Genet. Res., Camb. (2010), **92**, *pp.* 331–348.
doi:10.1017/S0016672310000601

Caenorhabditis elegans as a platform for molecular quantitative genetics and the systems biology of natural variation

BRYN E. GAERTNER AND PATRICK C. PHILLIPS*
Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, OR 97403, USA

(*Received 10 October 2010 and in revised form 11 November 2010*)

Summary

Over the past 30 years, the characteristics that have made the nematode *Caenorhabditis elegans* one of the premier animal model systems have also allowed it to emerge as a powerful model system for determining the genetic basis of quantitative traits, particularly for the identification of naturally segregating and/or lab-adapted alleles with large phenotypic effects. To better understand the genetic underpinnings of natural variation in other complex phenotypes, *C. elegans* is uniquely poised in the emerging field of quantitative systems biology because of the extensive knowledge of cellular and neural bases to such traits. However, perturbations in standing genetic variation and patterns of linkage disequilibrium among loci are likely to limit our ability to tie understanding of molecular function to a broader evolutionary context. Coupling the experimental strengths of the *C. elegans* system with the ecological advantages of closely related nematodes should provide a powerful means of understanding both the molecular and evolutionary genetics of quantitative traits.

1. Introduction

Established as a model organism by Sydney Brenner in the late 1960s, the nematode *Caenorhabditis elegans* has been used to study cellular differentiation, cellular and neural development, apoptosis, meiosis, aging and behaviour, generating six Nobel laureates during the last decade. This microscopic, transparent worm is a natural pick for addressing these biological questions, as its developmental stereotypy contributes to the ease of identifying genetic mutations central to these fundamental biological processes. Brenner (1974) established the standard method for mapping mutations using a forward-genetic approach by identifying hundreds of strains with abnormal and trackable phenotypes, thus enabling classic genetic two- and three-point mapping of novel mutations (Jorgensen & Mango, 2002). Since this time, this approach has evolved into a systematic procedure of mapping a mutation to chromosomal location, rescuing the phenotype by genetic transformation with a cosmid obtained from the physical map, and cloning the gene via fine-scale manipulation of the cosmid DNA sequence (Fay, 2006). The identity of putative causal loci can also be confirmed using interfering RNA (RNAi; Rual *et al*., 2004).

An alternative, albeit underutilized strategy for identifying the genetic basis to complex traits in *C. elegans* is via quantitative trait locus (QTL) mapping. There are two main motivations for taking this type of approach. First, when working with any complex phenotype, a quantitative approach allows simultaneous estimation of effect size and direction of multiple alleles across the genome. This runs contrary to Brenner's standard, which by necessity isolates mutations to a single locus and can be compromised in the case of dominant, epistatic or small effect alleles. The second motivation revolves around identifying the ultimate mechanisms of quantitative traits, which is to understand how natural genetic variation shapes phenotypes, thereby placing *C. elegans* into an appropriate evolutionary and ecological context. Although early quantitative genetic studies focused on the former motivation and were ultimately frustrated because inappropriate strains were used, more recent studies have been motivated by understanding the nature of genetic variation itself. These studies have not only generated *C. elegans* QTL success stories, but have also shed light on interesting characteristics of this model organism in general (Table 1).

* Corresponding author: e-mail: pphil@uoregon.edu

Table 1. *Quantitative genetic studies in* C. elegans. *RIL, recombinant inbred line; RIP, recombinant inbred population; RIAIL, recombinant inbred advanced intercrossed lines; MA, mutation accumulation. Asterisk* (*) *indicates a mutant in that background, as opposed to wild-type, was used*

Citation	Type of experiment	Strains used	Phenotype
Variance estimation			
Johnson & Wood (1982)	RIL	N2 × BO	Lifespan
Johnson (1987)	RIL	N2 × BO	Locomotion, lifespan, reproduction
Johnson & Hutchinson (1993)	RIL	N2 × BO, other wild isolates	Longevity, fertility, heterosis, G × E for type of media
Delattre & Felix (2001)	Variation	Wild Isolates	Vulval development
Jovelin *et al*. (2003)	Quantitative genetics	Wild Isolates	Chemosensation, locomotion
Viney *et al*. (2003)	Variation	Wild isolates	Dauer formation
Teotónio *et al*. (2006)	Variation	Wild isolates	Males mating, non-disjunction
Anderson *et al*. (2007)	Variation	N2, CB4856	Thermal preference and locomotion
Harvey & Viney (2007)	Variation	N2, DR1350	Growth, reproduction, G × E for temperature
Braendle & Felix (2008)	Variation	Wild isolates	Vulval development, G × E for multiple environments
Standard mapping			
Hodgkin & Doniach (1997)	Association mapping	Wild Isolates	Plugging
Friedman & Johnson (1988)	Forward genetics	N2*	Lifespan
de Bono & Bargmann (1998)	Forward genetics	N2 and RC301, other wild isolates	Clumping
Quantitative trait mapping			
Brooke & Johnson (1991)	RIL	N2 × BO	Lifespan, reproduction
Ebert *et al*. (1993)	RIP	N2 × BO	Lifespan
Ebert *et al*. (1996)	RIP	N2 × DH424	Lifespan, oxidative stress
Shook *et al*. (1996)	RIL	N2 × BO	Bagging, reproduction, lifespan, G × E for temperature
van Swinderen *et al*. (1997)	RIL	N2 × BO	Locomotion, paralysis, halothane resistance
van Swinderen *et al*. (1998*a*)	RIL	N2 × BO	Male mating, locomotion, paralysis, anaesthesia resistance
van Swinderen *et al*. (1998*b*)	RIL	N2 × BO	Locomotion and movement, isoflurane, halothane and ethanol resistance
Shook & Johnson (1999)	RIL	N2 × BO	Survival, reproduction, G × E for growth media
Ayyadevara *et al*. (2000*b*)	RIP	BO × RC301	Lifespan, reproduction
Knight *et al*. (2001)	RIL	N2 × BO	Body size, reproduction
Wicks *et al*. (2001)	RIP	N2 × CB4856	Dye-filling
Ayyadevara *et al*. (2003)	RIP	BO × CL2a (CB4857)	Lifespan, reproduction
Li *et al*. (2006)	RIL	N2 × CB4856	Genetical genomics, G × E temperature
Shmookler-Reis *et al*. (2006)	RIP	N2 × BO	Lifespan, UV resistance, heat stress, oxidative stress
Gutteling *et al*. (2007*a*)	RIL	N2 × CB4856	Reproduction, growth, plasticity, G × E temperature
Gutteling *et al*. (2007*b*)	RIL	N2 × CB4856	Reproduction, body size, body mass, G × E temperature

Kammenga *et al*. (2007)	RIL	N2 × CB4856	Body size, G × E temperature
Harvey *et al*. (2008)	RIL	N2 × DR1350	Reproduction, dauer formation, G × E environmental stress
Palopoli *et al*. (2008)	RIL	N2 × CB4856, N2 × CB4855	Copulatory plugging
Siedel *et al*. (2008)	RIAIL	N2 × CB4856	Genetic incompatibility
Doroszuk *et al*. (2009)	NIL	N2 × CB4856	Lifespan, pharyngeal pumping
Harvey (2009)	RIL	N2 × DR1350	Locomotion
Harvey *et al*. (2009)	RIL	N2 × DR1350, other wild isolates	Gene expression, G × E for stressful environment
McGrath *et al*. (2009)	RIAIL	N2 × CB4856, other wild isolates	Oxygen sensing and response
Persson *et al*. (2009)	RIL	N2* × CB4856, wild isolates	Oxygen sensing and response
Rockman & Kruglyak (2009)	Association	Wild isolates	Copulatory plugging
Chandler (2010)	RIL	N2* × CB4856, other wild isolates	Male development via permissive mutations, G × E temperature
Viñuela *et al*. (2010)	RIL	N2 × CB4856	Gene expression, aging
Li *et al*. (2010)	RIL	N2 × CB4856	Alternative splicing
Rockman *et al*. (2010)	RIAIL	N2 × CB4856	Gene expression
Mutation accumulation			
Keightley & Caballero (1997)	MA	N2	Lifespan, reproduction
Vassilieva & Lynch (1999)	MA	N2	Survival to maturity, longevity
Denver *et al*. (2000)	MA	N2	Mutation rate (mitochondrial)
Azevedo *et al*. (2002)	MA	N2	Body size
Estes & Lynch (2003)	MA	N2	Fitness recovery
Denver *et al*. (2004)	MA	N2	Gene expression, mutation rate
Estes *et al*. (2004)	MA	N2	Reproduction, life history (elevated mutation rate)
Ajie *et al*. (2005)	MA	N2	Chemosensation
Baer *et al*. (2005)	MA	N2, *C. briggsae*, *Oscheius myriophila*	Fitness
Estes *et al*. (2005)	MA	N2	Life history, body size, behaviour
Estes & Phillips (2006)	MA	N2	Life history
Baer *et al*. (2006)	MA	N2, *C. briggsae*	Temperature-dependent fitness
Ostrow *et al*. (2007)	MA	N2, PB306, *C. briggsae*, *O. myriophila*	Body size
Vassilieva *et al*. (2007)	MA	N2	Fitness
Baer (2008)	MA	N2, PB306, *C. briggsae*, *O. myriophila*	Body size
Denver *et al*. (2009)	MA	N2	Mutation rate (genomic)
Joyner-Matos *et al*. (2009)	MA	N2, PB306	Lifespan
Phillips *et al*. (2009)	MA	N2, PB306, *C. briggsae*	Mutation rate
Salomon *et al*. (2009)	MA	N2, PB306, *C. briggsae*	Fitness, body size
Baer & Denver (2010)	MA	N2	Gene expression
Braendle *et al*. (2010)	MA	N2, PB306, *C. briggsae*	Vulval development
Denver *et al*. (2010)	MA	N2	Fitness recovery (genomic)

In principle, the attributes that make *C. elegans* such a productive model organism for forward genetics are the same ones that make it an excellent metazoan for quantitative genetics. The goal of any quantitative genetic study is to identify the proportion of heritable phenotypic variation versus environmental variation and, in the case of QTL or association studies, to identify genetic intervals that contribute to this heritable variation. In crosses for most metazoan quantitative genetic studies, biological attributes of the experimental system such as inbreeding depression and slow generation time only permit an F2 or backcross population to be used for mapping purposes. Because unique genotypes can only be phenotyped once, genotype and environment are automatically confounded under these experimental designs. Recombinant inbred lines (RILs) are a powerful solution to the problem of the environmental and individual variance introduced in a mapping cross, provided that the lines can be maintained indefinitely. In such crosses, filial progeny are inbred until they are homozygous at every locus (Hartl & Clark, 2007), yet each line is a mosaic of their contributing parents across the genome.

