population. This result shows similar findings to McGrath *et al.,* (2011), where they found dauer larvae formation in response to ascaroside C3 was greatly reduced, in a standard dauer larvae assay (McGrath *et al.,* 2011). As these *srg* genes confer a resistance to the ascaroside C3 component of the dauer pheromone the worms will be in contact with all other parts in a growing population, a reason why dauer larvae numbers are significantly lower than N2, though still present in a growing population assay.

Here we have demonstrated that analysis of QTL regions results in large numbers of potential candidate genes for further investigation (Tables 5.1 and 5.2). With the identification of genes known to be associated with dauer larvae development found in 20 of the 24 QTL. Candidate genes taken from this analysis and further investigated have shown that genes known to affect dauer larvae development in standard dauer assays, using a single cohort of age matched individuals, show similar results to a growing population assay. An *npr-1* effect on dauer larvae development in growing populations has also been demonstrated where an effect using a single cohort of age matched individuals

showed nothing (Viney *et al.,* 2003). This indicates that it is important to analyse the other alleles associated with laboratory domestication. With the number of potential genes underlying a QTL it is improbable to investigate them all, however, it is clear that each QTL needs to be further investigated, including the genes which are known to affect dauer larvae formation, as this style of dauer larvae analysis can reveal differences not seen when using single cohorts of age matched individuals.

CHAPTER SIX: General discussion and future directions

PRINCIPLE FINDINGS

Growing population assays:

- are a feasible way to investigate dauer larvae development, and compliment other methods
- allow extensive variation in life-history traits to be uncovered
- demonstrate the high genomic complexity of dauer larvae development through the identification of a large number of QTLs for the trait
- link novel genes to dauer larvae development, previously not known to be involved in the trait
- provide advanced insight into the ecology of *C. elegans*

This thesis describes the development of methods that allow the analysis of dauer larvae development in growing populations of *C. elegans* (Chapter Two). Analysis of the canonical *C. elegans* isolate N2 indicated that food availability, dauer pheromone (a measure of conspecific population density) and temperature affect dauer larvae development in growing populations as would be predicted from analyses of single synchronised cohorts of worms, the standard way to examine dauer larvae formation. This is important as it validates the assay. Critically, these analyses also demonstrated both that dauer larvae are formed prior to patch exhaustion once a certain critical level of food is exceeded and that the number of dauer larvae present increases after the patch is exhausted, *i.e.* worms that had not completed development as dauer larvae when the food was exhausted continue development in the absence of bacterial food.

The work shown in this thesis also indicates that dauer larvae which complete development after the exhaustion of the bacterial food patch have a reduced reproductive fitness in comparison with dauer larvae that develop prior to patch exhaustion. Given that fitness of arrested dauer larvae is known to decline over time (Klass and Hirsh, 1974; Kim & Paik, 2008), it would be good to see if this dauer survival interacts with the fitness effects observed here.

Using the methods developed for the analysis of growing populations, extensive natural genetic variation in dauer larvae development within growing populations across multiple wild isolates is uncovered (Chapter Three). Interestingly, this variation presents a different picture to that seen in analyses of single synchronised cohorts of worms (Viney *et al*., 2003), in that here many of the wild isolates form more dauer larvae than N2. This observation suggests that there may be more complex interactions between the production of pheromone by an isolate and the perception of the pheromone.

To examine the genetic basis of variation of dauer larvae development in growing populations, introgression lines (ILs) derived from the genetically divergent isolates N2 (Bristol, UK) and CB4856 (Hawaii) were analysed (Chapter 3). Analysis revealed a total of 24 QTL affecting dauer larvae formation; 10 QTLs identified through bin mapping, 18 QTLs identified through single IL analysis and 20 QTLs identified through sequential pairwise analysis, representing the most QTLs identified in a single screen to date in *C. elegans*. Further analysis of the QTLs (Chapter Five) showed that they are variable across environments with negative effect QTLs detectable under a wider range of conditions and some positive effect QTLs only being identified with the highest volume of food. However, most of the QTLs are confirmed in multiple tests of individual genotypes. These findings

validate the QTLs detected, and demonstrate the complex genetic architecture of variation in dauer larvae formation in *C. elegans*. The environmental interactions demonstrated here, particularly in the way that the positive and negative effect QTLs differ in their response, represent an interesting avenue for future research.

To further investigate the genetic basis of dauer larvae development, three RIL panels, two constructed with different *C. elegans* parental isolates (CB4856 x N2 and DR1350 x N2) and one from *C. briggsae* (HK104 x AF16)*,* were examined for dauer larvae development in growing populations (Chapter Four). These analyses show extensive variation in dauer formation in the CB4856 x N2 RIL panel, similar to the analysis of the Chapter Three CB4856 x N2 ILs. In contrast, while there is variation in dauer formation in the DR1350 x N2 RIL panel, it is considerably less. Mapping of these data also revealed differences between the two panels; the CB4856 x N2 RILs identifying two QTLs, one each on chromosomes *II* and *IV*, the DR1350 X N2 RILs identifying one QTL on the *X* chromosome. Comparison of these two genetically divergent isolates suggests that there are differences due to the selective history, DR1350 being more genetically related to N2 than CB4856 due to their genetic isolation (Koch *et al.,* 2000; Andersen *et al.,* 2012). Comparison of the CB4856 x N2 RILs with the ILs from Chapter Three indicate that there are also differences due to the methodologies. This can be explained by the differences in composition of their introgressions, and shows that the power of ILs in identifying genomic regions controlling dauer larvae formation is greater than that of RILs and again demonstrates the complexity of dauer larvae development in growing populations. Another consequence of the methodologies is a comparison between growing populations and the standard dauer larvae formation analysis, demonstrated by the difference in QTLs detected in a previous study of

dauer larvae formation using the DR1350 x N2 RILs (Harvey *et al.,* 2008) where three QTLs were identified, compared to the single QTL identified in this study. The *C. briggsae* RIL panel was analysed as a comparative study of dauer larvae development in growing populations with four QTLs for dauer larvae formation in growing populations identified in this panel.

Of the 24 QTLs identified, analysis showed that 20 contain genes known to be associated with dauer larvae development, these QTLs could therefore be a consequence of variation at the loci; out of the 47 potential genes, 27 of them have a polymorphism between N2 and CB4856. These analyses have also identified regions which contain alleles associated with domestication in the laboratory in the N2 genotype in 5 QTLs, and also regions which have genes not present in the CB4856 genotype (Chapter Five). Three candidate genes; *npr-1*, *srg-36* and *srg-37* were further analysed for their effect on dauer larvae development in growing populations. Analysis of *npr-1* shows that variation at *npr-1* acts to reduce dauer larvae development in growing populations, with the CB4856 allele acting to lower the number of dauer larvae. Additional analysis indicates that the bordering phenotype, known to be caused by variation of *npr-1*, does not correlate with dauer larvae formation in wild isolates, suggesting that aggregation caused by attraction to and avoidance of O_2 and CO_2 does not affect dauer larvae formation. NILs containing deletions of *srg-36* and *srg-37* show reduced dauer larvae development in growing populations. RNAi analysis of *srg-36* and *srg-37* are inconclusive due to the life history differences observed between the mutant lines of differing RNAi sensitivity. These analyses therefore indicate that at least one domestication allele affects dauer larvae development in growing populations, suggesting that several

other QTLs could be explained by similar domestication alleles, and that analysis of complex traits by RNAi in *C. elegans* is complicated by the difference between lines.

GENERAL DISCUSSION AND SIGNIFICANCE

It has been established that population level analyses of *C. elegans* are feasible and that these analyses support previous studies of the environmental factors affecting dauer larvae development. Extensive variation in dauer larvae formation has been demonstrated, as well as a fitness loss of dauer larvae formed in different circumstances. Analysis of the quality of their food source suggests there is also variation which is likely to represent adaptation to differing quantities or qualities of food, as well as an adaptive benefit for variation between isolates. The ecology of *C. elegans* is still poorly understood, and as such, these findings help to understand the ecological relevance of the variation between isolates in their sensitivity to dauer inducing conditions. Comparing the data on growing population assays and the recent data on natural propagating populations, it is clear there are similarities. Both the laboratory assays and the wild populations can maintain a large number of nematodes of all life stages, with a large percentage of them being dauer larvae (Félix and Duveau, 2012). The naturally occurring populations have a larger percentage of dauer larvae formed than the growing populations bred in the laboratory (Félix and Duveau, 2012). As the number of dauer larvae formed in a growing population in a 2D environment is fewer than those formed in a 3D environment, it could be inferred that 1. the 3D assay is a nearer approximation of the natural environment of *C. elegans* and 2. there are other variables found in nature that affect, and increase, dauer larvae formation. One potential variable that would be interesting for further examination would be to investigate the role of natural

food sources, either singularly or as a combination of potential food sources. Another potential variable for further examination is temperature, as temperatures in the wild vary considerably throughout the day, as opposed to the constant temperatures when cultured in a laboratory. The reproductive difference represents one aspect of dauer larvae fitness, and other aspects, such as fitness loss with age of dauer larvae and any potential maternal effects would also need to be investigated.

That the genetic basis of the extensive variation in dauer larvae development in growing populations can be examined with the use of ILs and RILs has also been demonstrated. Such an extensive analysis of a phenotype has not been done in nematodes, resulting in an unprecedented number of QTLs for a single trait from one mapping panel, and has been useful in assessing the power of analysis using ILs and RILs for a complex trait. Dauer larvae development in growing populations is highly variable, and is under large amount of environmental constraint which affects the detection of QTLs. One complication of the results obtained in the QTL analysis is that some QTLs are likely to be a consequence of Dobzhansky-Muller incompatibilities (Haerty and Singh, 2006; Orr and Turelli, 2007; Seidel *et al.,* 2008), caused by incompatibilities that reduce population growth rates, for example, fecundity and reproductive timing (Snoek *et al.,* 2014), which will affect dauer larvae development.

The establishment of a validated assay for the investigation of dauer larvae development in a growing population has added a useful tool for the study of *C. elegans* dauer larvae development, and more generally, for the study of the genetics of complex traits. A comparison of the methods for genetic analysis revealed that the use of ILs is far more successful in identifying QTLs for dauer larvae development that using RILs, and that analysis

of the QTLs can be followed through to low numbers of potential genes. Using different combinations of *C. elegans* strains in the RIL panels did not reveal significant data to suggest that, although adapted to laboratory conditions, N2 and CB4856 should be abandoned in studying complex traits. The selfing mode of reproduction of *C. elegans* is not likely to be a factor in the study of complex traits which are not sex biased, such as dauer larvae development, as analysis of male/female reproduction using *fog-2* mutants did not reveal any significant different in dauer larvae development in a growing population (unpublished data).

A number of candidate genes for dauer larvae development in growing populations underlying the QTLs were identified and further analysed. In investigating *npr-1*, a locus of interest as a laboratory domesticated allele in N2, I demonstrated an effect to reduce the number of dauer larvae formed. Investigation of *srg-36* and *-37*, both important for sensing the small molecule signals in the dauer pheromone, also showed an effect to reduce the numbers of dauer larvae formed. These analyses have, respectively, shown that a gene which has previously been shown to have no effect on dauer larvae development with a single cohort of worms has an effect in a growing population, and that genes which are known to effect dauer larvae development with a single cohort of worms also have an effect in growing populations. This indicates that both assay styles complement each other as either is likely to identify subtle differences in dauer larvae induction which the other cannot. These results suggest that other alleles linked to domestication in N2, should be analysed in growing populations, as well as analysing all genes known to effect dauer larvae development in standard dauer assays (Green *et al.,* 2014).

Together, this analysis on dauer larvae development in growing populations shows that there is extensive natural variation in this complex trait. This study also shows that the genetic architecture of dauer larvae development in a growing population is highly complex, matching original studies of dauer larvae development within a single cohort of worms. An issue of interest both because of our limited understanding of the ecology of *C. elegans* and this trait represented a potentially tractable system for the analysis of the genetics of complex traits, a question of more general significance. Understanding the genetic architecture and control of complex traits is very important as it allows us to examine how they have evolved. This has ecological, as well as human health, implications as many complex traits result in disease.

FUTURE DIRECTIONS

- To identify all genes associated with dauer larvae development in growing populations, with a comparison to genes linked to dauer larvae development using the standard dauer assay
- To discover the causal allelic variation for the observed difference in phenotype
- To compare the mapped incompatibilities of N2 and CB4856
- To investigate dauer larvae development, in ILs, RILs and/or RIAILS of different parental isolates (neither N2 or CB4856) and in a large collection of natural isolates allowing GWAS analysis, under a range of environmental conditions
- To investigate dauer larvae fitness after exposure to consequential pressures, such as heat shock, poisoning and pathogens

- To perform robust comparisons of laboratory grown and naturally propagating populations
- To investigate potential aspects of pheromone use on rivals and kin

Future directions of the work shown in this thesis are to identify all genes affecting dauer larvae development in growing populations which underlie the QTLs found. Linking to this, dauer larvae development using the ILs, though under the standard dauer assay, i.e. investigating the likelihood of forming dauer larvae, will show any overlapping QTLs and genes similar in both assays. Having these two sets of data, it will be possible to discern some of the components affecting the dauer larvae development trait, i.e. the same genes recognised in both assays would indicate that they are food and/or pheromone perception chemosensory genes. Any genes which are not familiar in both assays could then suggest that other life-history traits, such as reproductive timing and life-time fecundity, could be consequentially causing the variation in dauer larvae development in growing populations. From here, work would focus on discovering the alleles which cause variation in the phenotype.

It would be beneficial to compare the mapped incompatibilities (Snoek *et al.,* 2014) to ascertain the true dauer larvae development QTLs, forgoing QTLs that are a consequence of reduced fitness caused by any incompatibilities due to the distant genetic relatedness of the two parental isolates, such as reproductive output. Since there are a number of incompatibilities associated with inbred lines from N2 and CB4856 it would be very beneficial to investigate ILs and RILs of different parental lines, using recently sampled wild isolates that have also not had an opportunity to become adapted to the laboratory

environment. This would improve our knowledge of the genetic architecture of natural genetic variation. Investigating dauer larvae development in a growing population under a wider range of environmental conditions would also help towards understanding how the control of variation in dauer larvae development has evolved. Analysis of a larger group of wild isolates would also be advantageous as a full GWAS mapping analysis could then be performed.