C. elegans is particularly amenable to mapping using RILs because of its absence of inbreeding depression (Johnson & Hutchinson, 1993), large brood size, rapid 3–4 day generation time and self-compatible mating system. This androdioecious mating system of self-fertile hermaphrodites and rare males allows rapid inbreeding, and homozygous offspring can be cryopreserved in their larval form to minimize deterioration of RILs due to new mutations, genetic drift or lab adaptation. This is not the case in any other metazoan model organism. Further, although as a rule *C. elegans* has only one meiotic recombination per generation (Meneely *et al*., 2002), the process of inbreeding when generating RILs affords many opportunities for recombination, resulting in an expansion of the genetic map and increasing the precision of interval mapping (Lander & Botstein, 1989). While early studies used only moderately dense polymorphic markers (Williams *et al*., 1992), current polymorphic markers are as dense as one per 70 kb, with the potential to increase to more than one per 10 kb (Baird *et al*., 2008). This, combined with advanced intercrossing for further expansion of the genetic map (Rockman & Kruglyak, 2008), creates the tantalizing possibility of generating a QTL map with precision to the tens of genes instead of the current hundreds-of-genes interval. Thus, although there are relatively few studies to date, *C. elegans* has the potential to be a premier system for establishing the emerging field of molecular quantitative genetics.

Here, we review the history of quantitative genetic analysis in *C. elegans*, discussing the modest gains of early studies with possible explanations for their lack of progress. We then highlight the several more recent 'success stories' in this model organism in which the nucleotide basis of quantitative traits have been identified, and discuss the insights that genomic approaches have provided to both mutation accumulation and mapping studies. We conclude by discussing future directions for quantitative genetics in *C. elegans* and suggest that the biology of this nematode can be much better understood by applying quantitative genetics to systems biology. Ultimately, however, this species' mating system and ecology is likely to limit any substantial functional insights into the evolutionary context of the traits being studied, and researchers interested in these ultimate mechanisms may be better served by studying closely related species.

2. The early years: good questions, insufficient tools

Two broad categories of traits particularly suited to being studied in a quantitative genetic framework are life history and behaviour. Life history refers broadly to any measurable traits related to life cycle of an organism, particularly ones salient to reproduction such as brood size, proxies for brood health, age to first reproduction and longevity. Behaviour, likewise, can be loosely defined as an organism's motor response to sensory stimuli and is likely to be influenced by incremental differences that accumulate along that organism's nervous system. A quantitative genetics approach is particularly appropriate for these characters because they are the epitome of complex traits, being influenced by nearly every functional system within an organism. As importantly, both life history and behaviour can be examined within a rigorous theoretical context that allows specific hypotheses to be tested (Lande, 1982; Toth & Robinson, 2007). It is perhaps not surprising, then, that these were also the first traits to be examined in a quantitative genetics framework within *C. elegans*.

Johnson & Wood (1982) first demonstrated that there was a highly heritable basis to natural differences in lifespan using a set of RILs generated from crosses between the canonical lab strain, N2, and a French isolate Bergerac-BO (hereafter, BO), used in part because differences in the number of transposable elements could permit association mapping once appropriate resources were developed (Emmons *et al*., 1983). These lines were also used to identify genetic bases to components of senescence, motility, mortality and reproduction. Analysis of these lines suggested that in *C. elegans* genes specifying the length of a reproductive period are independent of those specifying lifespan (Johnson, 1987), a finding supported by mutant studies and forward genetics (see for example, Huang *et al*., 2004).

https://doi.org/10.1017/S0016672310000601 Published online by Cambridge University Press

It was not until 1991, however, that the first QTL for a life-history trait (or any other trait) was identified (Brooke & Johnson, 1991). This study used single-marker association mapping to identify positive associations between markers on LG IV and LG II and differences in fertility and lifespan. With only 14 RILs and three polymorphic markers, this study was not sufficient to make any definitive conclusions about the genetic architecture of aging. However, it did demonstrate the potential of the *C. elegans* system to identify loci related to life history traits, provided that sufficient polymorphic markers could be developed.

Just one year later, Williams *et al.* (1992) generated a dense genetic and physical map by exploiting the differences in Tc1 transposable elements between BO and N2, identifying 41 informative polymorphic markers that covered 55% of the genome. This set the stage for a series of papers over the next decade that identified multiple QTLs for life-history traits in different experimental conditions (Ebert *et al.*, 1993, 1996; Shook *et al.*, 1996; Shook & Johnson, 1999; Knight *et al.*, 2001). Virtually all of these experiments utilized an N2 × BO cross as the basis for RILs or recombinant inbred populations genotyped using Tc1 transposable elements as polymorphic markers. The frequent occurrence of conflicting results provide an opportunity to examine the role of environmental variation, represented here by different experimental conditions (e.g. different cultivation temperature, agar versus liquid, etc.) on differences in life-history traits, as well as to identify those QTLs that are robust to environmental variation and may be common among other strains (Shmookler-Reis *et al.*, 2006). In particular, different studies have repeatedly found QTL for lifespan and fertility in the centre of chromosome IV, and fertility in the centre of chromosomes III and V, although little progress towards cloning these QTLs was made from these lines.

In the mean time, the very labs studying life-history in a quantitative genetic framework were also using forward genetics to identify mutations affecting life-history traits (Friedman & Johnson, 1988). Various mutant screens identified worms with exceptionally long lifespans, extending post-reproductive longevity three-fold. Other mutants show exceptional stress resistance or susceptibility (reviewed in Johnson, 2007). These mutants laid the groundwork for revolutionary findings in the field of aging research, in particular that these metabolic pathways are conserved across metazoans and have important implications for the effects of oxidative stress, caloric intake and reproduction on aging.

Unlike life-history mutations, until 2001 there were no anaesthetic-resistant *C. elegans* mutants identified so as to build a biochemical pathway for anaesthesia. Four years before this, however, multiple highly heritable QTLs were identified for halothane and isoflurane resistance (van Swinderen *et al.*, 1997, 1998*a*, 1998*b*). Behavioural QTLs relevant to halothane resistance were found on chromosomes I and V, with epistasis (non-additive genetic variation) between these two loci as well as others on chromosomes II, IV and X. Isoflurane resistance co-localizes with QTL on chromosomes I and V. Interestingly, two anaesthesia mutants were later identified on chromosomes I and V in close proximity to the previously identified QTL (van Swinderen *et al.*, 2001; Wang *et al.*, 2001). So, though in this case QTL mapping was not useful for identifying specific alleles that cause variation in anaesthetic susceptibility, the fact that mutants co-localize so strongly with these QTLs indicate the potential for quantitative genetics studies to map variants where mutagenesis was initially unsuccessful.

3. Little genetic diversity, little progress in quantitative genetics

Genotyping polymorphic markers were the rate-limiting step in these early quantitative genetic studies, so the presence of easily traceable transposable elements between the Bergerac (BO) and Bristol (N2) lines was advantageous. However, the same mutational processes that generate these markers can also reduce the certainty of any experiments conducted with these lines. The issues with using a mutator strain (BO) were recognized early on (Moerman & Waterston, 1984) and reiterated in the discussion sections of most QTL papers. First, the genetic components responsible for active transposition in one parental strain would also be active in a subset of the RILs (see, for example, Mori *et al.*, 1988, 1990), thereby reducing confidence in the assumed stability of the genotypes. This may reduce the heritability of traits measured because genetic differences due to transposition would be misattributed to environmental variance. Second, if the transposable elements actively disrupt genetic and cellular processes, the differences in phenotype would be due to recent transposition within a mutator line, rather than arising as variation within a natural population. This limits the conclusions that can be drawn from any such study. In particular, despite vast differences in the number of transposon insertions between the two strains (Williams *et al.*, 1992), the N2 and BO strains are actually quite closely related, with about one polymorphism per 10 000 bp (Denver *et al.*, 2003; Koch *et al.*, 2000). This suggests that any functional variation between them may in fact be due to transposable elements themselves. This is especially the case for chromosomes I, II and III, which more recent genetic studies have shown harbour almost no N2–BO genetic variation, and chromosomes V and X, which show variation only on the chromosome tips.

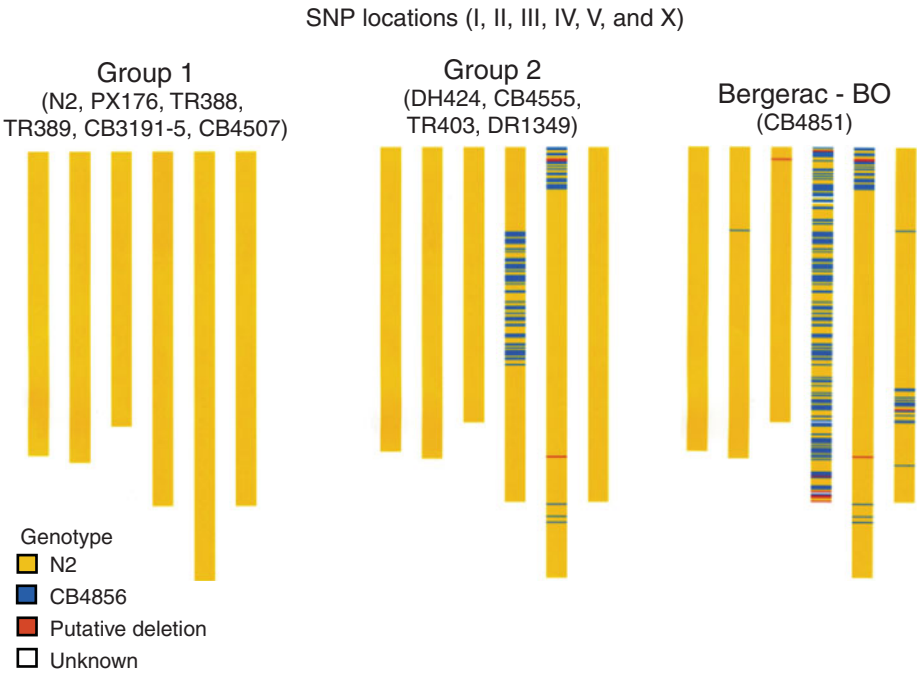

Fig. 1. Analysis of polymorphisms in isolates closely related to the standard *C. elegans* strain, N2. 'Group 1' strains are over 99% identical to N2 when analysed at 1454 N2/CB4856 SNPs. 'Group 2' strains have an SNP pattern consistent with meiotic recombination from a cross between N2 and Bergerac (BO). Modified with permission from McGrath *et al.* (2009).

Indeed, virtually all of the genetic variation between N2 and BO is contained on chromosome IV (Fig. 1), so it is perhaps not surprising that most of the repeatedly detectable and the only genetically verified QTL were localized to that chromosome (Knight *et al.*, 2001). During this period there were efforts to map QTL with other strains (Ebert *et al.*, 1996), but unfortunately the same problems remained as one of the parents (DH424) used appears to be the result of a cross between N2 and BO (Fig. 1; Egilmez *et al.*, 1995; McGrath *et al.*, 2009).

How critical are these problems? It depends on the questions being asked. If a transposable element (TE) disrupts a gene that is central to a metabolic or aging network and the researcher is interested in constructing a basic biochemical pathway that describes that network, then the results from mapping studies between nearly isogenic strains with variable transposable element activity are potentially quite informative. However, the past 30 years have demonstrated that if this type of network construction is the goal, forward genetics is the most efficient approach and yields faster results. In contrast, if the goal is understanding natural processes that effect small quantitative changes in life-history traits or any other phenotype, then mapping using an N2 × BO cross is a poor approach. Using a mutator strain in a mapping cross has the potential to release any transposition-suppressive effects that have co-evolved within the strain, dramatically increasing the mutagenic effect in RILs. Because these potentially disruptive TEs are novel, their effects cannot be directly assayed, but only inferred based on their proximity to previously identified TEs, and the genetic map based on these elements covered only 55% of the genome. To date, no gene has been cloned from a QTL identified in the N2 × BO RILs, and their use essentially disappeared from the literature almost a decade ago.

4. From genotype to phenotype using extensive genetic diversity

Using a quantitative genetics approach to identify the genetic basis of life-history traits fell out of favour around the turn of the century, perhaps due in part to the successes of traditional forward-genetics approaches in addition to the inherent limitations of a quantitative approach given the strains being used. The dearth of *C. elegans* quantitative genetics papers in the first half of the 21st century marks a transition from a research goal of understanding the genetic basis of complex traits to a goal of understanding the genetic basis of natural variation in complex traits (Fig. 2*a*). The shift is subtle but important: in the world of natural variation, mutagenesis is uninformative, as the questions are fundamentally concerned with natural processes that shape quantitative traits wherein the role of mutations is countered by other population genetic processes. Additionally, mutations of small or epistatic effect are unlikely to be

https://doi.org/10.1017/S0016672310000601 Published online by Cambridge University Press

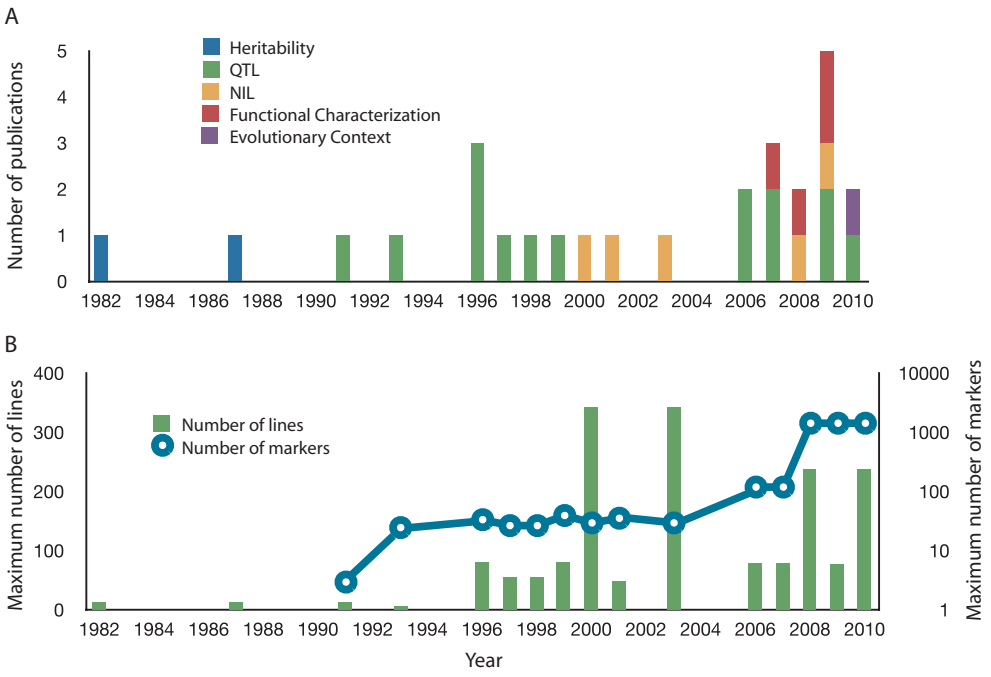

Fig. 2. Progress on specifying the genotype–phenotype map with *C. elegans*. Progress is rank-ordered from the analysis of heritability (low) to identifying the evolutionary context for a cloned gene (high). Bars are derived from publications cited in Table 1 in which the objective of the paper was to identify the genetic basis to a complex trait using quantitative genetics. Much of this progress has been generated by an increase in the number of lines and markers used over time, as shown in (B). For each year, the publication with the most RILs and markers used was plotted. For 2000 and 2003, recombinant inbred populations and cohort selection were used, so the uniqueness of each line's genotype cannot be certain, and the total number of lines therefore is somewhat exaggerated.

localized using standard mapping strategies as these effects become diminished over repeated backcrosses. However, with sufficiently distinct phenotypic variation and sufficient marker coverage in carefully controlled experiments, these small-effect and epistatic interactions can be mapped in natural populations. Interest in these questions served to recruit a new wave of biologists interested in using the power of the *C. elegans* system to address longstanding questions in quantitative genetics.

With the re-emergence of *C. elegans* quantitative genetics, came new lines, new crossing strategies, more markers and more success in identifying the genetic basis of quantitative traits. Underlying this re-emergence was the rapid development of molecular genetic tools: more efficient genotyping of Tc1 elements (Ayyadevara *et al.*, 2000*a*), a sequenced genome (*C. elegans* Sequencing Consortium, 1998), a second sequenced genome (Wicks *et al.*, 2001) and a set of restriction-fragment-length polymorphism (RFLP)-based high-density markers (Wicks *et al.*, 2001). Shortly after the N2 genome was sequenced and the cost of sequencing declined, more genetically and phenotypically diverse strains could be used in mapping crosses, which is likely the key factor in the increasing successes of quantitative genetics studies in *C. elegans*. This success ranges from the basic task of identifying a causal relationship between a trait and its associated locus, to having the power to detect the environment's influence on a phenotype, to finding the nucleotide basis of differences in a phenotype, and finally to using these differences to build a quantitative network.

5. Using introgression lines to test QTL hypotheses

In any mapping study, the ultimate outcome is identifying a statistically significant relationship between genotype and phenotype. However, this relationship is purely correlative and can be influenced by extraneous factors such as environmental variance and segregation distortion, creating a spurious association where none exists. Spurious or miss-localized QTL can also appear due to incomplete marker coverage or in the presence of several linked QTLs (Bost *et al.*, 2001). While this is a problem in most model organisms, the rapid generation time of *C. elegans* permits extensive backcrossing into a parental line such that a QTL-containing chromosomal region of one parental strain is isolated in the contragenic background, generating a nearly isogenic line (NIL). Any phenotypic difference between a NIL and its parent is thus caused by the specific chromosomal segment captured by this introgression. The presence of a phenotype in

https://doi.org/10.1017/S0016672310000601 Published online by Cambridge University Press

introgression lines demonstrates causation from QTL data and, like three-point mapping in forward genetics, are an important step in identifying the specific nucleotides that cause variation in a phenotype.

Ayyadevara *et al*. (2000*b*, 2003) were the first to use NILs in *C. elegans*, and they did so in order to explore the influence of different genetic backgrounds on life history variation, thereby moving beyond the N2 × BO paradigm. NILs were generated from regions identified in a QTL mapping cross in a BO × RC301 (Ayyadevara *et al*., 2000*b*) and a BO × CL2a (CB4857) RIL panel (Ayyadevara *et al*., 2003). In the former case, presence of BO introgression at two separate loci on chromosomes III and X reduced lifespan consistent with the predicted effect from mapping; presence of an RC301 introgression into BO at those same loci increased lifespan (Ayyadevara *et al*., 2000*b*). In the latter case, two sets of NILs were made from QTL on chromosomes IV and V, again verifying the allelic effect predicted in the mapping study. Unfortunately, the introgressions were several megabases, making finer-scale molecular work difficult.

Although these papers demonstrated that fine-mapping approaches are possible in a *C. elegans* QTL study, the drawbacks of using a mutator strain (BO) still remained, and with genotyping costs rapidly falling, there was no longer any reason to keep using this strain simply for the ease of genotyping. A Hawaiian isolate, CB4856, emerged as the strain most genetically distant from N2, and quickly a set of markers based on polymorphisms affecting restriction endonuclease sites (RFLPs) was developed (Wicks *et al*., 2001). These 493 markers spanned the entire genome with an average of one marker every 0·6 cM, enabling rapid mapping of polymorphic traits and mutants. Given their increasing marker density and newer strategies for crossing to increase recombination and expand the genetic map (Darvasi & Soller, 1995; Rockman & Kruglyak, 2008), N2 × CB4856 RILs have been the predominant set of lines used for quantitative genetics over the past 5 years. This half-decade, perhaps not coincidentally, has also seen the most success in mapping specific alleles causing natural variation in phenotypes (Fig. 2*a*). However, this may not be the coup it initially appears to be, as each of these QTLs behave in an essentially Mendelian fashion. Nonetheless, the first gene cloned from a QTL in *C. elegans* was functionally characterized in 2007 (Kammenga *et al*., 2007), followed closely by three more (Palopoli *et al*., 2008; McGrath *et al*., 2009; Persson *et al*., 2009). Each of these cases yield insights into the strategies for mapping genetic variants in *C. elegans*, and provide a cautionary tale against drawing broad conclusions from only one strain.