Investigating the causes of fitness differences between dauer larvae would be interesting; are they due to provisioning (e.g. lipid storage levels) or if there are other differences, for example epigenetic effects? Exploring the fitness of choosing to form dauer larvae and the fitness of those dauer larvae due to natural environmental pressures, for example pathogens, dehydration, temperature fluctuations and competition would also be interesting. In combination, such analyses would allow a greater understanding of the likely long-term effects of the variation in dauer larvae fitness observed in this thesis.

As the natural habitat of *C. elegans* has only recently been discovered (Félix and Duveau, 2012) it would be valuable to compare naturally propagating and laboratory grown populations, with the associated intra- and interspecific relationships. The population sizes found in this thesis are larger than the ones identified by Félix and Duveau, 2012, though the ratio of dauer larvae to the total number of worms found is much larger in their work, up to 70% dauer larvae in one natural population (Félix and Duveau, 2012). This could be explained by a number of, potentially interconnected, environmental factors such as the difference between a constant laboratory temperature and the fluctuating temperatures found in their natural environment, the presence of pathogenic bacteria and which bacteria they use as a food source. When worms are fed on dead bacteria in the laboratory, there

are drastic differences in the population and the number of dauer larvae formed in a growing population, resulting in a much larger ratio of dauer larvae to the total number of worms (Appendix Fig 4). Also, heat shock induces the formation of dauer larvae (Ailion and Thomas, 2000), touching on the effects of living in a harsh environment.

Finally, interactions between isolates competing for resources would be interesting to investigate further, as the dauer pheromone might represent a way in which worms could recognise and respond to kin or rivals (for some discussion of this question, see Viney and Franks 2004). The findings of Mayer and Sommer (2011) when investigating dauer larvae formation in *P. pacificus*, show that an isolates response to their own pheromone is different to another's pheromone, suggest this is a possibility in *C. elegans*.

BIBLIOGRAPHY

- Aidoo M, Terlouw DJ, Kolczak MS, McElroy PD, ter Kuile FO, Kariuki S, Nahlen BL, Lal AA and Udhayakumar V (2002). Protective effects of the sickle cell gene against malaria morbidity and mortality. *The Lancet* **359**: 1311-1312
- Ailion M and Thomas JH (2000). Dauer formation induced by high temperatures in *Caenorhabditis elegans*. *Genetics* **156**: 1047–1067
- Albert PS and Riddle DL (1983). Developmental alterations in sensory neuroanatomy of the *Caenorhabditis elegans* dauer larva. *Journal of Comparative Neurology* **219**: 461-481
- Alcedo J and Kenyon C (2004). Regulation of *C. elegans* longevity by specific gustatory and olfactory neurons. *Neuron* **41**: 45-55
- Altermatt F and Ebert D (2008). The influence of pool volume and summer desiccation on the production of the resting and dispersal stage in a *Daphnia* metapopulation. *Oecologia* **157**: 441-452
- Altermatt F and Ebert D (2010). Populations in small, ephemeral habitat patches may drive dynamics in a *Daphnia magna* metapopulation. *Ecology* **91**: 2975-2982
- Andersen EC, Bloom JS, Gerke JP and Kruglyak L (2014). A variant in the neuropeptide receptor *npr-1* is a major determinant of *Caenorhabditis elegans* growth and physiology. *PLoS Genetics* **10(2):** e1004156
- Andersen EC, Gerke JP, Shapiro JA, Crissman JR, Ghosh R, Bloom JS, Félix MA and Kruglyak L (2012). Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. *Nature Genetics* **44**: 285-290
- Anderson P (1995). Mutagenesis. *Methods in Cell Biology* **48**: 31-58
- Angilletta Jr. MJ, Steury TD and Sears MW (2004). Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integrative and Comparative Biology* **44**: 498-509
- Anholt RRH and Mackay TFC (2004). Quantitative genetic analyses of complex behaviours in *Drosophila*. *Nature Review Genetics* **5**: 838-849
- Arendt JD (2010). Size-fecundity relationships, growth trajectories, and the temperature-size rule for ectotherms. *Evolution* **65**: 43-51

- Arnold FH, Wintrode PL, Miyazaki K and Gershenson A (2001). How enzymes adapt: lessons from directed evolution. *Trends in Biochemical Sciences* **26**: 100-106
- Ashton FT, Bhopale VM, Holt D, Smith G, Schad GA (1998). Developmental switching in the parasitic nematode *Strongyloides stercoralis* is controlled by the ASF and ASI amphidial neurons. *The Journal of Parasitology* **84**: 691-695
- Avery L (1993). The genetics of feeding in *Caenorhabditis elegans*. *Genetics* **133**: 897-917
- Ayroles JF, Carbone MA, Stone EA, Jordan KW, Lyman RF, Magwire MM, Rollmann SM, Duncan LH, Lawrence F, Anholt RRH and Mackay TFC (2009). Systems genetics of complex traits in *Drosophila melanogaster*. *Nature Genetics* **41**: 299-307
- Ayyadevara S, Ayyadevara R, Hou S, Thaden JJ and Shmookler Reis RJ (2001). Genetic Mapping of Quantitative Trait Loci Governing Longevity of *Caenorhabditis elegans* in Recombinant-Inbred Progeny of a Bergerac-BO 3 RC301 Interstrain Cross. *Genetics* **157**: 655–666
- Ayyadevara S, Ayyadevara R, Vertino A, Galecki A, Thaden JJ and Reis RJS (2003). Genetic loci modulating fitness and life span in *Caenorhabditis elegans*: categorical trait interval mapping in CL2a X Bergerac-BO recombinant-inbred worms. *Genetics* **163**: 557–570
- Bach L, Jörgen R and Lundberg P (2007). On the evolution of conditional dispersal under environmental and demographic stochasticity. *Evolutionary Ecology Research* **9**: 663-673
- Bargmann CI and Horvitz HR (1991). Control of larval development by chemosensory neurons in *Caenorhabditis elegans*. *Science* **251**: 1243-1246
- Bargmann CI, Hartwieg E and Horvitz HR (1993). Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell* **74**: 515–527
- Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, Plagnol V, Pociot F, Schuilenburg H, Smyth DJ, Stevens H, Todd JA, Walker NM, Rich SS and The Type 1 Diabetes Genetics Consortium (2009). Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nature Genetics* **41**: 703-707

* Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM , Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ, the NIDDK IBD Genetics Consortium, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E, the Belgian-French IBD Consortium, the Wellcome Trust Case Control Consortium, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M and Daly MJ (2008). Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nature Genetics* **40**: 955-962
* Barrière A and Félix MA (2005). High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. *Current Biology* **15**: 1176-1184
* Barrière A and Félix MA (2007). Temporal dynamics and linkage disequilibrium in natural *Caenorhabditis elegans* populations. *Genetics* **176**: 999-1011
* Basten CJ, Weir BS and Zeng ZB (2002). QTL Cartographer, Version 1.16. Department of Statistics, North Carolina State University, Raleigh, NC
* Beavis WD (1998). *QTL analyses: power, precision, and accuracy*. Cited in Molecular dissection of complex traits. CRC Press LLC, USA. Pages 145-162
* Belknap JK, Hitzemann R, Crabbe JC, Phillips TJ, Buck KJ and Williams RW (2001). QTL analysis and genome-wide mutagenesis in mice: Complementary genetic approaches to the dissection of complex traits. *Behavior Genetics* **31**: 5-15
* Bengtsson J (1998). Which species? What kind of diversity? Which ecosystem function? Some problems in studies of relations between biodiversity and ecosystem function. *Applied Soil Ecology* **10**: 191-199
* Berns K, Hijmans EM, Mullenders J, Brummelkamp TR, Velds A, Helmerikx M, Kerkhoven RM, Madiredo M, Nijkamp W, Weigelt B, Agami R, Ge W, Cavet G, Linsley PS, Beijersbergen RL and Bernards R (2004). A Large-scale RNAi screen in human cells identifies new components of the p53 pathway. *Nature* **428**: 431-437

- Blacker D, Bertram L, Saunders AJ, Moscarillo TJ, Albert MS, Wiener H, Perry RT, Collins JS, Harrell LE, Go RCP, Mahoney A, Beaty T, Fallin MD, Avramopoulos D, Chase GA, Folstein MF, McInnis MG, Bassett SS, Doheny KJ, Pugh EW, Tanzi RE and The NIMH Genetics Initiative Alzheimer's Disease Study Group (2003). Results of a high-resolution genome screen of 437 Alzheimer's Disease families. *Human Molecular Genetics* **12**: 23-32
- Boer MP, Wright D, Feng L, Podlich DW, Luo L, Cooper M and van Eeuwijk FA (2007). A mixed-model quantitative trait loci (qtl) analysis for multiple-environment trial data using environmental covariables for qtl-by-environment interactions, with an example in maize. *Genetics* **177**: 1801–1813
- Bowler DE and Benton TG (2005). Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews* **80**: 205-225
- Bowler DE and Benton TG (2008). Maternal effects mediated by maternal age: from life histories to population dynamics. *Journal of Animal Ecology* **77**: 1038-1046
- Bowler DE and Benton TG (2009). Impact of dispersal on population growth: the role of inter-patch distance. *Oikos* **118**: 403-412
- Boyd WA, McBride SJ and Freedman JH (2007). effects of genetic mutations and chemical exposures on *Caenorhabditis elegans* feeding: evaluation of a novel, high-throughput screening assay. *PLoS One* **12:** e1259
- Brenner S (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**: 7-94
- Burke DT, Kozloff KM, Chen S, West JL, Wilkowski JM, Goldstein SA, Miller RA and Galecki AT (2012). Dissection of complex adult traits in a mouse synthetic population. *Genome Research* **22**: 1549-1557
- Butcher RA, Fujita M, Schoeder FC and Clardy J (2007). Small-molecule pheromones that control dauer development in *Caenorhabditis elegans*. *Nature Chemical Biology* **3**: 420-422
- Butcher RA, Ragains JR, Kim E and Clardy J (2008). A potent dauer pheromone component in *Caenorhabditis elegans* that acts synergistically with other components. *Proceedings of the National Academy of Sciences* **105**: 14288-14292

- Butcher RA, Ragains JR, Li W, Ruvkun G, Clardy J and Mak HY (2009). Biosynthesis of the *Caenorhabditis elegans* dauer pheromone. *Proceedings of the National Academy of Sciences* **106:** 1875-1879
- Byerly, L., Cassada, R. C. and Russell, R.L. 1976 The life cycle of the nematode *Caenorhabditis elegans*. I. Wild-type growth and reproduction. *Developmental Biology* **51:** 23-33
- Cassada RC and Russell RL (1975). The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. *Developmental Biology* **46**: 326-342
- Castelletto ML, Massey HC Jr and Lok JB (2009). Morphogenesis of *Strongyloides stercoralis* infective larvae requires the DAF-16 ortholog FKTF-1. *PLoS Pathogens* **5:** e1000370
- Caswell-Chen EP, Chen J, Lewis EE, Douhan GW, Nadler SA and Carey JR (2005). Revising the standard wisdom of *C. elegans* natural history: ecology of longevity. *Science's SAGE KE* **40:** pe30
- Chen J and Caswell-Chen EP (2004). Facultative vivipary is a life-history trait in *Caenorhabditis elegans*. *Journal of Nematology* **36**: 107-113
- Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SGF, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK and Stevens RC (2007). High resolution crystal structure of an engineered human β2-adrenergic g protein-coupled receptor. *Science* **318**: 1258-1265
- Chown SL, Hoffmann AA, Kristensen TN, Angilletta Jr MJ, Stenseth NC and Pertoldi C (2010). Adapting to climate change: a perspective from evolutionary physiology. *Climate Research* **43**: 3
- Clotuche G, Navajas M, Mailleux AC and Hance T (2013) Reaching the ball or missing the flight? collective dispersal in the two-spotted spider mite *Tetranychus urticae*. *PLoS One* **8**: e77573
- Clutton-Brock TH and Lukas D (2011). The evolution of social philopatry and dispersal in female mammals. *Molecular Ecology* **21**: 472-492
- Coleman DL and Eicher EM (1990). Fat (fat) and tubby (tub): Two autosomal recessive mutations causing obesity syndromes in the mouse. *Journal of Heredity* **81**: 424-427

- Conde L, Bracci PM, Richardson R, Montgomery SB and Skibola CF (2013). Integrating GWAS and expression data for functional characterization of disease-associated SNPs: An application to follicular lymphoma. *The American Journal of Human Genetics* **92**: 126-130
- Cookson W, Liang L, Abecasis G, Moffatt M and Lathrop M (2009). Mapping complex disease traits with global gene expression. *Nature Reviews Genetics* **10**: 184-194
- Cornfield J, Haenszel W, Hammond EC, Lilienfeld AM, Shimkin MB and Wynder EL (2009). Smoking and lung cancer: recent evidence and a discussion of some questions. *International Journal of Epidemiology* **38**: 1175-1191
- Cornils A, Gloeck M, Chen Z, Zhang Y and Alcedo J (2011). Specific insulin-like peptides encode sensory information to regulate distinct developmental processes. *Development* **138**: 1183-1193
- Cousyn C, De Meester L, Colbourne JK, Brendonck L, Verschuren D and Volckaert F (2001). Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. *Proceedings of the National Academy of Sciences* **98**: 6256-6260
- Crane YM and Gelvin SB (2007). RNAi-mediated gene silencing reveals involvement of *Arabidopsis* chromatin-related genes in *Agrobacterium*-mediated root transformation. *Proceedings of the National Academy of Sciences* **104**: 15156-15161
- Crossan J, Paterson S and Fenton A (2006). Host availability and the evolution of parasite life-history strategies. *Evolution* **61**: 675-684
- Curtsinger JW and Khazaeli AA (2002). Lifespan, QTLs, age-specificity, and pleiotropy in *Drosophila*. *Mechanisms of Ageing and Development* **123**: 81–93
- Darvasi A and Pisanté-Shalom A (2002). Complexities in the genetic dissection of quantitative trait loci. *Trends in Genetics* **18**: 489-491
- de Bono M and Bargmann CI (1998). Natural variation in a neuropeptide Y receptor homolog modifies social behaviour and food response in *C. elegans*. *Cell* **94**: 679-689
- De Stasio EA and Dorman S (2001). Optimization of ENU mutagenesis of *Caenorhabditis elegans*. *Mutation Research* **495**: 81-88