6. A Mendelian polymorphism with an evolutionary context

Hodgkin & Doniach (1997) observed several phenotypic differences among wild isolates of *C. elegans*, including presence or absence of a copulatory plug and tendency to burrow into agar or clump on a plate. Although the copulatory plugging locus was localized to a large region on LGIII, no progress was made for over a decade in identifying the precise mechanism for this Mendelian polymorphism. The gene responsible for differences in copulatory plugging was ultimately cloned and characterized using two separate RILs, one generated from a cross between N2 and CB4855 enriched for recombination on chromosome III, and another from an advanced intercross between N2 and CB4856 (Palopoli *et al*., 2008). Non-plugging strains, including N2, have a retrotransposon insertion in the open reading frame, inhibiting production of a mucin protein that generates the plug.

Variation in copulatory plugging is interesting from an evolutionary perspective because it represents a type of sexual selection in the form of passive mate-guarding, and its polymorphic state in the wild suggests different selective pressures for male *C. elegans*, whose frequency varies in natural populations (Teotónio *et al*., 2006; Anderson *et al*., 2010) and whose utility is indispensable (Morran *et al*., 2009) despite their mysterious indifference to hermaphrodite mating cues (Chasnov *et al*., 2007). From a lab perspective, because the plugging allele is dominant and specifically caused by a retrotransposon insertion in the N2 background, this locus could not have been mapped without using a phenotypically divergent natural isolate. This is worth taking into consideration for other genes in the N2 background whose functions are unknown: perhaps natural processes have rendered these genes non-functional in this particular background, but another background could provide better insights into their biological roles.

7. Explicitly testing the environmental influence on a phenotype

The role of the environment in shaping phenotypic variation is often acknowledged but rarely tested. In traditional quantitative genetic theory, all variations in a phenotype are by definition partitioned into that which can be accounted for by genetics (i.e. heritable differences) and that which cannot (i.e., environmental variation). Because 'environment' is so broadly defined, the cause of environmental variation can range from epigenetic intracellular variation to stochastic processes during maturation and adulthood. Some of the earliest studies of variation among *C. elegans* natural isolates combined detailed knowledge of *C. elegans* development with questions related

https://doi.org/10.1017/S0016672310000601 Published online by Cambridge University Press

to the robustness of those developmental systems to environmental variation. Delattre & Felix (2001) investigated variation within and between strains in one of the best characterized developmental systems in *C. elegans*: the hermaphrodite vulva. They were able to demonstrate individual variation down to the level of a single cell lineage. Subsequent work showed that the 'error rate' of vulval development, while very low overall, can be altered by environmental effects (e.g. starvation) in a strain-specific manner (Braendle & Felix, 2008, 2009). Additional among-strain (or 'cryptic') genetic variation for vulval development can be revealed by sensitizing the strains by backcrossing in mutations that disrupt genetic pathways known to govern normal vulval development (Milloz *et al*., 2008). Because it is a trait that varies among strains, this sensitivity to environmental effects can in principle be mapped (i.e. using variation *per se* as the trait, e.g. Chandler, 2010).

As ectotherms, temperature is one aspect of the environment that has potential to drive huge phenotypic differences among isolates of *C. elegans*. The genetic basis for these differences is identified statistically by testing for an interaction effect between genotype and environment (a G × E interaction). Because this is a measurable phenotype, it can be mapped just like any other: indirectly by comparing two QTL maps derived from different environments, or directly by generating a QTL map using the interaction effect term as the phenotype. RILs allow the same genotype to be measured multiple times, thereby increasing the precision of the genetic estimates. This allows specific hypotheses about the nature of the environment's influence on phenotypic variation to be directly addressed. Jan Kammenga and colleagues have identified important factors relating temperature and genotype to life history traits using a set of 80 RILs between N2 and CB4856 genotyped at 121 polymorphic markers (Gutteling *et al*., 2007*a*, *b*). Traditional QTL mapping of life history phenotypes, including fertility, egg size and growth rate, produced QTL at different genomic locations at different temperatures, suggesting a plastic response (Gutteling *et al*., 2007*a*). Testing explicitly for an interaction effect, 23% of these QTLs had a significant G × E component (Gutteling *et al*., 2007*b*), which, though fewer in number than expected based on other organisms (see e.g. Vieira *et al*., 2000), indicate that the environment plays a large role in the evolution of quantitative traits and that detection of QTL relies on assays in multiple conditions.

Explicitly using the genotype–environment interaction term allowed Kammenga *et al*. (2007) to clone the first QTL in *C. elegans*. The N2 strain, like most ectotherms, follows a temperature-size rule where ectotherms raised at cooler temperatures tend to be larger than those raised at warmer temperatures, though the mechanism of this size difference was unclear. Body size in CB4856 is not significantly affected by temperature, however, and by using RILs this G × E interaction was mapped to intervals on both chromosomes III and IV. The distal chromosome IV QTL contained a promising candidate gene, *tra-3*, which is known to influence cell size in mammals. Complementation testing verified that an F96L SNP acts in the CB4856 background to inhibit calcium binding in the TRA-3 regulatory protease, keeping this strain smaller in lower temperatures.

Similar to temperature, food availability can have profound effects on *C. elegans* development. Dauer formation is an alternative stress-resistant phase of development for *C. elegans*, and one of the biochemical pathways follows the highly conserved insulin-like signalling pathway (Hu, 2007). There is natural variation in the propensity to form dauer larvae (Viney *et al*., 2003), which has been attributed to several QTLs (Harvey *et al*., 2008). Because this is a measure of phenotypic plasticity, it is likely that the plasticity is under transcriptional control, and as such can be examined by comparing gene expression in different wild isolates and RILs (Harvey *et al*., 2009). Although findings from this study were not sufficient to map loci responsible for such plasticity, this work did demonstrate that large suites of genes are differentially expressed among lines in the presence and absence of dauer pheromone, and that many of these genes possess an excess of binding sites for DAF-16, a dauer-induced transcription factor. A more thorough investigation of these phenotypes may uncover novel dauer response targets, as well as specific genetic elements controlling plasticity in this response.

8. Perturbation of the genetic map as nuisance – and as data

In QTL mapping, evidence for linkage between polymorphic markers and a locus causing differences in a quantitative trait is driven by a statistical relationship between genotype and phenotype. As with any other statistical test, this relationship can be biased if sample size is uneven between groups. This can arise if, for any given marker, one genotype is significantly more common than another in a mapping population. If this pattern persists over multiple markers in the same genomic region, then it is likely to be a signature of segregation distortion, which could indicate hybrid incompatibility or meiotic drive.

Such a pattern was observed on the left arm of chromosome I during construction of N2 × CB4856 recombinant inbred advanced intercross lines (RIAILs) (Siedel *et al*., 2008), where only 5 of 239 lines carried the CB4856 allele; unlikely considering the extensive opportunities for recombination as the lines were generated. By taking advantage of

https://doi.org/10.1017/S0016672310000601 Published online by Cambridge University Press

high-density markers and extensive recombination in an advanced intercross, these tightly linked loci of incompatibility were mapped to a 62-kb region, or roughly 0·23 cM on the standard *C. elegans* genetic map. When explored further, Seidel *et al.* found that this segregation distortion was the result of two tightly linked loci acting non-additively between the male parent and the zygote to cause embryonic lethality: the N2 paternal allele of one locus (*peel-1*) is lethal if it interacts with the CB4856 allele of the second locus (*zeel-1*), though the converse is not true: offspring sired by spermatozoa carrying the CB4856 *peel-1* allele have an embryonic lethality no different from wild-type.

This type of interaction puts N2 males at a significant reproductive disadvantage, which is particularly astonishing considering that the N2 allele of *peel-1* is segregating in populations carrying the CB4856 *zeel-1* allele, with a population genetic pattern consistent with balancing selection or counterbalancing positive selection on a tightly linked locus. Consistent with the enigmatic ecology of this nematode, the precise mechanism of this balancing selection is currently unclear. The segregation of a major incompatibility locus at high frequencies within populations really only makes sense if self-fertilization is the predominant mode of reproduction within *C. elegans*. Continuous selfing generates strong linkage disequilibrium, which in turn allows alleles with strong epistatic effects among loci to enter the population.

9. A digenic basis to oxygen sensing

In addition to differences in copulatory plugging, vulval development, temperature-dependent growth rates and reproductive compatibility, wild isolates of *C. elegans* have different responses to increases in oxygen or carbon dioxide by modulating either speed (Persson *et al.*, 2009) or turning frequency (McGrath *et al.*, 2009). Much of the variation in this response was found to map to a gene called *npr-1*, which distinguishes other behaviours among wild isolates (de Bono & Bargmann, 1998). Researchers used RILs between either N2 and CB4856 (McGrath *et al.*, 2009) or CB4856 and AX613, an N2 mutant with a CB4856 *npr-1* allele (Persson *et al.*, 2009), and found that in addition to *npr-1*, a region of chromosome V was strongly associated with differences in this phenotype. McGrath *et al.* (2009) tested the effects of this locus by generating NILs in the N2 background and took a candidate gene approach to map the polymorphism responsible. Persson *et al.* (2009) mapped and sequenced the same interval, testing associations where all wild isolates with one SNP slowed down with decreased oxygen, and all the isolates with another SNP did not change their speed with a change in oxygen. Both groups identified a duplication event in the N2 allele of *glb-5*, a globin gene that can bind oxygen and carbon dioxide. The duplication causes a frame-shift and mis-splicing of the gene product, substantially reducing its activity as evidenced by neural activity assays.

These papers found examples of seemingly naturally segregating variation causing completely different behaviours, where the allelic effect of two genes, *npr-1* and *glb-5*, acting together explained the variance observed between two wild isolates. Like previous studies, NILs verified mapping data, resulting not only in a cloned gene but also giving a functional context to that natural variation. While these papers successfully characterized the genetic basis of a quantitative trait, all were performed using an N2 as one of the parents. This is reasonable starting point, as virtually all molecular tools including transgenic lines were developed in the N2 background. However, as McGrath *et al.* (2009) found, using N2 to make inferences about the effects of natural genetic variation ignores massive amounts of lab adaptation that has taken place in this strain.

10. A major locus of lab adaptation

McGrath *et al.*'s (2009) analysis identified a disturbing pattern of nucleotide variation around the *npr-1* locus, one of the two genes responsible for the observed variation in response to changes in oxygen and carbon dioxide concentration. Over a decade earlier, this gene was identified as the causal agent in variation among isolates of 'clumping' and burrowing on Petri dish or engage in 'social feeding' by using mutagenesis and standard mapping strategies (de Bono & Bargmann, 1998). When it was initially cloned, *npr-1* was heralded as one of the first loci to demonstrate a naturally segregating allelic basis to differences in behaviour (Thomas, 1998). NPR-1, a G protein-coupled neuropeptide receptor, has since been found to influence virtually all behavioural variation among wild isolates when compared with N2 (Macosko *et al.*, 2009), including foraging behaviour, innate immunity (Reddy *et al.*, 2009), gustation (Pocock & Hobart, 2010), oxygen- and carbon dioxide-avoidance (Persson *et al.*, 2009; McGrath *et al.*, 2009), pheromone cues (Macosko *et al.*, 2009) and ethanol responses (Davies *et al.*, 2004).

When isolates were analysed at all N2/CB4856 SNPs in addition to the *npr-1* allele, however, many of the adaptive stories that had been spun around this polymorphism began to unwind. As N2 and CB4856 are the most genetically distinct strains for this set of markers, any wild isolates should have some mix of N2 and CB4856 SNPs; the extent to which the balance shifts towards more N2 or more CB4856 SNP frequency reflects their phylogenetic relationship (Rockman & Kruglyak, 2009). If variation at *npr-1*

were locally adaptive, one expectation is that the clumping phenotype would arise repeatedly in different backgrounds, such that variation at *npr-1* (or, multiple *npr-1* alleles) would be uncorrelated with phylogenetic relationships. Alternatively, perhaps the *npr-1* allele arose only once but was so strongly selected for that it swept to fixation in certain geographic regions or environmental conditions, in which case we would expect *npr-1* allelism to be in strong linkage disequilibrium with SNPs surrounding that locus.

Although the actual findings are consistent with an adaptive selective sweep of sorts, the *npr-1* N2 allele is actually the result of lab adaptation. Phylogenetic analysis of so-called wild isolates with N2-like clumping behaviour attributed to the *npr-1* locus revealed that these isolates are either genetically identical to N2 and may be recaptured N2 escapees or lab contaminants, or (disturbingly) are recaptured escapees from an N2 × BO cross (Fig. 1). Further analysis showed that the N2 *npr-1* allele arose during lab adaptation before N2 was cryopreserved (McGrath *et al*., 2009). Thus, despite the utility of N2 and the mapping success using N2 × CB4856 RILs for mapping Mendelian traits, many of the differences between these two strains, especially behavioural ones, are likely due to the large effect of *npr-1* and associated mutations that arose in the N2 background as it lived for thousands of generations in the lab.

11. Mutation accumulation in a post-genomic era

If nothing else, laboratory adaptation can generate a strong signal that allows one to directly map the transition from genotype to phenotype. This feature actually makes *C. elegans* a powerful system for experimental evolution. The first area to capitalize on this in a quantitative genetic framework exploited the selfing aspect of the *C. elegans*' mating system to construct multiple genetically homogeneous lines in order to study the accumulation of spontaneous mutations. The work of Peter Keightley and Michael Lynch and their colleagues has provided estimates for the rate of mutation accumulation for a variety of traits including reproduction and longevity (Keightley & Caballero, 1997; Vassilieva & Lynch, 1999; Estes *et al*., 2005; Vassilieva *et al*., 2007; Joyner-Matos *et al*., 2009), body size (Azevedo *et al*., 2002), development (Braendle *et al*., 2010), gene expression (Denver *et al*., 2004) and behaviour (Ajie *et al*., 2005). Overall, these studies show that all traits accumulate spontaneous mutations that contribute to natural genetic variation at approximately the same rate (10^{-3}–10^{-4} of the environmental variance each generation). While this information is important for understanding many fundamental aspects of quantitative variation, in the context of identifying the genetic basis of quantitative traits, these studies are particularly useful because they all begin in a single common genetic background. This provides the potential to identify the actual changes in DNA sequences that have led to the novel quantitative variation for the trait of interest.

The first steps along this road were taken by sampling random parts of the genome in order to estimate the actual per-nucleotide mutation rate in order to compare it to the estimated mutation rate observed at the level of phenotype (Keightley & Eyre-Walker, 2000; Denver *et al*., 2000, 2004; Estes *et al*., 2004). Each of these studies suggest that perhaps less than 1% of mutations have phenotypic effects that are observable at the level of resolution possible within the laboratory. Coupled with the very large average effect size for new mutations that tends to be estimated in mutation accumulation studies, this suggests that the distribution of mutational effects is very L-shaped, with a very large number of mutations of small effect and a much smaller number of mutations with fairly large effects. Recent advances in sequencing technology now allow entire genomes to be sequenced at fairly low cost. This approach has been used to good effect for the mutation accumulation lines (Denver *et al*., 2009). These comprehensive sequencing approaches have confirmed the earlier observations that the per nucleotide mutation rate is of the order of 10^{-9}.

A different class of mutation accumulation experiments is possible when one begins the process using a low fitness genotype that is then allowed to adapt to either original laboratory conditions or to a novel environment. Whereas the mutation accumulation experiments described above are largely focused on deleterious mutations, these so-called 'fitness recovery' and laboratory adaptation experiments focus on new beneficial mutations. Estes & Lynch (2003) conducted a series of such experiments that suggested that most of these mutations are compensatory in nature, 'fixing' the deleterious effects of the initial mutations. Denver *et al*. (2010) have recently sequenced the genomes of a number of replicates from a similar experiment and have been able to identify a relatively small set of mutations that must be at least partially responsible for the fitness recovery. Their analysis suggests that there must be very strong selection on a small subset of these mutations and that the majority of them probably reach fixation via genetic hitchhiking, which should be very strong within self-fertilizing populations, as we discuss below. Interestingly, in keeping with the hypothesized compensatory nature of the evolutionary response, a number of the observed mutations appear to interact directly with some of the mutations that led to the low fitness of the ancestral population. However, most of the observed mutations arose late in the experiment,

whereas most of the fitness recovery was observed early in the experiment. This suggests either that some classes of mutations went unobserved under whole-genome re-sequencing (e.g. duplications and deletions) or that the populations were in a state of constant flux, with multiple substitutions replacing one another over time.

None of these whole genome re-sequencing projects has yet to establish a causal relationship between a specific observed mutation and a phenotypic outcome. Thus, while this approach appears promising, more work is needed to directly tie genotype to phenotype. The increasing popularity of *C. elegans* as a system for experimental evolution studies (e.g. Morran *et al*., 2009) should provide a rich set of starting materials for re-sequencing efforts. The existence of a reference genome and the increasing ease of large-scale sequencing should make whole-genome association studies realistic within the species. Such approaches have already been attempted using lower resolution marker analyses (Rockman & Kruglyak, 2009). As we discuss below, however, extensive linkage disequilibrium and the highly fragmented population genomic structure within *C. elegans* may preclude particularly high-resolution analyses for large sections of the genome.

12. Gene expression as an intermediate phenotype

In the quest to understand how a genotype produces quantitative variation in a phenotype, the golden age of *C. elegans* is emerging now. However, it must be said that for all the success in mapping the nucleotide basis of quantitative traits in the past 5 years, all of the loci display Mendelian segregation with large effect alleles, and if more than one locus is involved, the effect of multiple loci are largely additive (although interactions can be formally detected, McGrath *et al*., 2009). This is likely due to ascertainment bias, as virtually all phenotype QTLs cloned in metazoans are those of the low-hanging fruit variety: big effect sizes that are robust to environmental variation. Regardless of whether these QTLs are the result of rapid adaption (for example, Chan *et al*., 2010; Steiner *et al*., 2007) or are lab-derived (McGrath *et al*., 2009), we can hardly say that these are an accurate representation of all adaptive processes. Surely at least some of the variation we see within populations is due to the aggregate effect of small-effect alleles (e.g. Rebeiz *et al*., 2009), but how can these loci be identified considering their detectability may be overwhelmed by environmental variation?

One of the ways to tease apart small effect QTL from stochastic environmental variation is to nest the QTL analysis in multiple phenotypic scales, from gene expression to morphology and behaviour. By assaying variation in gene expression among RILs, effects of locally and distantly acting elements can be inferred. In these studies, when the genetic location of QTL and the genomic location of genes are compared, if a QTL co-localizes with a gene, it is assumed that the variation in that gene's expression is due to locally acting factors. Alternatively, variation in multiple genes' transcript abundances may link distantly to a regulatory 'hot spot,' which is likely the signature of a regulatory element with multiple downstream targets (Rockman & Kruglyak, 2006). These effects are usually interpreted as being in either *cis* or *trans*, respectively, but in fact mapping resolution is usually too low to be particularly firm in this regard, especially for *cis*-acting factors (Rockman & Kruglyak, 2006).

Using this approach, Li *et al*. (2006) assessed the genetical genomics underlying phenotypic plasticity to temperature in life history traits. N2 × CB4856 RILs were used to measure genome-wide gene expression at two different temperatures, testing for expression QTL (eQTL), temperature QTL and plasticity QTL (pQTL), which was scored as the interaction between expression levels and temperature. Although there were hundreds of strain- and temperature-specific eQTL, which is consistent with any type of protective heat-shock response, there were only a few pQTL, most of which are *trans*-acting and contain genes that are strong candidates for plasticity regulators. Further testing with either higher-density markers or under permissive conditions could lead to cloning genetic determinants of phenotypic plasticity. Using similar genetical genomics approaches, Li *et al*. (2010) investigated genetic variation controlling alternative splicing, reporting very few examples of heritable variation in alternative splicing (22 transcripts). This suggests that the regulatory mechanism of alternative splicing in *C. elegans* is robust with respect to genetic variation at the genome-wide scale. Similarly, Viñuela *et al*. (2010) reported on the genome-wide gene expression regulation as a function of genotype and age in an N2 × CB4856 RIL population and demonstrated that eQTL patterns are strongly affected by age, suggesting that the integrity of the gene network declines with age. Despite the intriguing insights that these studies provide, however, the evolutionary conclusions that can be drawn from these types of studies may be limited (Rockman *et al*., 2010).