- Diaz SA, Brunet V, Lloyd-Jones G, Spinner W, Wharam B and Viney M (2014). Diverse and manipulative signalling with ascarosides in the model nematode *C. elegans*. *BMC Evolutionary Biology* **14**: 46
- Dietzl G, Chen D, Schnorrer F, Su K, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblauer S, Couto A, Marra V, Keleman K and Dickson BJ (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. *Nature* **448**: 151-156
- Dodson S (1989). Predator-induced reaction norms. *Bioscience* **39**: 447-452
- Dorman JB, Albinder B, Shroyer T and Kenyon C (1995). The *age-1* and *daf-2* genes function in a common pathway to control the lifespan of *Caenorhabditis elegans*. *Genetics* **141**: 1399-1406
- Doroszuk A, Snoek LB, Fradin E, Riksen J and Kammenga J (2009). A genome-wide library of CB4856/N2 introgression lines of *Caenorhabditis elegans*. *Nucleic Acids Research* **37:** e110
- Duraisingh MT and Lodish HF (2012). Sickle cell microRNAs Inhibit the malaria parasite. *Cell Host and Microbe* **12**: 127-128
- Dytham C (2003). *Choosing and using statistics*: *A Biologists Guide*. 2nd Edn. Blackwell Science, Oxford
- Dytham C and Travis JMJ (2006). Evolving dispersal and age at death. *Oikos* **113**: 530-538
- Econopouly BF, McKay JK, Westra P, Lapitan NLV, Chapman PL and Byrne PF (2011). backcrossing provides an avenue for gene introgression from wheat to jointed goatgrass (*Aegilops cylindrica*) in the U.S. great plains. *Weed Science* **59**: 188-194
- Edelsparre AH, Vesterberg A, Lim JH, Anwari M and Fitzpatrick MJ (2014). Alleles underlying larval foraging behaviour influence adult dispersal in nature. *Ecology Letters* **17**: 333-339
- Edwards CA (1983). Earthworm ecology in cultivated soils. Earthworm Ecology. Springer Netherlands. 123-137
- Elvin M, Snoek L, Frejno M, Klemstein U, Kammenga J and Poulin G (2011). A fitness assay for comparing RNAi effects across multiple *C. elegans* genotypes. *BMC Genomics* **12**: 510

- Emery P (2007). Mutagenesis with *Drosophila*. *Methods in Molecular Biology* **362**: 187-195
- Esteller M (2011). Non-coding RNAs in human disease. *Nature Reviews Genetics* **12**: 861-874
- Félix MA and Braendle C (2010). The natural history of *Caenorhabditis elegans*. *Current Biology* **20**: 965-969
- Félix MA and Duveau F (2012). Population dynamics and habitat sharing of natural populations of *Caenorhabditis elegans* and *C. briggsae. BMC Biology* **10**: 59
- Félix MA, Jovelin R, Ferrari C, Han S, Cho YR, Andersen EC, Cutter AD and Braendle C (2013). Species richness, distribution and genetic diversity of Caenorhabditis nematodes in a remote tropical rainforest. *BCM Evolutionary Biology* **13**: 10
- Fellous S, Quillery E, Duncan AB and Kaltz O (2010). Parasitic infection reduces dispersal of ciliate host. *Biology Letters* **7**: 327-329
- Fields PA (2001) Review: Protein function at thermal extremes: Balancing stability and flexibility. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **129**: 417–431
- Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE and Mello CC (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans. Nature* **391**: 806–811
- Fisher RA (1918). The correlation between relatives on the supposition of Mendelian inheritance. *Transactions of the Royal Society of Edinburgh* **52**: 399-433
- Fjerdingstad EJ, Schtickzelle N, Manhes P, Gutierrez A and Clobert J (2007). Evolution of dispersal and life history strategies – *Tetrahymena* ciliates. *BCM Evolutionary Biology* **7**: 133
- Forbes VE, Olsen M, Palmqvist A and Calow P (2010). Environmentally sensitive life-cycle traits have low elasticity: implications for theory and practice. *Ecological Applications* **20**: 1449–1455
- Gaertner BE, Parmenter MD, Rockman MV, Kruglyak L and Phillips PC (2012). More than the sum of its parts: a complex epistatic network underlies natural variation in thermal preference behavior in *Caenorhabditis elegans*. *Genetics* **192**: 1533-1542

- Gao P, Jiao Y, Xiong Q, Wang CY, Gerling I and Gu W (2008). Genetic and molecular basis of QTL of diabetes in mouse: genes and polymorphisms. *Current Genomics* **9**: 324-337
- Gems D and Partridge L (2013). Genetics of longevity in model organisms: debates and paradigm shifts. *Annual Review of Physiology* **75**: 621-44
- Gems D and Riddle DL (2000). Defining wild-type life span in *Caenorhabditis elegans*. *Journal of Gerontology Series A: Biological Sciences and Medical Sciences* **55**: 215-219
- Gidalevitz T, Wang N, Deravaj T, Alexander-Floyd J and Morimoto RI (2013). Natural genetic variation determines susceptibility to aggregation or toxicity in a *C. elegans* model for polyglutamine disease. *BMC Biology* **11**: 100
- Gloria-Soria A and Azevedo RB (2008). *npr-1* regulates foraging and dispersal strategies in *Caenorhabditis elegans*. *Current Biology* **18**: 1694-1699
- Golden JW and Riddle DL (1982). A pheromone influences larval development in the nematode *Caenorhabditis elegans*. *Current Biology* **218**: 578-580
- Golden JW and Riddle DL (1984a). A pheromone-induced developmental switch in *Caenorhabditis elegans*: Temperature-sensitive mutants reveal a wild-type temperature-dependent process. *Proceedings of the National Academy of Sciences* **81**: 819-823
- Golden JW and Riddle DL (1984b). The *Caenorhabditis elegans* dauer larva: Developmental effects of pheromone, food, and temperature. *Developmental Biology* **102**: 368-378
- Golden JW and Riddle DL (1985). A gene affecting production of the *Caenorhabditis elegans* dauer-inducing pheromone. *Molecular and General Genetics* **198**: 534-6
- Goranson NC, Ebersole JP and Brault S (2005). Resolving an adaptive conundrum: reproduction in *Caenorhabditis elegans* is not sperm-limited when food is scarce. *Evolutionary Ecology Research* **7:** 325-333
- Gottlieb S and Ruvken G (1994). *daf-2*, *daf-16* and *daf-23*: Genetically interacting genes controlling dauer formation in *Caenorhabditis elegans*. *Genetics* **137**: 107-120
- Gowaty PA (1993). Differential dispersal, local resource competition and sex ratio variation in birds. *The American Naturalist* **141**: 263-280

- Green JWM and Harvey SC (2012). *Caenorhabditis elegans* dauer larvae development in growing populations. *Nematology* **14**: 165-173
- Green JWM, Snoek LB, Kammenga JE and Harvey SC (2013). Genetic mapping of variation in dauer larvae development in growing populations of *Caenorhabditis elegans*. *Heredity* **111**: 306-313
- Green JWM, Stastna JJ, Orbidans HE and Harvey SC (2014). Highly polygenic variation in environmental perception determines dauer larvae formation in growing populations of *Caenorhabditis elegans*. *PLoS One* **9**: e112830
- Greenawalt DM, Dobrin R, Chudin E, Hatoum IJ, Suver C, Beaulaurier J, Zhang B, Castro V, Zhu J, Sieberts SK, Wang S, Molony C, Heymsfield SB, Kemp DM, Reitman ML, Lum PY, Schadt EE and Kaplan LM (2011). A survey of the genetics of stomach, liver, and adipose gene expression from a morbidly obese cohort. *Genome Research* **21**: 1008-1016
- Greenwood PJ *(*1980*).* Mating systems, philopatry and dispersal in birds and mammals*. Animal Behaviour* **28***:* 1140–1162
- GuhaThakurta D, Palomar L, Stormo GD, Tedesco P, Johnson TE, Walker DW, Lithgow G, Kim S, and Link CD (2002). Identification of a novel cis-regulatory element involved in the heat shock response in *Caenorhabditis elegans* using microarray gene expression and computational methods. *Genome Research* **12**: 701-712
- Guisbert E, Herman C, Lu CZ, and Gross CA (2004). A chaperone network controls the heat shock response in *E. coli*. *Genes and Development* **18**: 2812-2821
- Gutteling EW, Doroszuk A, Riksen JAG, Prokop Z, Reszka J and Kammenga JE (2007a). Environmental influence on the genetic correlations between life-history traits in *Caenorhabditis elegans*. *Heredity* **98**: 206–213
- Gutteling EW, Riksen JAG, Bakker J and Kammenga JE (2007b). Mapping phenotypic plasticity and genotype–environment interactions affecting life-history traits in *Caenorhabditis elegans*. *Heredity* **98**: 28–37
- Haag ES (2005). The evolution of nematode sex determination: *C. elegans* as a reference point for comparative biology. *WormBook: the online review of* C. elegans *Biology* **1**

- Haerty W and Singh RS (2006). Gene regulation divergence is a major contributor to the evolution of Dobzhansky–Muller Incompatibilities between species of *Drosophila*. *Molecular Biology and Evolution* **23**: 1707-1714
- Hansen MM, Olivieri I, Waller DM and Nielsen EE (2012). Monitoring adaptive genetic responses to environmental change. *Molecular Ecology* **21**: 1311-1329
- Hanski I (1998). Metapopulation dynamics. *Nature* **365**: 41-49
- Hanski I and Beverton RJH (1994). Spatial Scale, patchiness and population dynamics on land. *Philosophical Transactions: Biological Sciences* **343**: 19-25
- Hanski I and Gilpin M (1991). Metapopulation dynamics: brief history and conceptual domain. *Biological Journal of the Linnean Society* **42**: 3–16
- Harbison ST, Chang S, Kamdar KP and Mackay TFC (2005). Quantitative genomics of starvation stress resistance in *Drosophila*. *Genome Biology* **6**: R36
- Harbison ST, Yamamoto AH, Fanara JJ, Norga KK and Mackay TFC (2004). Quantitative trait loci affecting starvation resistance in *Drosophila melanogaster*. *Genetics* **166**: 1807-1823
- Harper JM, Galecki AT, Burke DT, Pinkosky SL and Miller RA (2003). Quantitative trait loci for insulin-like growth factor I, leptin, thyroxine, and corticosterone in genetically heterogeneous mice. *Physiological Genomics* **15**: 44–51
- Hart AC, Sims S and Kaplan JM (1995). Synaptic code for sensory modalities revealed by C. elegans GLR-1 glutamate receptor. *Nature* **378**: 82 – 85
- Harvey SC (2009). Non-dauer Larval Dispersal in *Caenorhabditis elegans*. *Journal of Experimental Zoology* **312**: 224–230
- Harvey SC and Viney ME (2001). Sex determination in the parasitic nematode *Strongyloides ratti*. *Genetics* **158**: 1527-1533
- Harvey SC, Shorto A and Viney MA (2008). Quantitative genetic analysis of life-history traits of *Caenorhabditis elegans* in stressful environments. *BMC Evolutionary Biology* **8**: 15
- Hillier LW, Miller RD, Baird SE, Chinwalla A, Fulton LA, Koboldt DC and Waterston RH (2007). Comparison of *C. elegans* and *C. briggsae* Genome Sequences Reveals Extensive Conservation of Chromosome Organization and Synteny. *PLoS Biology* **5:** e167

- Hindorff LA, MacArthur J, Morales J, Junkins HA, Hall PN, Klemm AK, and Manolio TA. A catalog of published genome-wide association studies. Available at: www.genome.gov/gwastudies. Accessed [25/08/2013]
- Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS and Manolio TA (2009). Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proceedings of the National Academy of Sciences* **106**: 9362-9367
- Hodgkin J (1987). Primary sex determination in the nematode *C. elegans*. *Development* **101**: 5-16
- Hodgkin J and Barnes TM (1991). More is not better: Brood size and population growth in a self-fertilizing nematode. *Proceedings of the Royal Society B: Biological Sciences* **246**: 19-24
- Hodgkin J and Doniach T (1997). Natural variation and copulatory plug formation in *Caenorhabditis elegans*. *Genetics* **146**: 149-164
- Hodgkin JA and Brenner S (1977). Mutations causing transformation of sexual phenotype in the nematode *Caenorhabditis elegans*. *Genetics* **86**: 275-287
- Hoffmann AA and Willi Y (2008). Detecting genetic responses to environmental change. *Nature Reviews Genetics* **9**: 421-432
- Hoffmann MH (2005). Evolution of the realized climatic niche in the genus: *Arabidopsis* (brassicaceae). *Evolution* **59**: 1425-1436
- Holzenberger M, Dupont J, Ducos B, Leneuve P, Géloën A, Even PC, Cervera and Le Bouc Y (2002). IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* **421**: 182-187
- Honda S, Ishii N, Suzuki K and Matsuo M (1993). Oxygen-dependent perturbation of life span and aging rate in the nematode. *Journal of gerontology* **48**: B57-B61
- Howad W, Yamamoto T, Dirlewanger E, Testolin R, Cosson P, Cipriani G, Monforte AJ, Georgi L, Abbott AG and Arús (2005). Mapping with a few plants: using selective mapping for microsatellite saturation of the *Prunus* reference map. *Genetics* **171**: 1305-1309
- Hu PJ (2007). Dauer. *WormBook: the online review of* C. elegans *Biology* **1**