Although it was already known that genomic patterns of nucleotide diversity in *C. elegans* was driven in part by its mating system and in part by genetic hitchhiking or background selection (Cutter & Payseur, 2003), the effect that this diversity had on gene expression and other phenotypes was unknown. Rockman *et al*. (2010) quantified variation in transcript abundance for over 15 000 genes across a panel of advanced-intercross RILs between N2 and CB4856

genotyped at more than 1400 marker loci. The first main conclusion from this study is that most of the pattern of transcript abundance in these lines is due to eQTL in linkage disequilibrium with the gene, meaning it is likely due to *cis*-regulatory variation. The second conclusion is that *npr-1* underlies one of the three *trans*-acting linkage 'hotspots,' which is consistent with the possibility that that this lab-derived allele has as broad influence on all aspects of *C. elegans* biology. However, the fact that different studies have identified different regulatory hotspots (e.g. Li *et al*., 2006) suggests that micro-environmental differences experienced by individuals during development may play an equally important role.

A third, extremely important conclusion emerged from this paper that has broader implications for *C. elegans* quantitative genetics. Due to its mating system there is limited noticeable recombination and low genetic variation locked in long haplotype blocks in the centres of chromosome, and short haplotype blocks on the chromosome arms (Cutter, 2006; Rockman & Kruglyak, 2009). As a rule, deleterious mutations are rapidly purged from populations along with any neutral variation to which they are linked. In a process known as background selection, when SNPs are in tight linkage with new deleterious mutations, they can be rapidly driven out of the population, reducing the overall level of polymorphism (Charlesworth *et al*., 1993). Because of extensive, chromosome-wide linkage disequilibrium, this has the potential to be a particular problem within *C. elegans*. Rockman *et al*. (2010) found that the pattern of variation in gene expression was almost entirely explained by a model of background selection, suggesting that it is unlikely that observed differences in gene expression are the result of adaptive processes. Thus, while *C. elegans* is often described as having low genetic diversity, in reality its average diversity is low, but with extremely low diversity and reduced recombination in the gene-rich middle 2/3 of the chromosome and moderately high genetic diversity and recombination at the chromosome ends. While linkage can drive a decrease in variation due to deleterious mutations in background selection, it can also deplete variation under positive selection during a selective sweep of a new advantageous mutation. There is therefore a strong possibility that chromosome location may affect the evolutionary potential (or evolvability) a gene. We might predict, then, that much of at least the initial response to selection to a novel environment might involve genes at the chromosome ends despite the fact there may be fewer functional genetic targets within these regions. The relationship between chromosome architecture and genetic architecture for adaptive change is a question that more comprehensive whole-genome approaches should be able to address in other organisms in the near future, but Rockman *et al*. (2010) have shown that this likely to be a general feature for patterns of genetic variation within *C. elegans*.

13. Looking ahead: a quantitative approach to proximate and ultimate causation–systems quantitative genetics

While there are clearly problems with the natural context of its genetic variation, the features of *C. elegans* that make it so amenable to being a model organism, specifically fast generation time, large broods and cryopreservation, are the same elements necessary to conduct the most powerful functional quantitative genetics studies possible. Additionally, because of its mating system and the genetic resources already available, it is remarkably easy to generate and genotype novel sets of RILs using any parental strains, including transgenic ones, and avoid the pitfalls of lab adaptation. These features have also allowed the generation of NIL panels, which are a powerful complement to any mapping study (Doroszuk *et al*., 2009).

As high-throughput phenotyping becomes less cost-prohibitive, it is possible to engage in comprehensive phenotyping on sets of RILs in multiple environmental contexts. For example, microfluidic approaches can be used to measure body size and sex (Rohde *et al*., 2007), motor and neural response to sensory stimuli (Chalasani *et al*., 2007), locomotion (Lockery *et al*., 2008) longevity and potentially fecundity (Rohde *et al*., 2007). QTL identified in using these approaches have the potential to reveal small-effect and epistatic interactions among loci that are difficult to dissociate using traditional forward genetics. Additionally, combining multiple sets of phenotype data can be used to reveal pleiotropic alleles or pathways. This comprehensive phenotyping can also be performed in the context of different environments or developmental conditions, thus testing the effect of early life history on behaviour or life history traits in a precise and high-throughput manner.

Despite the problems of lab adaptation, the N2 strain is invaluable in the worm community because virtually all mutants were derived in this background. These mutant lines still have their uses in a quantitative genetic framework. For example, quantitative genetics can be performed in the context of a modifier screen, i.e. in the presence of an already-characterized mutation (e.g. Milloz *et al*., 2008; Chandler, 2010) to identify the effect that mutation has on genome-wide gene expression as well as on the target phenotype (Schadt *et al*., 2003). Because several scales of phenotypes are being measured concurrently, the connections between phenotypes can be quantified and can also identify novel candidate interactors, which

can then be functionally characterized (Chen *et al*., 2008). This approach can be used to generate a robust quantitative gene interaction network that can be used to address questions of proximate causation in any given phenotype (Rockman, 2008).

14. Patterns and process of natural variation

Because background selection and other linkage-dependent factors are likely to overpower most other selective processes (Rockman *et al*., 2010), *C. elegans* is hardly an ideal organism to understand patterns of population genetic processes. However, several labs are currently developing species closely related to *C. elegans* as model organisms to address the relationships between natural genetic variation and population genetics processes (Jovelin *et al*., 2003; Phillips, 2006; Kammenga *et al*., 2008). While *C. elegans* is often found commensal with humans, other species are often found in association with invertebrates in more natural habitats (Baird, 1999), so the evolutionary and ecological context of any variation in phenotype can potentially be tested.

For example, *Caenorhabditis briggsae*, which diverged from *C. elegans* approximately 80–110-million years ago (with roughly the same total nucleotide divergence as observed between mice and humans, Kiontke *et al*., 2004), shares many of the same strengths for quantitative genetic studies as *C. elegans*. *C. briggsae* is an emerging model for identifying the genetic basis for natural variation in the vulval cell lineage (Dolgin *et al*., 2008), temperature-dependent fecundity (Prasad *et al*., 2010) and male tail morphology (Baird *et al*., 2005). However, because they share the same (though independently derived) mating system that is partially responsible for unusual patterns of genomic nucleotide diversity observed in *C. elegans*, it is unclear to what extent variant phenotypes in *C. briggsae* will pose the same problems for adaptive inference as is currently found in *C. elegans*.

Other related species, particularly those with dioecious, obligately outcrossing mating systems, have orders of magnitude more genetic variation than *C. elegans* (Graustein *et al*., 2002; Jovelin *et al*., 2003; Jovelin *et al*., 2009; Cutter *et al*., 2006; Wang *et al*., 2010), creating an opportunity for association mapping in cases where RILs are not feasible. High-density polymorphic markers can be rapidly generated (e.g. Baird *et al*., 2008) and aligned against one of many sequenced *Caenorhabditis* species, allowing the same high-powered quantitative genetics experiments to be conducted in animal species more representative of the global population of metazoans. Thus, the future of quantitative genetics within this genus seems to be to perform functional analyses within *C. elegans*, but to explore questions related to natural variation within other closely related species.

We thank Cori Bargmann, Asher Cutter, Marie-Anne Felix, Simon Harvey, Tom Johnson, Jan Kammenga, Leonid Kruglyak, Matt Rockman, Robert Shmookler-Reis, Mark Viney, the members of the Phillips lab, and two anonymous reviewers for helpful comments and discussions. Cori Bargmann graciously provided materials for one of the figures. This work was supported by grants from the National Science Foundation (DEB-0641066 and IOS-0909816), the National Institutes of Health (P01-AG022500) and the Ellison Medical Foundation.

References

Ajie, B. C., Estes, S., Lynch, M. & Phillips, P. C. (2005). Behavioral degradation under mutation accumulation in *Caenorhabditis elegans*. *Genetics* **170**, 655–660.

Anderson, J. L., Albergotti, L., Proulx, S., Peden, C., Huey, R. B. & Phillips, P. C. (2007). Thermal preference of *Caenorhabditis elegans*: a null model and empirical tests. *Journal of Experimental Biology* **210**, 3107–3116.

Anderson, J. L., Morran, L. T. & Phillips, P. C. (2010). Outcrossing and the maintenance of males within *C. elegans* populations. *Journal of Heredity* **101**, S62–S74.

Ayyadevara, A., Thaden, J. J. & Shmookler-Reis, R. J. (2000*a*). Anchor polymerase chain reaction display: a high-throughput method to resolve, score and isolate dimorphic genetic markers based on interspersed repetitive DNA elements. *Analytical Biochemistry* **284**, 19–28.

Ayyadevara, S., Ayyadevara, R., Hou, S., Thaden, J. J. & Shmookler-Reis, R. J. (2000*b*). Genetic mapping of quantitative trait loci governing longevity of *Caenorhabditis elegans* in recombinant-inbred progeny of a Bergerac-BO × RC301 interstrain cross. *Genetics* **157**, 655–666.

Ayyadevara, S., Ayyadevara, R., Vertino, A., Galecki, A., Thaden, J. J. & Shmookler-Reis, R. J. (2003). Genetic loci modulating fitness and life span in *Caenorhabditis elegans*: categorical trait interval mapping in CL2a × Bergerac-BO recombinant-inbred worms. *Genetics* **163**, 557–570.

Azevedo, R. B. R., Keightley, P. D., Lauren-Maatta, C., Vassilieva, L. L., Lynch, M. & Leroi, A. M. (2002). Spontaneous mutational variation for body size in *Caenorhabditis elegans*. *Genetics* **162**, 755–765.

Baer, C. F. (2008). Quantifying the de-canalizing effects of spontaneous mutations in Rhabditid nematodes. *American Naturalist* **172**, 272–281.

Baer, C. F. & Denver, D. R. (2010). Spontaneous mutations decrease sensitivity of gene expression to random environmental variation in *Caenorhabditis elegans*. *PLoS ONE* **5**, e8750.

Baer, C. F., Phillips, N., Ostrow, D., Avalos, A., Blanton, D., Boggs, A., Keller, T., Levy, L. & Mezerhand, E. (2006). Cumulative effects of spontaneous mutations for fitness in *Caenorhabditis*: role of genotype, environment and stress. *Genetics* **174**, 1387–1395.

Baer, C. F., Shaw, F., Steding, C., Baumgaartner, M., Hawkins, A., Houppert, A., Mason, N., Reed, M., Simonelic, K., Woodard, W. & Lynch, M. (2005). Comparative evolutionary genetics of spontaneous mutations affecting fitness in rhabditid nematodes. *Proceedings of the National Academy of Sciences of the USA* **102**, 5785–5790.

Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., Selker, E. U., Cresko, W. A. & Johnson, E. A. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE* **3**, e3376.

Baird, S. E. (1999). Natural and experimental association of *Caenorhabditis remanei* with *Trachelipus rathkii* and other terrestrial isopods. *Nematology* **1**, 471–475.

Baird, S. E., Davidson, C. R. & Bohrer, J. C. (2005). The genetics of ray pattern variation in *Caenorhabditis briggsae*. *BMC Evolutionary Biology* **5**, 3.

Braendle, C., Baer, C. & Felix, M. (2010). Bias and evolution of the mutationally accessible phenotypic space in a developmental system. *PLoS Genetics* **6**, e1000877.

Braendle, C. & Felix, M. (2008). Plasticity and errors of a robust developmental system in different environments. *Developmental Cell* **15**, 714–724.

Braendle, C. & Felix, M. (2009). The other side of phenotypic plasticity: a developmental system that generates an invariant phenotype despite environmental variation. *Journal of Bioscience* **34**, 543–551.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94.

Bost, B., de Vienne, D., Hospital, F., Moreau, L. & Dillman, C. (2001). Genetic and nongenetic bases for the L-shaped distribution of quantitative trait loci effects. *Genetics* **157**, 1773–1787.

Brooke, A. & Johnson, T. E. (1991). Genetic specification of life span and self-fertility in recombinant-inbred strains of *Caenorhabditis elegans*. *Heredity* **67**, 19–28.

C. elegans Sequencing Consortium. (1998). Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* **282**, 2012–2018.

Chalasani, S. H., Chronia, N., Tsunozaki, M., Gray, J. M., Ramot, D., Goodman, M. B. & Bargmann, C. I. (2007). Dissecting a circuit for olfactory behavior in *Caenorhabditis elegans*. *Nature* **450**, 63–70.

Chan, Y. F., Marks, M. E., Jones, F. C., Villarreal, G., Shapiro, M. D., Brady, S. D., Soutwick, A. M., Absher, D. M., Grimwood, J., Schmutz, J., Myers, R. M., Petrov, D., Jonsson, B., Schluter, D., Bell, M. A. & Kingsley, D. M. (2010). Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a *Pitx-1* enhancer. *Science* **327**, 302–305.

Chandler, C. H. (2010). Cryptic intraspecific variation in sex determination in *Caenorhabditis elegans* revealed by mutations. *Heredity* **105**, 473–482.

Charlesworth, B., Morgan, M. T. & Charlesworth, D. (1993). The effect of deleterious mutations on neutral molecular variation. *Genetics* **134**, 1289–1303.

Chasnov, J. R., So, W. K., Chan, C. M. & Chow K. L. (2007). The species, sex & stage specificity of a *Caenorhabditis* sex pheromone. *Proceeedings of the National Academy of Sciences of the USA* **104**, 6730–6735.

Chen, Y., Zhu, J., Lum, P. Y., Yang, Z., Pinto, S., MacNeil, D. J., Zhang, C., Lamb, J., Edwards, S., Sieberts, S. K., Leonardson, A., Castellini, L. W., Wang, S., Champy, M. F., Zhang, B., Emilsson, V., Doss, S., Ghazalpour, A., Horvath, S., Drake, T., Lusis, A. J. & Schadt, E. E. (2008). Variations in DNA elucidate molecular networks that cause disease. *Nature* **452**, 429–435.

Cutter, A. D. (2006). Nucleotide polymorphism and linkage disequilibrium in wild populations of the partial selfer *Caenorhabditis elegans*. *Genetics* **172**, 171–184.

Cutter, A. D., Baird, S. E. & Charlesworth, D. (2006). High nucleotide polymorphism and rapid decay of linkage disequilibrium in wild populations of *Caenorhabditis remanei*. *Genetics* **174**, 901–913.

Cutter, A. D. & Payseur, B. A. (2003). Selection at linked sites in the partial selfer *Caenorhabditis elegans*. *Molecular Biology and Evolution* **20**, 665–673.

Darvasi, A. & Soller, M. (1995). Advanced intercross lines, an experimental population for fine genetic mapping. *Genetics* **141**, 1199–1207.

Davies, A. G., Bettinger, J. C., Thiele, T. R., Judy, M. E. & McIntire, S. L. (2004). Natural variation in the *npr-1* gene modifies ethanol responses of wild strains of *C. elegans*. *Neuron* **42**, 731–743.

de Bono, M. & Bargmann, C. I. (1998). Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. *Cell* **94**, 679–689.

Delattre, M. & Felix, M. A. (2001). Polymorphism and evolution of vulval precursor cell lineages within two nematode genera, *Caenorhabditis* and *Oscheius*. *Current Biology* **11**, 631–643.

Denver, D. R., Dolan, P. C., Wilhelm, L. J., Sung, W., Lucas-Lledo, J. I., Howe, D. K., Lewis, S. C., Okamoto, K., Thomas, W. K., Lynch, M. & Baer, C. F. (2009). A genome-wide view of *Caenorhabditis elegans* base-substitution mutation processes. *Proceedings of the National Academy of Sciences of the USA* **106**, 16310–16314.

Denver, D. R., Howe, D. K., Wilhelm, L. J., Palmer, C. A., Anderson, J. L., Stein, K. C., Phillips, P. C. & Estes, S. (2010). Selective sweeps and parallel mutation in the adaptive recovery from deleterious mutation in *Caenorhabditis elegans*. *Genome Research*, in press.

Denver, D. R., Morris, K., Kewalramani, A., Harris, K. E., Chow, A., Estes, S., Lynch, M. & Thomas, W. K. (2004). Abundance, distribution and mutation rates of homopolymeric nucleotide runs in the genome of *Caenorhabditis elegans*. *Journal of Molecular Evolution* **58**, 587–595.

Denver, D. R., Morris, K., Lynch, M., Vassilieva, L. L. & Thomas, W. K. (2000). High direct estimate of the mutation rate in the mitochondrial genome of *Caenorhabditis elegans*. *Science* **289**, 2342–2344.

Denver, D. R., Morris, K. & Thomas, W. K. (2003). Phylogenetics in *Caenorhabditis elegans*: an analysis of divergence and outcrossing. *Molecular Biology and Evolution* **20**, 393–400.

Dolgin, E. S., Felix, M. A. & Cutter, A. D. (2008). Hakuna Nematoda: genetic and phenotypic diversity in African isolates of *Caenorhabditis elegans* and *C. briggsae*. *Heredity* **100**, 304–315.

Doroszuk, A., Snoek, L. B., Fradin, E., Riksen, J. & Kammenga, J. (2009). A genome-wide library of CB4856/N2 introgression lines of *Caenorhabditis elegans*. *Nucleic Acids Research* **37**, e110.

Ebert, R. H., Cherkasova, V. A., Dennis, R. A., Wu, J. H., Ruggles, S., Perrin, T. E. & Shmookler-Reis, R. J. (1993). Longevity-determining genes in *Caenorhabditis elegans*: chromosomal mapping of multiple noninteractive loci. *Genetics* **135**, 1003–1010.

Ebert, R. H., Shammas, M. A., Sohal, B. H., Sohal, R. S., Egilmez, N. K., Ruggles, S. & Shmookler-Reis, R. J. (1996). Defining genes that govern longevity in *Caenorhabditis elegans*. *Developmental Genetics* **18**, 131–143.

Egilmez, N. K., Ebert, R. H. & Shmookler-Reis, R. J. (1995). Strain evolution in *Caenorhabditis elegans*: transposable elements as markers of interstrain evolutionary history. *Journal of Molecular Evolution* **40**, 372–381.

Emmons, S. W., Yesner, L., Ruan, K. & Katzenberg, D. (1983). Evidence for a transposon in *Caenorhabditis elegans*. *Cell* **32**, 55–65.

Estes, S., Ajie, B. C., Lynch, M. & Phillips, P. C. (2005). Spontaneous mutational correlations for life-history, morphological and behavioral characters in *Caenorhabditis elegans*. *Genetics* **170**, 645–653.

Estes, S. & Lynch, M. (2003). Rapid fitness recovery in mutationally degraded lines of *Caenorhabditis elegans*. *Evolution* **57**, 1022–1030.

Estes, S. & Phillips, P. C. (2006). Variation in pleiotropy and the mutational underpinnings of the G-matrix. *Evolution* **60**, 2655–2660.

Estes, S., Phillips, P. C., Denver, D. R., Thomas, W. K. & Lynch, M. (2004). Mutation accumulation in populations of varying size: implications for the distribution of mutational effects on fitness. *Genetics* **166**, 1269–1279.

Fay, D. (2006). Genetic mapping and manipulation: Chapter 5-SNPs: Three-point mapping., WormBook, ed. The *C. elegans* Research Community, WormBook. doi:10·1895/wormbook.1.94.1. Available at http://www.wormbook.org.

Friedman, D. B. & Johnson, T. E. (1988). A mutation in the *age-1* gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics* **118**, 75–86.

Graustein, A., Gaspar, J. M., Walters, J. R. & Palopoli, M. F. (2002). Levels of DNA polymorphism vary with mating system in the nematode genus *Caenorhabditis*. *Genetics* **161**, 99–107.

Gutteling, E. W., Doroscuk, A., Riksen, J. A. G., Prokop, Z., Reszka, J. & Kammenga, J. E. (2007*a*). Environmental influence on the genetic correlations between life-history traits in *Caenorhabditis elegans*. *Heredity* **98**, 206–213.