- Huang XQ and Röder MS (2011). High-density genetic and physical bin mapping of wheat chromosome 1D reveals that the powdery mildew resistance gene *Pm24* is located in a highly recombinogenic region. *Genetica* **139**: 1179-1187
- Hubner N, Wallace CA, Zimdahl H, Petretto E, Shulz H, Maciver F, Mueller M, Hummel O, Monti J, Zidek V, Musilova A, Kren V, Causton H, Game L, Born G, Schmidt S, Müller A, Cook SA, Kurtz TW, Whittaker J, Pravenec M and Aitman TJ (2005). Integrated transcriptional profiling and linkage analysis for identification of genes underlying disease. *Nature Genetics* **37**: 243 – 253
- Hughes SE, Evason K, Xiong C and Kornfeld K (2007). Genetic and pharmacological factors that influence reproductive aging in nematodes. *PLoS Genetics* **3**: e25
- Innocent TM, Abe J, West SA and Reece SE (2010). Competition between relatives and the evolution of dispersal in a parasitoid wasp. *Journal of Evolutionary Biology* **23:** 1374–1385
- Izrayelit Y, Srinivasan J, Campbell SL, Jo Y, von Reuss SH, Genoff MC, Sternberg PW and Schroeder FC (2012). Targeted metabolomics reveals a male pheromone and sex-specific ascaroside biosynthesis in *Caenorhabditis elegans*. *ACS Chemical Biology* **7**: 1321-1325
- Jackson AU, Galecki AT, Burke DT and Miller RA (2002). Mouse loci associated with life span exhibit sex-specific and epistatic effects. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **57**: 9-15
- Jang H, Kim K, Neal SJ, Macosko E, Kim D, Butcher RA, Zeiger DM, Bargmann CI and Sengupta P (2012). Neuromodulatory state and sex specify alternative behaviors through antagonistic synaptic pathways in *C. elegans*. *Neuron* **75**: 585-592
- Jenkins NL, McColl G and Lithgow GJ (2004). Fitness cost of extended lifespan in *Caenorhabditis elegans*. *Proceedings of the Royal Society B* **271**: 2523-2526
- Jensen VL, Bialas NJ, Bishop-Hurley SL, Molday LL, Kida K, Nguyen PAT, Blacque OE, Molday RS, Leroux MR and Riddle DL (2010a). Localization of a guanylyl cyclase to chemosensory cilia requires the novel ciliary MYND domain protein DAF-25. *PLoS Genetics* **6:** e1001199

- Jensen VL, Simonsen KT, Lee YH, Park D and Riddle DL (2010b). RNAi screen of DAF-16/FOXO target genes in *C. elegans* links pathogenesis and dauer formation. *PLoS One* **5**: e15902
- Jeong PW, Jung M, Yim YH, Kim H, Park M, Hong E, Lee W, Kim YH, Kim K and Paik YK (2005). Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-inducing pheromone. *Nature* **433**: 541-545
- Jordan KW and Mackay TFC (2006). Quantitative trait loci for locomotor behavior in *Drosophila melanogaster*. *Genetics* **174**: 271-284
- Jordan KW, Carbone MA, Yamamoto A, Morgan TJ and Mackay TFC (2007). Quantitative genomics of locomotor behavior in *Drosophila melanogaster*. *Genome Biology* **8**: R172
- Juilfs DM, Fülle HJ, Zhao AZ, Houslay MD, Garbers DL and Beavo JA (1997). A subset of olfactory neurons that selectively express cGMP-stimulated phosphodiesterase (PDE2) and guanylyl cyclase-D define a unique olfactory signal transduction pathway. *Proceedings of the National Academy of Sciences* **94**: 3388-3395
- Jump AS and Peñuelas J (2005). Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters* **8**: 1010-1020
- Justice MJ, Noveroske JK, Weber JS, Zheng B and Bradley A (1999). Mouse ENU mutagenesis. *Human Molecular Genetics* **8**: 1955-1963
- Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A Le Bot N, Moreno S, Sohrmann M, Welchman DP, Zipperlen P and Ahringer J (2003). Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* **421**: 231-237
- Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG and Ahringer J (2000). Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. *Genome Biology* **2**: 0002-1
- Kammenga JE, Doroszuk A, Riksen JAG, Hazendonk E, Spiridon L, Petrescu AJ, Tijsterman M, Platerk RHA and Bakker J (2007) A *Caenorhabditis elegans* wild type defies the temperature–size rule owing to a single nucleotide polymorphism in *tra-3*. *PLoS Genetics* **3:** e34

- Kammenga JE, Phillips PC, de Bono M and Doroszuk A (2008). Beyond induced mutants: using worms to study natural variation in genetic pathways. *Trends in Genetics* **24**: 178-185
- Kaplan F, Alborn HT, von Reuss SH, Ajredini R, Ali JG, Akyazi F, Stelinski LL, Edison AS, Schroeder FC and Teal PE (2012). Interspecific nematode signals regulate dispersal behaviour. *PLoS One* **7**: e38735
- Kaplan F, Srinivasan J, Mahanti P, Ajredini R, Durak O, Nimalendran R, Sternberg PW, Teal PEA, Schroeder FC, Edison AS and Alborn HT (2011). Ascaroside expression in *Caenorhabditis elegans* is strongly dependent on diet and developmental stage. *PLoS One* **6**: e17804
- Kaufman K, Pajoro A and Angenent GC (2010). Regulation of transcription in plants: mechanisms controlling developmental switches. *Nature Reviews Genetics* **11**: 830-842
- Kenyon C (2010). The genetics of ageing. *Nature* **464**: 504-512
- Keurentjes JJB, Bentsink L, Alonso-Blanco C, Hanhart CJ, Blankestijn-De Vries H, Effgen S, Vreugdenhil D and Koornneef M (2007). Development of a near-isogenic line population of *Arabidopsis thaliana* and comparison of mapping power with a recombinant inbred line population. *Genetics* **175**: 891-905
- Kim K, Sato K, Shibuya M, Zeiger DM, Butcher RA, Ragains JR Clardy J, Touhara K and Sengupta P (2009). Two chemoreceptors mediate developmental effects of dauer pheromone in *C. elegans*. *Science* **326**: 994-998
- Kim S and Paik YK (2008). Developmental and reproductive consequences of prolonged non-aging dauer in *Caenorhabditis elegans*. *Biochemical and Biophysical Research Communicatio*ns **368**: 588-592
- Kimura KD, Tissenbaum HA, Liu YX and Ruvkun G (1997). *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* **277**: 942–946
- King EG, Merkes CM, McNeil CL, Hoofer SR, Sen S, Broman KW, Long AD and Macdonald SJ (2012). Genetic dissection of a model complex trait using the Drosophila Synthetic Population Resource. Genome Research **22**: 1558-1566

- Kiontke KC, Félix M, Ailion M, Rockman MV, Braendle C Pénigault J and Fitch DHA (2011). A phylogeny and molecular barcodes for *Caenorhabditis*, with numerous new species from rotting fruits. *BMC Evolutionary Biology* **11**: 339
- Klass M and Hirsh D (1976). Non-ageing developmental variant of *Caenorhabditis elegans*. *Nature* **260**: 523-525
- Klass MR (1977). Aging in the nematode *Caenorhabditis elegans*: Major biological and environmental factors influencing life span. M*echanisms of Ageing and Development* **6**: 413-429
- Kloosterman B, Oortwijn M, uitdeWilligen J, America T, de Vos R, Visser RGF and Bachem CWB (2010). From QTL to candidate gene: Genetical genomics of simple and complex traits in potato using a pooling strategy. *BMC Genomics* **11**: 158
- Koch R, van Luenen HGAM, van de Horst M, Thijssen KL and Plasterk RHA (2000). Single nucleotide polymorphisms in wild isolates of *Caenorhabditis elegans*. *Genome Research* **10**: 1690-1696
- Koopmann TT, Adriaens ME, Moerland PD, Marsman RF, Westerveld ML, Lal S, Zhang T, Simmons CQ, Baczko I, Remedios CD, Bishopric NH, Varro A, George Jr AL, Lodder EM and Bezzina CR (2014). Genome-wide identification of expression quantitative trait loci (eQTLs) in human heart. *PLoS One* **9**: e97380
- Kover PX, Valdar W, Trakalo J, Scarcelli N, Ehrenreich IM, Purugganan MD, Durrant C and Mott R (2009). A multiparent advanced generation inter-cross to fine-map quantitative traits in *Arabidopsis thaliana*. *PLoS Genetics* **5**: e1000551
- Kraus PR, Boily MJ, Giles SS, Stajich JE, Allen A, Cox GM, Dietrich FS, Perfect JR, and Heitman J (2004). Identification of *Cryptococcus neoformans* temperature-regulated genes with a genomic-DNA microarray. *Eukaryotic Cell* **3**: 1249-1260
- L'Etoile ND and Bargmann CI (2000). Olfaction and odor discrimination are mediated by the *C. elegans* guanylyl cyclase ODR-1. *Neuron* **25**: 575-586
- Lai CQ, Parnell LD, Lyman RF, Ordovas JM and Mackay TF (2007). Candidate genes affecting *Drosophila* life span identified by integrating microarray gene expression analysis and QTL mapping. *Mechanisms of Ageing and Development*. **128**: 237-249
- Lakowski B and Hekimi S (1998). The genetics of caloric restriction in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences* **95**: 13091-13096

- Lande R and Shannon (1996). The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* **50**: 434-437
- Lander ES and Botstein D (1989). Mapping medelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**: 185-199
- Lang DH, Gerhard GS, Griffith JW, Vogler GP, Vandenbergh DJ, Blizard DA, Stout JT, Lakoski JM and McClearn GE (2010). Quantitative trait loci (QTL) analysis of longevity in C57BL/6J by DBA/2J (BXD) recombinant inbred mice. Ag*ing clinical and experimental research* **22**: 8-19
- Lavelle P, Decaëns T, Aubert M, Barot S, Blouin M, Bureau F, Margerie P, Mora P and Rossi JP (2006). Soil invertebrates and ecosystem services. *European Journal of Soil Ecology* **42**: 3-15
- Lawson-Handley LJ and Perrin N (2007). Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology* **16**: 1559-1578
- Lebigre C, Alatalo RV and Siitari H (2010). Female-biased dispersal alone can reduce the occurrence of inbreeding in black grouse (*Tetrao tetrix*). *Molecular Ecology* **19**: 1929-1939
- LeDeaux JR, Graham GI and Stuber CW (2006). Stability of QTLs involved in heterosis in maize when mapped under several stress conditions. *Maydica* **51**: 151-167
- Leduc MS, Hageman RS and Yuan R (2010). Identification of genetic determinants of IGF-1 levels and longevity among mouse inbred strains. *Aging Cell* **9**: 823-836
- Lee DL, editor (2002). The biology of nematodes. New York, Taylor and Francis.
- Leibel RL, Chung WK and Chua SC (1997). The Molecular genetics of rodent single gene obesities. *The Journal of Biological Chemistry* **272**: 31937-31940
- Leips J and Mackay TFC (2000). Quantitative trait loci for lifespan in *Drosophila melanogaster*: Interactions with genetic background and larval density. *Genetics* **155**: 1773-1788
- Lewis JA and Hodgkin JA (1977). Specific neuroanatomical changes in chemosensory mutants of the nematode *Caenorhabditis elegans*. *Journal of Comparative Neurology* **172**: 489-510
- Li Y, Alvarez OA, Gutteling EW, Tijsterman M, Fu J, Riksen JAG, Hazendonk E, Prins P, Plasterk RHA, Jansen RC, Breitling R and Kammenga JE (2006). Mapping

determinants of gene expression plasticity by genetical genomics in *C. elegans*. *PLoS Genetics* **2:** e222

- Li Y, Breitling R, Snoek LB, van der Velde KJ, Swertz MA, Riksen J, Jansen RC and Kammenga JE (2010). Global genetic robustness of the alternative splicing machinery in *Caenorhabditis elegans*. *Genetics* **186**: 405-410
- Limousin D, Streiff R, Courtois B, Dupuy V, Alem S and Greenfield MD (2012). Genetic architecture of sexual selection: QTL mapping of male song and female receiver traits in an acoustic moth. *PLoS One* **7**: e44554
- Lin K, Dorman JB, Rodan A and Kenyon C (1997). *daf-16*: An HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. *Science* **278**: 1319-1322
- Lin K, Hsin H, Libina N and Kenyon C (2001). Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nature Genetics* **28**: 139-145
- Lisec J, Steinfath M, Meyer RC, Selbig J, Melchinger AE, Willmitzer L and Altmann T (2009). Identification of heterotic metabolite QTL in *Arabidopsis thaliana* RIL and IL populations. *The Plant Journal* **59**: 777-788
- Liu K, Yu D, Cho YY, Bode AM, Ma W, Yao K, Li S, Li J, Bowden GT, Dong Z and Dong Z (2013). Sunlight UV-induced skin cancer relies upon activation of the p38α signalling pathway. *Cancer Research* **73**: 2181-2188
- Lublin A and Link C (2013). Alzheimer's Disease drug discovery: In-vivo screening using *C. elegans* as a model for β-amyloid peptide-induced toxicity. *Drug Discovery Today Technologies* **10**: e115-e119
- Mackay TF (2001a). Quantitative trait loci in *Drosophila*. *Nature Reviews Genetics* **2**: 11-20
- Mackay TF (2004). The genetic architecture of quantitative traits: lessons from *Drosophila. Current Opinion in Genetics and Development* **14**: 253-257
- Mackay TFC (1985). A quantitative genetic analysis of fitness and its components in *Drosophila melanogaster*. *Genetic Research* **47**: 59-70
- Mackay TFC (2001b). The genetic architecture of quantitative traits. *Annual Review Genetics* **35**: 303-339

- Mackay TFC and Anholt RRH (2006). Of flies and man: *Drosophila* as a model for human complex traits. *The Annual Review of Genomics and Human Genetics* **7**: 339-367
- Mackay TFC, Heinsohn SL, Lyman RF, Moehring AJ, Morgan TJ and Rollmann SM (2005). Genetics and genomics of *Drosophila* mating behavior. *Proceedings of the National Academy of Sciences USA* **102**: 6622-6629
- Mackay TFC, Stone EA and Ayroles JF (2009). The genetics of quantitative traits: challenges and prospects. *Nature Reviews Genetics*. **10**: 565-577
- Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ ,McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TFC, McCarroll SA and Visscher PM (2009). Finding the missing heritability of complex diseases. *Nature* **461**: 747-753
- Maydan JA, Flibotte S, Edgley ML, Lau J, Selzer RR, Richmond TA, Pofahl NJ, Thomas JH and Moerman DG (2007). Efficient high-resolution deletion discovery in Caenorhabditis elegans by array comparative genomic hybridization. *Genome Research* **17**: 337–347
- Mayer MG and Sommer RJ (2011). Natural variation in *Pristionchus pacificus* dauer formation reveals cross-preference rather than self-preference of nematode dauer pheromones. *Proceedings of the Royal Society B: Biological Sciences* **278**: 2784-2790
- McGrath PT, Rockman MV, Zimmer M, Jang H, Macosko EZ, Kruglyak L and Bargmann CI (2009). Quantitative Mapping of a digenic behavioral trait implicates globin variation in *C. elegans* sensory behaviors. *Neuron* **61**: 692-699
- McGrath PT, Xu Y, Ailion M, Garrison JL, Butcher RA and Bargmann CI (2011). Parallel evolution of domesticated *Caenorhabditis* species targets pheromone receptor genes. *Nature* **477**: 321-325
- Miao, XX, Xub SJ, Li MH, Li MW, Huang JH, Dai FY, Marino SW, Mills DR, Zeng P, Mita K, Jia SH, Zhang Y, Liu WB, Xiang H, Guo QH, Xu AY, Kong XY, Lin HX, Shi YZ, Lu G, Zhang X, Huang W, Yasukochi Y, Sugasaki T, Shimada T, Nagaraju J, Xiang ZH, Wang SY, Gioldsmith MR, Lu C, Zhao GP and Huang YP (2005). Simple sequence repeat-

based consensus linkage map of *Bombyx mori*. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 16303-16308

- Miller RA, Harper JM, Galeki A and Burke DT (2002). Big mice die young: early life body weight predicts longevity in genetically heterogeneous mice. *Aging Cell* **1**: 22-29
- Moehring AJ and Mackay TFC (2004). The quantitative genetic basis of male mating behavior in *Drosophila melanogaster*. *Genetics* **167**: 1249-1263
- Moiroux J, Lann CL, Seyahooei MA, Vernon P, Pierre JS, Van Baaren J and Van Alphen JJM (2010). Local adaptations of life-history traits of a *Drosophila* parasitoid, *Leptopilina boulardi*: does climate drive evolution? *Ecological Entomology* **35:** 727–736
- Monteiro A and Podlaha O (2009). Wings, horns, and butterfly eyespots: How do complex traits evolve? *PLoS Biology* **7**: e1000037
- Monteiro A and Prudic KL (2010). Multiple approaches to study color pattern evolution in butterflies. *Trends in Evolutionary Biology* **2**: 7-15
- Morgan TJ and Mackay TFC (2006). Quantitative trait loci for thermotolerance phenotypes in Drosophila melanogaster. Heredity **96**: 232-242
- Morris JZ, Tissenbaum H and Ruvken G (1996). A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. *Nature* **382**: 536-539
- Nadeau JH and Frankel WN (2000). The roads from phenotypic variation to gene discovery: mutagenesis versus QTLs. *Nature Genetics* **25**: 381-384
- Noblin X, Rojas NO, Westbrook J, Llorens C, Argentina M and Dumais J (2012). The fern sporangium: a unique catapult. *Science* **335:** 1322
- Nuzhdin SV, Khazaeli AA and Curtsinger JW (2005). Survival analysis of life span quantitative trait loci in *Drosophila melanogaster*. *Genetics* **170**: 719-731
- Nuzhdin SV, Pasyukova EG, Dilda CL, Zeng Z and Mackay TFC (1997). Sex-specific quantitative trait loci affecting longevity in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences* **94**: 9734-9739

- Ogawa A, Streit A, Antebi A and Sommer RJ (2009). A conserved endocrine mechanism controls the formation of dauer and infective larvae in nematodes. *Current Biology* **19**: 67-71
- Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, and Ruvkun GB (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* **389**: 994-9
- Orr HA and Turelli M (2007). The evolution of postzygotic isolation: Accumulating Dobzhansky-Muller incompatibilities. *Evolution* **55**: 1085-1094
- Pantin F, Simonneau T, Rolland G, Dauzat M and Muller B (2011). Control of leaf expansion: a developmental switch from metabolics to hydraulics. *Plant Physiology* **156**: 803-815
- Paradis S, Ailion M, Toker A, Thomas JH and Ruvken G (1999). A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in *Caenorhabditis elegans*. *Genes and Development* **13**: 1438–1452
- Park D, O'Doherty I, Somvanshi RK, Bethke A, Schroeder FC, Kumar U and Riddle DL (2012). Interaction of structure-specific and promiscuous G-protein–coupled receptors mediates small-molecule signaling in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences* **109**: 9917-9922
- Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Williams TN, Weatherall DJ and Hay SI (2010). Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis. *Nature Communications* **1**: 104
- Pigliucci M (2005). Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology and Evolution* **20:** 481-486
- Pollard DA (2012). Design and construction of recombinant inbred lines. *Methods in Molecular Biology* **871**: 31-39
- Pomerai DD (1990). From Gene to Animal: An introduction to the molecular biology of animal development. New York: Cambridge University Press.
- Poot M, Badea A, Williams RW and Kas MJ (2011). Identifying human disease genes through cross-species gene mapping of evolutionary conserved processes. *PLoS One* **6:** e18612

- Pungaliya C, Srinivasan J, Fox BW, Malik RU, Ludewig AH, Sternberg PW and Schroeder FC (2009). A shortcut to identifying small molecule signals that regulate behavior and development in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences* **106**: 7708-7713
- Pusey AE (1987). Sex-biased dispersal and inbreeding in birds and mammals. *Trends in Ecology and Evolutio*n *2*: 295-299
- Ramanathan R, Varma S, Ribeiro JMC, Myers TG, Nolan TJ, Abraham D, Lok JB and Nutman TB (2011). Microarray-based analysis of differential gene expression between infective and noninfective larvae of *Strongyloides stercoralis*. *PLoS Neglected Tropical Diseases* **5**: e1039
- Rayner A and Boddy L (1988). Fungal decomposition of wood: Its Biology and ecology. John Wiley and Sons, Bath, United Kingdom. 59-115
- Rea SL, Wu D, Cypser JR, Vaupel JW and Johnson TE (2005). A stress-sensitive reporter predicts longevity in isogenic populations of *Caenorhabditis elegans*. *Nature Genetics* **37**: 894-898
- Reddy KC, Andersen EC, Kruglyak L and Kim DH (2009). A polymorphism in *npr-1* is a behavioral determinant of pathogen susceptibility in *C. elegans. Science 323: 382-384*
- Reiner DJ, Ailion M, Thomas JH and Meyer BJ (2008). *C. elegans* anaplastic lymphoma kinase ortholog SCD-2 controls dauer formation by modulating TGF-beta signaling. *Current Biology* **18**: 1101-1109
- Ren P, Lim CS, Johnsen R, Albert PS, Pilgrim D and Riddle DL (1996). Control of *C. elegans* larval development by neuronal expression of a TGF-beta homolog. *Science* **274**: 1389–1391
- Rhodes SL, Sinsheimer JS, Bordelon Y, Bronstein JM and Ritz B (2010). Replication of GWAS associations for GAK and MAPT in Parkinson's Disease. *Annals of Human Genetics* **75**: 195-200
- Riddle DL (1977). A genetic pathway for dauer larva formation in *C. elegans*. *Stadler Genetics Symposium* **9**: 101-120
- Riddle DL, Blumenthal T, Meyer BJ and Priess JR (1997). *Introduction to* C. elegans *II*. Cold Spring Harbor Laboratory Press; New York

- Riddle DL, Swanson MM and Albert PS (1981). Interacting genes in nematode dauer larva formation. *Nature* **290**: 668-671
- Roayaie K, Crump GC, Sagasti A and Bargmann CI (1998). The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. *Neuron* **20**: 55-67
- Rockman MV (2012). The QTN program and the alleles that matter for evolution: all that's gold does not glitter. *Evolution* **66**: 1-17
- Rockman MV and Kruglyak L (2008). Breeding designs for recombinant inbred advanced intercross lines. *Genetics* **179**: 1069-1078
- Rockman MV and Kruglyak L (2009). Recombinational Landscape and population genomics of *Caenorhabditis elegans*. *PLoS Genetics* **5:** e1000419
- Rockman MV, Hahn MW, Soranzo N, Loisel DA, Goldstein DB and Wray GA (2004). Positive selection on MMP3 regulation has shaped heart disease risk. *Current Biology* **14**: 1531-1539
- Rockman MV, Skrovanek SS and Kruglyak L (2010). Selection at linked sites shapes heritable phenotypic variation in *C. elegans*. *Science* **330**: 372-376
- Rodriguez M, Snoek LB, Riksen JAG, Bevers RP and Kammenga JE (2012). Genetic variation for stress-response hormesis in *C. elegans* lifespan. *Experimental Gerontology* **47**: 581-587
- Rogina B, Reenan RA, Nilsen SP and Helfand SL (2000). Extended Life-Span Conferred by Cotransporter Gene Mutations in *Drosophila*. *Science* **290**: 2137-2140
- Ronce O and Olivieri I (1997). Evolution of reproductive effort in a metapopulation with local extinctions and ecological succession. *American Naturalist* **150**: 220-249
- Ronce O and Promislow D (2010). Kin competition, natal dispersal and the moulding of senescence by natural selection. *Proceedings of the Royal Society B: Biological Sciences* **277**: 3659-3667
- Ross JA, Koboldt DC, Staisch JE, Chamberlin HM, Gupta BP, Miller RD, Baird SE and Haag ES (2011). *Caenorhabditis briggsae* Recombinant inbred lines genotypes reveal inter-strain incompatibility and the evolution of recombination. *PLoS Genetics* **7:** e1002174

- Ruaud AF, Katic I and Bessereau JL (2011). Insulin/insulin-like Growth factor signaling controls non-dauer developmental speed in the nematode *Caenorhabditis elegans. Genetics 187: 337-343*
- Sargent DJ, Cipriani G, Vilanova S, Gil-Ariza D, Arús P, Simpson DW, Tobutt KR and Monfort A (2008). The development of a bin mapping population and the selective mapping of 103 markers in the diploid *Fragaria* reference map. *Genome* **51**: 120-127
- Sax K (1923). The Association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics* **8**: 552-560
- Sega GA (1984). A review of the genetic effects of ethyl methanesulfonate. *Mutation Research* **134**: 113-142
- Seidel HS, Rockman MV and Kruglyak L (2008). Widespread genetic incompatibility in *C. elegans* maintained by balancing selection. *Science* **319**: 589-594
- Shao H, Sinasac DS, Burrage LC, Hodges CA, Supelak PJ, Palmert MR, Moreno C, Cowley Jr AW, Jacob HJ and Nadeau JH (2010). Analyzing complex traits with congenic strains. *Mammalian Genome* **21**: 276-286
- Shastry BS (2009). SNPs: impact on gene function and phenotype. *Methods in Molecular Biolog*y **578**: 3-22
- Shaw WM, Luo S, Landis J, Ashraf J and Murphy CT (2007). The *C. elegans* TGF-beta dauer pathway regulates longevity via insulin signalling. *Current Biology* **17**: 1635-1645
- Shulman JM, Chipendo P, Chibnik LB, Aubin C, Tran D, Keenan BT, Kramer PL, Schneider JA, Bennett DA, Feany MB and De Jager PL (2011). Functional Screening of Alzheimer pathology genome-wide association signals in *Drosophila*. *American Journal of Human Genetics* **88**: 232-238
- Simmer F, Moorman C, van der Linden AM, Kuijk E, van den Berghe PVE, Kamath RS, Fraser AG, Ahringer J and Plasterk RHA (2003). Genome-wide RNAi of *C. elegans* using the hypersensitive *rrf-3* strain reveals novel gene functions. *PloS Biology* **1**: e12
- Smith EN, Kruglyak L (2008). Gene–environment interaction in yeast gene expression. *PLoS Biology* **6**: e83

- Snoek BL, Orbidans HE, Stastna JJ, Aartse A, Rodriguez M, Riksen JAG, Kammenga JE and Harvey SC (2014). Widespread Genomic Incompatibilities in Caenorhabditis elegans. *G3*
- Soller M, Brody T and Genizi A (1976). On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. *Theoretical and Applied Genetics* **47**: 35-39
- Sommer RJ and Ogowa A (2011). Hormone signalling and phenotypic plasticity in nematode development and evolution. *Current Biology* **21**: 758-766
- Sørensen JG, Nielsen MM, Kruhøffer M, Justesen J and Loeschcke V (2005). Full genome gene expression analysis of the heat stress response in *Drosophila melanogaster.* Cell Stress and Chaperones **10**: 312–328
- Srinivasan J, von Reuss SH, Bose N, Zaslaver A, Mahanti P, Ho MC, O'Doherty OG, Edison AS, Sternberg PW and Schroeder FC (2012). A modular library of small molecule signals regulates social behaviors in *Caenorhabditis elegans*. *PLoS Biology* **10**: e1001237
- Stasiuk SJ, Scott MJ and Grant WN (2012). Developmental plasticity and the evolution of parasitism in an unusual nematode, *Parastrongyloides trichosuri. Evolution and Development* **3:** 1
- Stearns SC and Koella JC (1986). The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. *Evolution* **40**: 893-913
- Stein LD, Bao Z, Blasiar D, Blumenthal T, Brent MR, Chen N, Chinwalla A, Clarke L, Clee C, Coghlan A, Coulson A, D'Eustachio P, Fitch DHA, Fulton LA, Fulton RE, Griffiths-Jones S, Harris TW, Hillier LW, Kamath R, Kuwabara PE, Mardis ER, Marra MA, Miner TL, Minx P, Mullikin JC, Plumb RW, Rogers J, Schein JE, Sohrmann M, Spieth J, Stajich JE, Wei C, Willey D, Wilson RK, Durbin R and Waterston RH (2003). The genome sequence of *Caenorhabditis briggsae*: A Platform for comparative genomics. *PLoS Biology* **1**: e45
- Steinmetz LM, Sinha H, Richards DR, Spiegelman JI, Oefner PJ, McCusker JH and Davis RW (2002). Dissecting the architecture of a quantitative trait locus in yeast. *Nature* **416**: 326-330

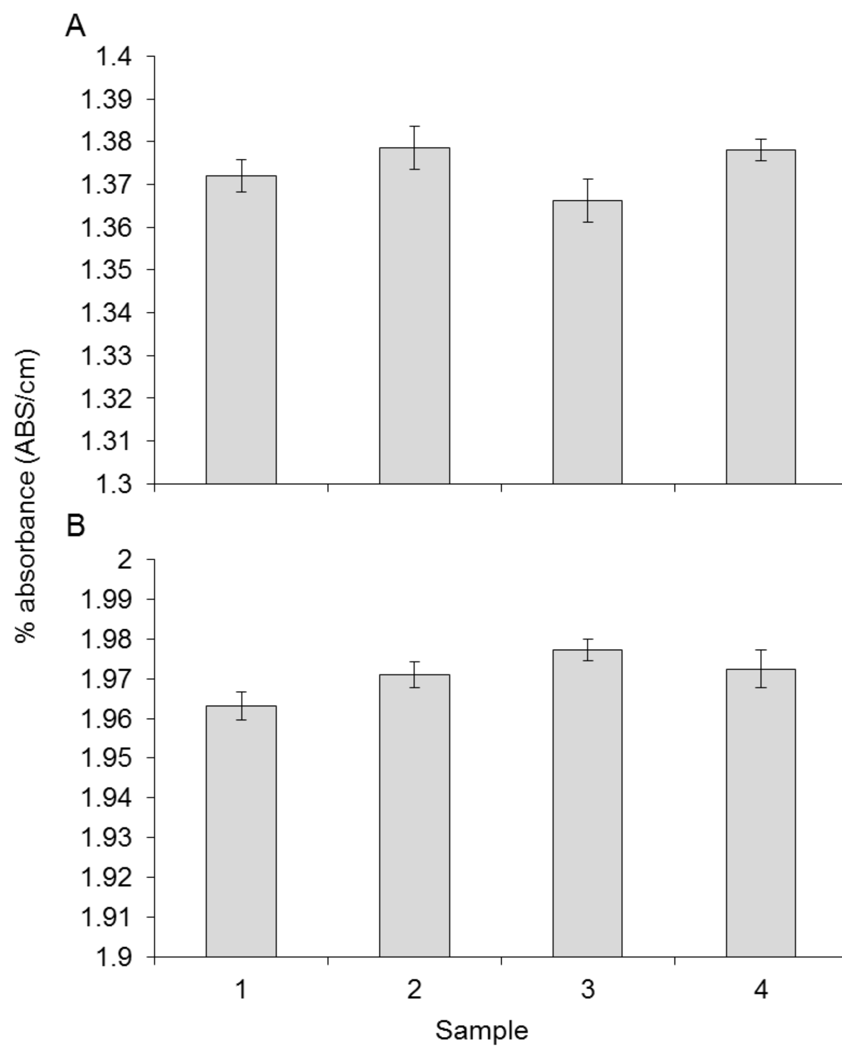
- Stern DL and Orgogozo V (2008). The loci of evolution: how predictable is genetic evolution? *Evolution* **62**: 2155-2177
- Stiernagle, T. (2006) Maintenance of *C. elegans*. *WormBook: The online review of* C. elegans *Biology* **1**
- Stuart MJ and Nagel RL (2004). Sickle-cell disease. *The Lancet* **364**: 1343-1360
- Styer KL, Singh V, Macosko E, Steele SE, Bargmann CI and Aballay A (2008). Innate immunity in *Caenorhabditis elegans* is regulated by neurons expressing NPR-1/GPCR. *Science* **322**: 460-464
- Sulston JE and Brenner S (1974). The DNA of *Caenorhabditis elegans. Genetics* **77**: 95-104
- Summers K and Crespi B (2008). Molecular evolution of the prostate cancer susceptibility locus RNASEL: Evidence for positive selection. *Infection, Genetics and Evolution* **8**: 297-301
- Szalma SJ, Hostert BM, LeDeaux RJ, Stuber CW and Holland JB (2007). QTL mapping with near-isogenic lines in maize. *Theoretical and Applied Genetics* **114**: 1211-1228
- Thomas JA (1990). Genetic analysis of defecation in *Caenorhabditis elegans*. *Genetics* **124**: 855-872
- Timmons L and Fire A (1998). Specific interference by ingested dsRNA. *Nature* **395** 854- 854
- Timmons L, Court DL and Fire A (2001). Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. *Gene* **263**: 103-112
- Toma DP, White KP, Hirsch J and Greenspan RJ (2002). Identification of genes involved in *Drosophila melanogaster* geotaxis, a complex behavioral trait. *Nature Genetics* **31**: 349-353
- Tomioka M, Adachi T, Suzuki H, Kunitomo H, Schafer WR and Iino Y (2006). The insulin/PI 3-kinase pathway regulates salt chemotaxis learning in *Caenorhabditis elegans*. *Neuron* **51**: 613-25
- Travis JMJ and Dytham C (1999). Habitat persistence, habitat availability and the evolution of dispersal. *Proceedings of the Royal Society B: Biological Sciences* **266**: 723-728

- Treusch S, Hamamichi S, Goodman JL, Matlack KES, Chung CY, Baru V, Shulman JM, Parrado A, Bevis BJ, Valastyan JS, Han H, Lindhagen-Persson MM Reiman EM, Evans DA, Bennett DA, Olofsson A, DeJager PL, Tanzi RE, Caldwell KA, Caldwell GA and Lindquist S (2011). Functional links between Aβ toxicity, endocytic trafficking, and Alzheimer's Disease risk factors in yeast. *Science* **334**: 1241-1245
- Troemel ER, Kimmel BE and Bargmann CI (1997). Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. *Cell* **91**: 161–169
- Uchida O, Nakano H, Koga M and Ohshima Y (2003). The *C. elegans che-1* gene encodes and zinc finger transcription factor required for specification of the ASE chemosensory neurons. *Development* **130**: 1215-1224
- Vertino A, Ayyadevara S, Thaden JJ and Reis RJ (2011). A Narrow quantitative trait locus in *C. elegans* coordinately affects longevity, thermotolerance, and resistance to paraquat. *Frontiers in Genetics* **2**: 63
- Vieira C, Pasyukova EG, Zeng S, Hackett JB, Lyman RF and Mackay TFC (2000). Genotype-environment interaction for quantitative trait loci affecting lifespan in *Drosophila melanogaster*. *Genetics* **154**: 213-227
- Viney ME (1996). Developmental switching in the parasitic nematode *Strongyloides ratti*. *Proceedings of the Royal Society of London Series B*: *Biological Sciences* **263**: 201-208
- Viney ME, Gardner MP and Jackson JA (2003). Variation in *Caenorhabditis elegans* dauer larva development. *Development Growth and Differentiation* **45**: 389-396
- Viney ME, Thompson FJ and Crook M (2005). TGF-b and the evolution of nematode parasitism. *International Journal for Parasitology* **35**: 1473–1475
- Viñuela A, Snoek LB, Riksen JAG and Kammenga JE (2010). Genome-wide gene expression regulation as a function of genotype and age in *C. elegans*. *Genome Research* **20**: 929-937
- Viñuela A, Snoek LB, Riksen JAG and Kammenga JE (2012). Aging uncouples heritability and expression-QTL in *Caenorhabditis elegans*. *G3* **2:** 597-605
- Visscher PM, Brown MA, McCarthy MI and Yang J (2011). Five Years of GWAS discovery. *American Journal of Human Genetics* **90**: 7-24

- Volkman SK, Galecki AT, Burke DT, Miller RA and Goldstein SA (2004). Quantitative trait loci that modulate femoral mechanical properties in a genetically heterogeneous mouse population. *Journal of Bone and Mineral Research* **19**: 1497-1505
- Volkman SK, Galecki AT, Burke DT, Paczas MR, Moalli MR, Miller RA and Goldstein SA (2003). Quantitative trait loci for femoral size and shape in a genetically heterogeneous mouse population. *Journal of Bone and Mineral Research* **18**: 1497-1505
- Wakimoto BT, Lindsley DL and Herrera C (2004). Toward a comprehensive genetic analysis of male fertility in *Drosophila melanogaster*. *Genetics* **167**: 207-216
- Walker DW, McColl G, Jenkins NL, Harris J and Lithgow GJ (2000). Evolution of lifespan in *C. elegans*. *Nature* **405**: 296-297
- Ward S and Carrel JS (1979). Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. *Developmental Biology* **73**: 304-321
- Watson WL and Conte AJ (1954). Smoking and lung cancer. *American Cancer Society* **7**: 245-249
- Weber KP, De S, Kozarewa I, Turner DJ, Babu MM and de Bono M (2010). Whole genome sequencing highlights genetic changes associated with laboratory domestication of *C. elegans*. *PLoS One* **5**: e13922
- West-Eberhard MJ (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* **20**: 249-278
- Willcox BJ, Donlon TA, He Q, Chen R, Grove JS, Yano K, Masaki KH, Willcox DC, Rodriguez B and Curb JD (2008). FOXO3A genotype is strongly associated with human longevity. *Proceedings of the National Academy of Sciences* **105**: 13987-13992
- Wolf N, Galecki A, Lipman R, Chen S, Smith-Wheelock M, Burke D and Miller R (2004). Quantitative trait locus mapping for age-related cataract severity and synechia prevalence using four-way cross mice. *Investigative Ophthalmology and Visual Science* **45**: 1922-1929

- Xue Y, Haas SA, Brino L, Gusnanto A, Reimers M, Talibi D, Vingron M, Ekwall K and Wright APH (2004). A DNA microarray for fission yeast: minimal changes in global gene expression after temperature shift. *Yeast* **21**: 25-39
- Yamamoto A, Zwarts L, Callaerts P, Norga K, Mackay TFC and Anholt RRH (2008). Neurogenetic networks for startle-induced locomotion in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences* **105**: 12393-12398
- Yan J, Yang X, Shah T, Sánchez-Villeda H, Li J, Warburton M, Zhou Y, Crouch JH and Xu Y (2010). High-throughput SNP genotyping with the GoldenGate assay in maize. *Molecular Breeding* **25**: 441-451
- Zhang X, Noguez JH, Zhou Y and Butcher RA (2013). Analysis of ascarosides from *Caenorhabditis elegans* using mass spectrometry and NMR spectroscopy. *Methods in Molecular Biology* **1068**: 71-92
- Zhang Y, Lu H and Bargmann CI (2005). Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. *Nature* **438**: 179-184
- Zhou D, Xue J, Chen J, Morcillo P, Lambert JD, White KP and Haddad GG (2007a). Experimental selection for *Drosophila* survival in extremely low O2 environment. *PLoS One* **2**: e490
- Zhou H, Falkenburger BH, Schulz JB, Tieu K, Xu Z and Xia XG (2007b). Silencing of the *Pink1* gene expression by conditional RNAi does not induce dopaminergic neuron death in mice. International Journal of Biological Sciences **3**: 242–250
- Zimmerman E, Palsson A and Gibson G (2000). Quantitative trait loci affecting components of wing shape in *Drosophila melanogaster*. *Genetics* **155**: 671-683
- Zou F, Gelfond JAL, Airey DC, Lu L, Manly KF, Williams RW and Threadgill DW (2005). Quantitative trait locus analysis using recombinant inbred intercrosses: Theoretical and empirical considerations. *Genetics* **170**: 1299-1311
- Zuo W, Moses ME, West GB, Hou C and Brown JH (2012). A general model for effects of temperature on ectotherm ontogenetic growth and development. *Proceedings of the Royal Society B: Biological Sciences* **7**: 1840-1846

Appendix 1



Appendix Figure 1: Standardising *E. coli* as food. Single colony of *E. coli* grown in LB broth for 24 hours (A) and 1% w/v solution of E. coli (from 24hr suspension) (B). Error bars signify standard errors.

Gene	Date identified	Reference
aap-1	2002	Wolkow, C. A., Munoz, M. J., Riddle, D. L., & Ruvkun, G. Insulin receptor substrate and p55 orthologous adaptor proteins function in the Caenorhabditis elegans daf-2/insulin-like signaling pathway. J Biol Chem, 277, 49591-7.
aex-6	2003	Ailion, M., & Thomas, J. H. (2003). Isolation and characterization of high-temperature-induced Dauer formation mutants in Caenorhabditis elegans. Genetics, 165, 127-44.
age-1	1999	Mihaylova VT, Borland CZ, Manjarrez L, Stern MJ, Sun H. The PTEN tumor suppressor homolog in Caenorhabditis elegans regulates longevity and dauer formation in an insulin receptor-like signaling pathway. Proc Natl Acad Sci U S A. 1999 Jun 22;96(13):7427-32
akt-1	2003	Ailion, M., & Thomas, J. H. (2003). Isolation and characterization of high-temperature-induced Dauer formation mutants in Caenorhabditis elegans. Genetics, 165, 127-44.
akt-2	1998	Scott Ogg and Gary Ruvkun. The C. elegans PTEN Homolog, DAF-18, Acts in the Insulin Receptor-like Metabolic Signaling Pathway. Molecular Cell 2: 887-893
asc-1	2000	Cherkasova V, Ayyadevara S, Egilmez N, Shmookler Reis R. Diverse Caenorhabditis elegans genes that are upregulated in dauer larvae also show elevated transcript levels in long-lived, aged, or starved adults. J Mol Bio, Jul 14;300(3):433-48
asna-1	2007	Kao, G., Nordenson, C., Still, M., Ronnlund, A., Tuck, S., & Naredi, P. (2007). ASNA-1 positively regulates insulin secretion in C. elegans and mammalian cells. Cell, 128, 577-87.
atg-7	2003	Melendez, A., Talloczy, Z., Seaman, M., Eskelinen, E. L., Hall, D. H., & Levine, B. (2003). Autophagy genes are essential for dauer development and life-span extension in C. elegans. Science, 301, 1387-91.
bec-1	2003	Alicia Meléndez, Zsolt Tallóczy, Matthew Seaman, Eeva-Liisa Eskelinen, David H. Hall and Beth Levine. Autophagy Genes Are Essential for Dauer Development and Life-Span Extension in C. elegans. Science 301: 1387-1391
cam-1	2003	Ailion, M., & Thomas, J. H. (2003). Isolation and characterization of high-temperature-induced Dauer formation mutants in Caenorhabditis elegans. Genetics, 165, 127-44.
che-10	1993	Thomas JH. Chemosensory regulation of development in C. elegans. BioEssays 15: 791-797
che-11	1992	Jennifer J. Vowels and JamesH . Thomas. Genetic Analysis of Chemosensory Control of Dauer Formation in Caenorhabditis elegans. Genetics 130: 105-123
che-2	1991	CI Bargmann and HR Horvitz. Control of larval development by chemosensory neurons in Caenorhabditis elegans. Science 251: 1243-1246
col-43	2005	Bando T, Ikeda T, Kagawa H. The homeoproteins MAB-18 and CEH-14 insulate the dauer collagen gene col-43 from activation by the adjacent promoter of the Spermatheca gene sth-1 in Caenorhabditis elegans. J Mol Biol. 2005 Apr 22;348(1):101-12
cut-6	2003	Muriel JM, Brannan M, Taylor K, Johnstone IL, Lithgow GJ, Tuckwell D.

		M142.2 (cut-6), a novel Caenorhabditis elegans matrix gene important for dauer body shape. Dev Biol. 2003 Aug 15;260(2):339-51
daf-1	2000	Inoue T, Thomas JH. Suppressors of transforming growth factor-beta pathway mutants in the Caenorhabditis elegans dauer formation pathway. Genetics. 2000 Nov;156(3):1035-46
daf-10	1990	Laura L. Georgi, Patrice S. Albert and Donald L. Riddle. daf-1, a C. elegans gene controlling dauer larva development, encodes a novel receptor protein kinase. Cell 61: 635-645
daf-11	2000	Deborah A. Birnby, Elizabeth Malone Link, Jennifer J. Vowels, Hong Tian, Patrick L. Colacurcio and James H. Thomas. A Transmembrane Guanylyl Cyclase (DAF-11) and Hsp90 (DAF-21) Regulate a Common Set of Chemosensory Behaviors in Caenorhabditis elegans. Genetics 155: 85-104
daf-12	2006	Fisher AL, Lithgow GJ. The nuclear hormone receptor DAF-12 has opposing effects on Caenorhabditis elegans lifespan and regulates genes repressed in multiple long-lived worms. Aging Cell. 2006 Apr;5(2):127-38
daf-14	1981	Riddle, D. L., & MacMorris, M. A. (1981). Critical periods in the development of the Caenorhabditis elegans dauer larva. Dev Biol, 84, 27-40
daf-15	2004	Jia, K., Chen, D., & Riddle, D. L. (2004). The TOR pathway interacts with the insulin signaling pathway to regulate C. elegans larval development, metabolism and life span. Development, 131, 3897-906.
daf-16	1994	Gottlieb S, Ruvkun G. daf-2, daf-16 and daf-23: genetically interacting genes controlling Dauer formation in Caenorhabditis elegans - Genetics. 1994 May;137(1):107-20.
daf-18	2005	Masse I, Molin L, Billaud M, Solari F. Lifespan and dauer regulation by tissue-specific activities of Caenorhabditis elegans DAF-18. Dev Biol. 2005 Oct 1;286(1):91-101
daf-2	1994	Gottlieb S, Ruvkun G. daf-2, daf-16 and daf-23: genetically interacting genes controlling Dauer formation in Caenorhabditis elegans - Genetics. 1994 May;137(1):107-20.
daf-22	2009	Joo HJ, Yim YH, Jeong PY, Jin YX, Lee JE, Kim H, Jeong SK, Chitwood DJ, Paik YK. Caenorhabditis elegans utilizes dauer pheromone biosynthesis to dispose of toxic peroxisomal fatty acids for cellular homoeostasis. Biochem J. 2009 Jul 29;422(1):61-71
daf-23	1994	Gottlieb S, Ruvkun G. daf-2, daf-16 and daf-23: genetically interacting genes controlling Dauer formation in Caenorhabditis elegans - Genetics. 1994 May;137(1):107-20.
daf-25	2010	Jensen, V. L., Bialas, N. J., Bishop-Hurley, S. L., Molday, L. L., Kida, K., Nguyen, P. A., Blacque, O. E., Molday, R. S., Leroux, M. R., & Riddle, D. L. Localization of a guanylyl cyclase to chemosensory cilia requires the novel ciliary MYND domain protein DAF-25. PLoS Genet, 6, e1001199.
daf-26	1990	W-H, Y., & Riddle, D. L. (1990). NEW DAUER-DEFECTIVE GENES DEFINING LATE STEPS IN THE GENETIC PATHWAY FOR DAUER LARVA FORMATION. Worm Breeder's Gazette, 11(3), 66.
daf-27	1990	W-H, Y., & Riddle, D. L. (1990). NEW DAUER-DEFECTIVE GENES DEFINING LATE STEPS IN THE GENETIC PATHWAY FOR DAUER LARVA FORMATION. Worm Breeder's Gazette, 11(3), 66.
daf-28	1994	Malone, E. A., & Thomas, J. H. (1994). A screen for nonconditional dauer-constitutive mutations in Caenorhabditis elegans. Genetics, 136, 879-86.
daf-3	1997	Patterson GI, Koweek A, Wong A, Liu Y, Ruvkun G.

		The DAF-3 Smad protein antagonizes TGF-beta-related receptor signaling in the Caenorhabditis elegans dauer pathway. Genes Dev. 1997 Oct 15;11(20):2679-90
daf-32	2009	Wang, Y., Ezemaduka, A. N., Tang, Y. and Chang, Z. (2009), Understanding the mechanism of the dormant dauer formation of C. elegans: From genetics to biochemistry. IUBMB Life, 61: 607–612.
daf-33	2009	Wang, Y., Ezemaduka, A. N., Tang, Y. and Chang, Z. (2009), Understanding the mechanism of the dormant dauer formation of C. elegans: From genetics to biochemistry. IUBMB Life, 61: 607–612.
daf-34	2009	Wang, Y., Ezemaduka, A. N., Tang, Y. and Chang, Z. (2009), Understanding the mechanism of the dormant dauer formation of C. elegans: From genetics to biochemistry. IUBMB Life, 61: 607–612.
daf-36	2006	Rottiers, V., Motola, D. L., Gerisch, B., Cummins, C. L., Nishiwaki, K., Mangelsdorf, D. J., & Antebi, A. (2006). Hormonal control of C. elegans dauer formation and life span by a Rieske-like oxygenase. Dev Cell, 10, 473-82.
daf-4	1993	Estevez, M., Attisano, L., Wrana, J. L., Albert, P. S., Massague, J., & Riddle, D. L. The daf-4 gene encodes a bone morphogenetic protein receptor controlling C. elegans dauer larva development. Nature, 365, 644-9
daf-5	2004	Li S. da Graca, Karen K. Zimmerman, Melissa C. Mitchell, Marianne Kozhan-Gorodetska, Kamila Sekiewicz, Yairani Morales and Garth I. Patterson. DAF-5 is a Ski oncoprotein homolog that functions in a neuronal TGFβ pathway to regulate C. elegans dauer development. Development 131, 435-446
daf-6	1981	Albert, P. S., Brown, S. J., & Riddle, D. L. (1981). Sensory control of dauer larva formation in Caenorhabditis elegans. J Comp Neurol, 198, 435-51
daf-7	1992	Vowels, J. J., & Thomas, J. H. Genetic analysis of chemosensory control of dauer formation in Caenorhabditis elegans. Genetics, 130, 105-23
daf-8	1981	Riddle, D. L., & MacMorris, M. A. (1981). Critical periods in the development of the Caenorhabditis elegans dauer larva. Dev Biol, 84, 27-40
daf-9	1988	Albert, P. S., & Riddle, D. L. (1988). Mutants of Caenorhabditis elegans that form dauer-like larvae. Dev Biol, 126, 270-93.
dyf-1, 5, 9, 12 and 13	1995	Todd A. Starich, Robert K. Herman, Claire K. Kari, Wen-Hui Yeh, Wendy S. Schackwitz, Marcia W. Schuyler, Joan Collet, James H. Thomas and Donald L Riddle. Mutations Affecting the Chemosensory Neurons of Caenorhabditis elegans. Genetics 139: 171-188
eak-3	2008	Zhang Y, Xu J, Puscau C, Kim Y, Wang X, Alam H, Hu PJ. Caenorhabditis elegans EAK-3 inhibits dauer arrest via nonautonomous regulation of nuclear DAF-16/FoxO activity. Dev Biol. 2008 Mar 15;315(2):290-302.
eak-4	2008	Yanmei Zhanga, Jinling Xub, Cristina Puscaua, Yongsoon Kima, Xi Wanga, Hena Alama and Patrick J. Hua. Caenorhabditis elegans EAK-3 inhibits dauer arrest via nonautonomous regulation of nuclear DAF-16/FoxO activity. Developmental Biology 315: 290-302
eak-6	2008	Yanmei Zhanga, Jinling Xub, Cristina Puscaua, Yongsoon Kima, Xi Wanga, Hena Alama and Patrick J. Hua. Caenorhabditis elegans EAK-3 inhibits dauer arrest via nonautonomous regulation of nuclear DAF-16/FoxO activity. Developmental Biology 315: 290-302
hbl-1	2008	Nicole Fielenbach and Adam Antebi.

		C. elegans dauer formation and the molecular basis of plasticity. Genes and Development 22:2149–2165
hid-1	2003	Ailion, M., & Thomas, J. H. (2003). Isolation and characterization of high-temperature-induced Dauer formation mutants in Caenorhabditis elegans. Genetics, 165, 127-44.
hid-2	2003	Ailion, M., & Thomas, J. H. (2003). Isolation and characterization of high-temperature-induced Dauer formation mutants in Caenorhabditis elegans. Genetics, 165, 127-44.
hid-4	2003	Ailion, M., & Thomas, J. H. (2003). Isolation and characterization of high-temperature-induced Dauer formation mutants in Caenorhabditis elegans. Genetics, 165, 127-44.
hid-7	2003	Ailion, M., & Thomas, J. H. (2003). Isolation and characterization of high-temperature-induced Dauer formation mutants in Caenorhabditis elegans. Genetics, 165, 127-44.
hpd-1	2003	Siu Sylvia Lee, Scott Kennedy, Andrew C. Tolonen and Gary Ruvkun. DAF-16 Target Genes That Control C. elegans Life-Span and Metabolism. Science 300: 644-647
ifta-2	2006	Schafer, J. C., Winkelbauer, M. E., Williams, C. L., Haycraft, C. J., Desmond, R. A., & Yoder, B. K. (2006). IFTA-2 is a conserved cilia protein involved in pathways regulating longevity and dauer formation in Caenorhabditis elegans. J Cell Sci, 119, 4088-100.
ins-1	2001	Pierce, S. B., Costa, M., Wisotzkey, R., Devadhar, S., Homburger, S. A., Buchman, A. R., Ferguson, K. C., Heller, J., Platt, D. M., Pasquinelli, A. A., Liu, L. X., Doberstein, S. K., & Ruvkun, G. (2001). Regulation of DAF-2 receptor signaling by human insulin and ins-1, a member of the unusually large and diverse C. elegans insulin gene family. Genes Dev, 15, 672-86.
ins-18	2001	Pierce, S. B., Costa, M., Wisotzkey, R., Devadhar, S., Homburger, S. A., Buchman, A. R., Ferguson, K. C., Heller, J., Platt, D. M., Pasquinelli, A. A., Liu, L. X., Doberstein, S. K., & Ruvkun, G. (2001). Regulation of DAF-2 receptor signaling by human insulin and ins-1, a member of the unusually large and diverse C. elegans insulin gene family. Genes Dev, 15, 672-86.
ins-9	2001	Pierce, S. B., Costa, M., Wisotzkey, R., Devadhar, S., Homburger, S. A., Buchman, A. R., Ferguson, K. C., Heller, J., Platt, D. M., Pasquinelli, A. A., Liu, L. X., Doberstein, S. K., & Ruvkun, G. (2001). Regulation of DAF-2 receptor signaling by human insulin and ins-1, a member of the unusually large and diverse C. elegans insulin gene family. Genes Dev, 15, 672-86.
ist-1	2002	Catherine A. Wolkow, Manuel J. Muñoz, Donald L. Riddle and Gary Ruvkun. Insulin Receptor Substrate and p55 Orthologous Adaptor Proteins Function in the Caenorhabditis elegans daf-2/Insulin-like Signaling Pathway. Journal of Biological Chemistry, 277, 49591-49597
mrp-1	2005	Tomoko Yabe, Norio Suzuki, Tatsuhiko Furukawa, Takeshi Ishihara, and Isao Katsura. Multidrug resistance-associated protein MRP-1 regulates dauer diapause by its export activity in Caenorhabditis elegans. Development132, 3197-3207
ncr-1	2004	Jie Li, Gemma Brown, Michael Ailion, Samuel Lee and James H. Thomas. NCR-1 and NCR-2, the C. elegans homologs of the human Niemann-Pick type C1 disease protein, function upstream of DAF-9 in the dauer formation pathways. Development 131, 5741-5752
ncr-2	2004	Jie Li, Gemma Brown, Michael Ailion, Samuel Lee and James H. Thomas. NCR-1 and NCR-2, the C. elegans homologs of the human Niemann-Pick type C1 disease protein, function upstream of DAF-9 in the dauer formation pathways. Development 131, 5741-5752
pcm-1	1997	Kagan, R. M., Niewmierzycka, A., & Clarke, S. (1997). Targeted gene

		disruption of the Caenorhabditis elegans L-isoaspartyl protein repair methyltransferase impairs survival of dauer stage nematodes. Arch Biochem Biophys, 348, 320-8
rop-1	2000	Labbé JC, Burgess J, Rokeach LA, Hekimi S. ROP-1, an RNA quality-control pathway component, affects Caenorhabditis elegans dauer formation. Proc Natl Acad Sci U S A. 2000 Nov 21;97(24):13233-8
scd-1	2000	Inoue, T., & Thomas, J. H. (2000). Suppressors of transforming growth factor-beta pathway mutants in the Caenorhabditis elegans dauer formation pathway. Genetics, 156, 1035-46.
scd-2	2000	Inoue, T., & Thomas, J. H. (2000). Suppressors of transforming growth factor-beta pathway mutants in the Caenorhabditis elegans dauer formation pathway. Genetics, 156, 1035-46.
scd-3	2000	Inoue, T., & Thomas, J. H. (2000). Suppressors of transforming growth factor-beta pathway mutants in the Caenorhabditis elegans dauer formation pathway. Genetics, 156, 1035-46.
srbc-64	2009	Kim, K., Sato, K., Shibuya, M., Zeiger, D. M., Butcher, R. A., Ragains, J. R., Clardy, J., Touhara, K., & Sengupta, P. (2009). Two chemoreceptors mediate developmental effects of dauer pheromone in C. elegans. Science, 326, 994-8. doi:10.1126/science.1176331
srf-6	2007	Olsen, D. P., Phu, D., Libby, L. J., Cormier, J. A., Montez, K. M., Ryder, E. F., & Politz, S. M. (2007). Chemosensory control of surface antigen switching in the nematode Caenorhabditis elegans. Genes Brain Behav, 6, 240-52.
srg-36	2011	McGrath, P. T., Xu, Y., Ailion, M., Garrison, J. L., Butcher, R. A., & Bargmann, C. I. (2011). Parallel evolution of domesticated Caenorhabditis species targets pheromone receptor genes. Nature, 477, 321-5.
srg-37	2011	McGrath, P. T., Xu, Y., Ailion, M., Garrison, J. L., Butcher, R. A., & Bargmann, C. I. (2011). Parallel evolution of domesticated Caenorhabditis species targets pheromone receptor genes. Nature, 477, 321-5.
tax-2	1998	C.M. Coburn, I. Mori, Y. Ohshima and C.I. Bargmann. A cyclic nucleotide-gated channel inhibits sensory axon outgrowth in larval and adult Caenorhabditis elegans: a distinct pathway for maintenance of sensory axon structure. Development 125: 249-258
tax-4	2003	Ailion, M., & Thomas, J. H. (2003). Isolation and characterization of high-temperature-induced Dauer formation mutants in Caenorhabditis elegans. Genetics, 165, 127-44.
unc-31	2003	Ailion, M., & Thomas, J. H. (2003). Isolation and characterization of high-temperature-induced Dauer formation mutants in Caenorhabditis elegans. Genetics, 165, 127-44.
unc-64	2003	Ailion, M., & Thomas, J. H. (2003). Isolation and characterization of high-temperature-induced Dauer formation mutants in Caenorhabditis elegans. Genetics, 165, 127-44.

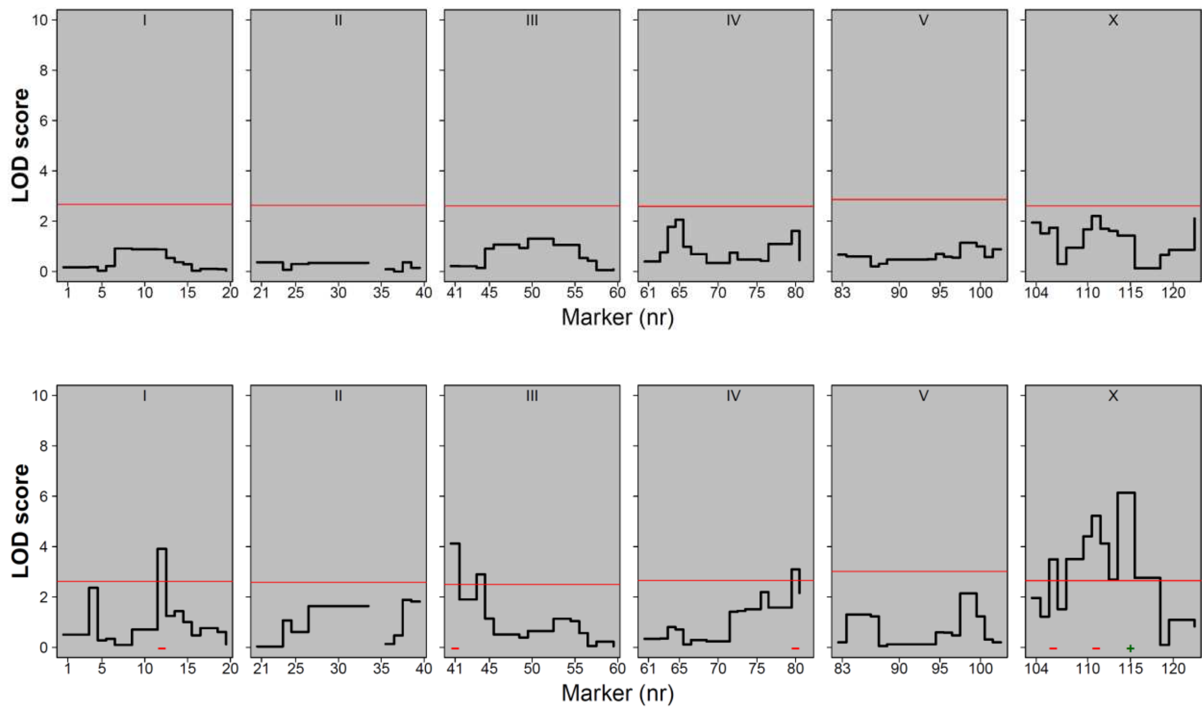
Appendix Table 1: A list of genes known to be involved in dauer larvae formation, as identified through the standard dauer larvae assay.

N2	Mushroom compost	Bristol
JU1494	Rotting stem	France
JU262	Compost	France
JU319	Soil	France
JU345	Arthropod	France
JU362	Compost	France
JU393	Compost	France
JU400	Compost	France
MY1	Compost	Germany
MY16	Compost	Germany
MY2	Compost	Germany
CB4853	Vegetable garden	North America
PX174	Soil	North America
PX179	Arthropod	North America
JU1400	Rotting fruit	Spain
JU1401	Snail	Spain
JU1409	Rotting fruit	Spain
JU1410	Dead snail	Spain
JU1411	Snail	Spain
JU1416	Arthropod	Spain
JU1442	Rotting fruit	Spain

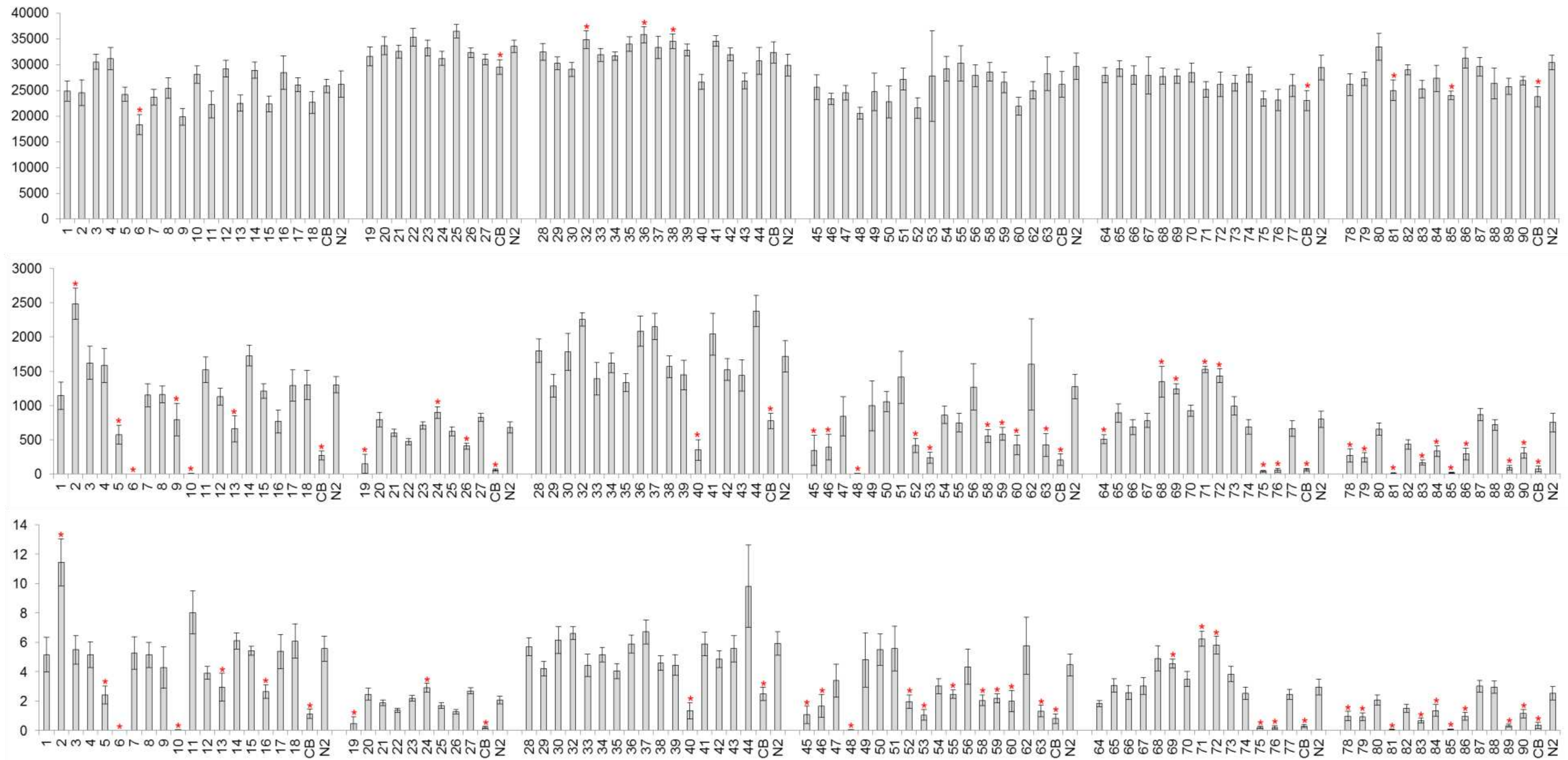
Appendix Table 2: Wild isolates and N2 and their original habitat of isolation (www.Wormbase.org).

Chromosome	Total number of dauer larvae	Population size	% dauer larvae	Single IL analysis threshold at a 0.05 Bonferroni correction (*p*)	Sequential IL analysis threshold at a 0.05 Bonferroni correction (*p*)
I	2.67	2.67	2.62	0.003	0.002
II	2.56	2.63	2.58	0.006	0.005
III	2.57	2.61	2.50	0.003	0.002
IV	2.89	2.59	2.66	0.003	0.002
V	2.94	2.86	3.02	0.004	0.003
X	2.80	2.61	2.65	0.004	0.003

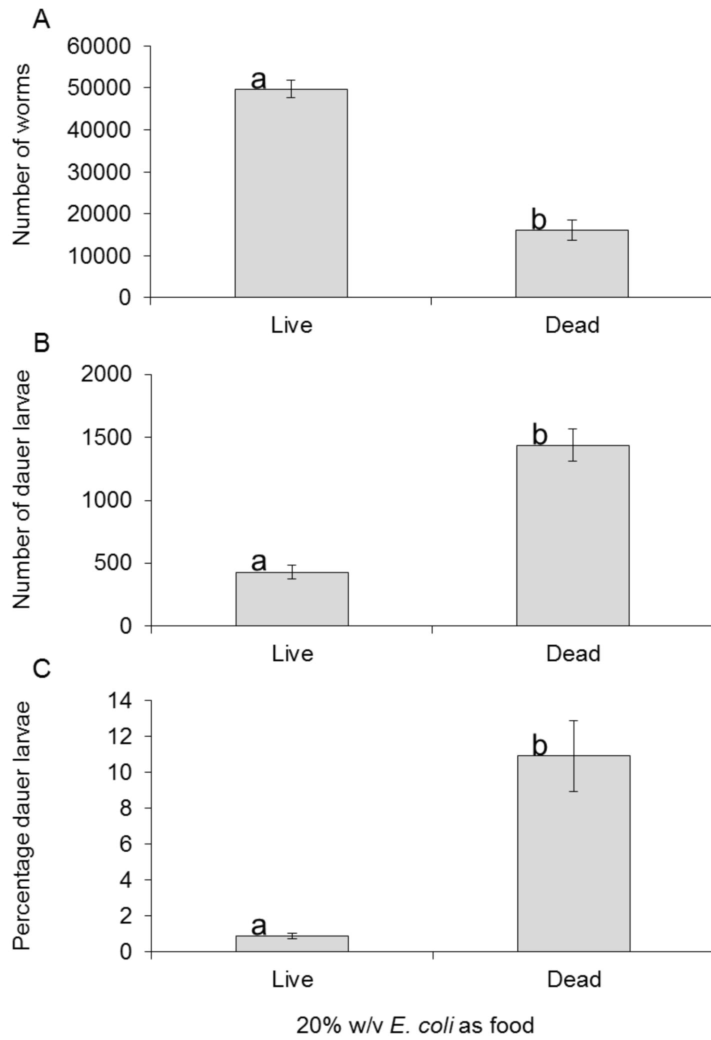
Appendix Table 3: -log10(p) threshold at a 0.05 FDR, for bin mapping the total number of dauer larvae, the population size and the percentage of dauer larvae (Column 2, 3 and 4 respectively) including thresholds for the different mapping methods for total number of dauer larvae per chromosome (column 5 and 6).



Appendix Figure 2: Bin mapping of population size (upper panel) and percentage of dauer larvae (lower panel). Chromosome specific threshold (FDR=0.05) shown by the horizontal line and significance per marker in black. Detected QTLs are shown by the"+" (positive CB4856 effect) and the "-" (negative CB4856 effect).



Appendix Figure 3: The population size (Top), number of dauer larvae (middle) and percentage dauer larvae (bottom) from a population grown on 20% w/v *E. coli* at 20°C. lines per chromosome are separated by the gap. Asterisk (*) signifies difference from N2 within the chromosome. Errors bars signify standard error.



Appendix Figure 4: The population size (A), number of dauer larvae formed (B) and the percentage of dauer larvae (C) at the point of food exhaustion for N2 grown on living or dead *E. coli* (20% w/v at 20°C). Different letters indicate statistically different from one another. Errors bars signify standard error.