Gutteling, E. W., Riksen, J. A. G., Bakker, J. & Kammenga, J. E. (2007*b*). Mapping phenotypic plasticity and genotype-environment interactions affecting life-history traits in *Caenorhabditis elegans*. *Heredity* **98**, 28–37.

Hartl, D. L. & Clark, A. G. (2007). *Principles of Population Genetics*, 4th edn. Sunderland, MA: Sinauer Associates, Inc.

Harvey, S. C. (2009). Non-dauer larval dispersal in *Caenorhabditis elegans*. *Journal of Experimental Zoology B: Molecular and Developmental Evolution* **312**, 224–230.

Harvey, S. C., Barker, G. L. A., Shorto, A. & Viney, M. E. (2009). Natural variation in gene expression in the early development of dauer larvae of *Caenorhabditis elegans*. *BMC Genomics* **10**, 325.

Harvey, S. C., Shorto, A. & Viney, M. E. (2008). Quantitative genetic analysis of life-history traits of *Caenorhabditis elegans* in stressful environments. *BMC Evolutionary Biology* **8**, 15.

Harvey, S. C. & Viney, M. E. (2007). Thermal variation reveals natural variation between isolates of *Caenorhabditis elegans*. *Journal of Experimental Zoology B: Molecular and Developmental Evolution* **308**, 409–416.

Hodgkin, J. & Doniach, T. (1997). Natural variation and copulatory plug formation in *Caenorhabditis elegans*. *Genetics* **146**, 149–164.

Hu, P. J. (2007). Dauer. WormBook, ed. The *C. elegans* Research Community, WormBook. doi:10.1895/wormbook.1.144.1. Available at http://www.wormbook.org.

Huang, C., Xiong, C. & Kornfeld, K. (2004). Measurements of age-related changes of physiological processes that predict lifespan of *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the USA* **101**, 8084–8089.

Johnson, T. E. (1987). Aging can be genetically dissected into component processes using long-lived lines of *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the USA* **84**, 2777–2781.

Johnson, T. E. (2007). *Caenorhabditis elegans* 2007: the premier model for the study of aging. *Experimental Gerontology* **43**, 10–4.

Johnson, T. E. & Hutchinson, E. W. (1993). Absence of strong heterosis for life span and other life history traits in *Caenorhabditis elegans*. *Genetics* **134**, 465–474.

Johnson, T. E. & Wood, W. B. (1982). Genetic analysis of life-span in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the USA* **79**, 6603–6607.

Jorgensen, E. M. & Mango, S. E. (2002). The art and design of genetic screens: *Caenorhabditis elegans*. *Nature Reviews Genetics* **3**, 356–369.

Jovelin, R., Ajie, B. C. & Phillips, P. C. (2003). Molecular evolution and quantitative variation for chemosensory behaviour in the nematode genus *Caenorhabditis*. *Molecular Ecology* **12**, 1325–1337.

Jovelin, R., Dunham, J. P., Sung, F. S. & Phillips, P. C. (2009). High nucleotide divergence in developmental regulatory genes contrasts with the structural elements of olfactory pathways in *Caenorhabditis*. *Genetics* **181**, 1387–1397.

Joyner-Matos, J., Upadhyay, A., Salomon, M. P., Grigaltchik, V. & Baer, C. F. (2009). Genetic (co)variation for life span in rhabditid nematodes: role of mutation, selection and history. *Journal of Gerontology* **64A**, 1134–1145.

Kammenga, J. E., Doroszuk, A., Riksen, J. A. G., Hazendonk, E., Spiridon, L., Petrescu, A. J., Tijsterman, M., Plasterk, R. H. A. & 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**, 0358–0366.

Kammenga, J. E., Phillips, P. C., de Bono, M. & Doroscuk, A. (2008). Beyond induced mutants: using worms to study natural variation in genetic pathways. *Trends in Genetics* **24**, 178–185.

Keightley, P. D. & Caballero, A. (1997). Genomic mutation rates for lifetime reproductive output and lifespan in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the USA* **94**, 3823–3827.

Keightley, P. D. & Eyre-Walker, A. (2000). Deleterious mutations and the evolution of sex. *Science* **290**, 331–333.

Kiontke, K., Gavin, N. P., Raynes, Y., Roehrig, C., Piano, F. & Fitch, D. H. A. (2004). *Caenorhabditis* phylogeny predicts convergence of hermaphroditism and extensive intron loss. *Proceedings of the National Academy of Sciences of the USA* **101**, 9003–9008.

Knight, C. G., Azvedo, R. B. R. & Leroy, A. M. (2001). Testing life-history pleiotropy in *Caenorhabditis elegans*. *Evolution* **55**, 1795–1804.

Koch, R., van Luenen, H. G., van der Horst, M., Thijssen, K. L. & Plasterk, R. H. (2000). Single nucleotide polymorphisms in wild isolates of *Caenorhabditis elegans*. *Genome Research* **10**, 1690–1696.

Lande, R. (1982). A quantitative genetic theory of life history evolution. *Ecology* **63**, 607–615.

Lander, E. S. & Botstein, D. (1989). Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185–189.

Li, Y., Alvarez, O. A., Gutteling, E. W., Tijsterman, M., Fu, J., Riksen, J. A. G., Hazendonk, E., Prins, P., Plasterk, R. H. A., Jansen, R. C., Breitling, R. & Kammenga, J. E. (2006). Mapping determinant of gene expression plasticity by genetical genomics in *C. elegans*. *PLoS Genetics* **2**, 2155–2161.

Li, Y., Breitling, R., Snoek, L. B., van der Velde, K. J., Swertz, M. A., Riksen, J., Jansen, R. C. & Kammenga, J. E. (2010). Global genetic robustness of the alternative splicing machinery in *Caenorhabditis elegans*. *Genetics* **186**, 405–410.

Lockery, S. R., Lawton, K. J., Doll, J. C., Faumont, S., Coulthard, S. M., Thiele, T. R., Chronis, N., McCormick, K. E., Goodman, M. B. & Pruitt, B. L. (2008). Artificial dirt: microfluidic substrates for nematode neurobiology and behavior. *Journal of Neurophysiology* **99**, 3136–3143.

Macosko, E. Z., Pokala, N., Feinberg, E. H., Chalasani, S. H., Butcher, R. A., Clardy, J. & Bagmann, C. I. (2009). A hub-and-spoke circuit drives pheromone attraction and social behaviour in *C. elegans*. *Nature* **458**, 1171–1175.

McGrath, P. T., Rockman, M. V., Zimmer, M., Jang, H., Macosko, E. Z., Kruglyak, L. & Bargmann, C. I. (2009). Quantitative mapping of a digenic behavioral trait implicates globin variation in *C. elegans* sensory behaviors. *Neuron* **61**, 692–699.

Meneely, P. M., Farago, A. F. & Kauffman, T. M. (2002). Crossover distribution and high interference for both the X chromosome and an autosome during oogenesis and spermatogenesis in *Caenorhabditis elegans*. *Genetics* **162**, 1169–1177.

Milloz, J., Duveau, F., Nuez, I. & Felix, M. A. (2008). Intraspecific evolution of the intercellular signaling network underlying a robust developmental system. *Genes and Development* **22**, 3064–3075.

Moerman, D. G. & Waterston, R. H. (1984). Spontaneous unstable *unc-122* IV mutation in *C. elegans* var. Bergerac. *Genetics* **108**, 859–877.

Mori, I., Moerman, D. G. & Waterson, R. H. (1988). Analysis of a mutator activity necessary for germline transposition and excision of Tc1 transposable elements in *Caenorhabditis elegans*. *Genetics* **120**, 397–407.

Mori, I., Moerman, D. G. & Waterston, R. H. (1990). Interstrain crosses enhance excision of Tc1 transposable elements in *Caenorhabditis elegans*. *Molecular and General Genetics* **220**, 251–255.

Morran, L. T., Parmenter, M. D. & Phillips, P. C. (2009). Mutation load and rapid adaptation favour outcrossing over self-fertilization. *Nature* **462**, 350–352.

Ostrow, D. G., Phillips, N., Avalos, A., Blanton, D., Boggs, A., Keller, T., Levy, L., Rosenbloom, J. & Baer, C. F. (2007). Mutational bias for body size in Rhabditid nematodes. *Genetics* **176**, 1653–1661.

Palopoli, M. F., Rockman, M. V., TinMaung, A., Ramsay, C., Curwen, W., Aduna, A., Laurita, J. & Kruglyak, L. (2008). Molecular basis of the copulatory plug polymorphism in *Caenorhabditis elegans*. *Nature* **454**, 1019–1023.

Persson, A., Gross, E., Laurent, P., Busch, K. E., Bretes, H. & de Bono, M. (2009). Natural variation in a neural globin tunes oxygen sensing in wild *Caenorhabditis elegans*. *Nature* **458**, 1030–1033.

Phillips, N., Salomon, M., Custer, A., Ostrow, D. & Baer, C. F. (2009). Spontaneous mutational and standing genetic (Co)variation at dinucleotide microsatellites in *Caenorhabditis briggsae* and *C. elegans*. *Molecular Biology and Evolution* **26**, 659–669.

Phillips, P. C. (2006). One perfect worm. *Trends in Genetics* **22**, 405–407.

Pocock, R. & Hobart, O. (2010). Hypoxia activates a latent circuit for processing gustatory information in *C. elegans*. *Nature Neuroscience* **13**, 610–614.

Prasad, A., Croydon-Sugarman, M. J. F., Murray, R. L. & Cutter, A. D. (2010). Temperature-dependent fecundity associates with latitude in *Caenorhabditis briggsae*. *Evolution*, in press.

Rebeiz, M., Pool, J. E., Kassner, V. A., Aquadro, C. F. & Carroll, S. B. (2009). Stepwise modification of a modular enhancer underlies adaptation in a *Drosophila* population. *Science* **326**, 1663–1667.

Reddy, K. C., Anderson, E. C., Kruglyak, L. & Kim, D. H. (2009). A polymorphism in *npr-1* is a behavioral determinant of pathogen susceptibility in *C. elegans*. *Science* **323**, 382–384.

Rockman, M. V. (2008). Reverse engineering the genotype-phenotype map with natural genetic variation. *Nature* **456**, 738–744.

Rockman, M. V. & Kruglyak, L. (2006). Genetics of global gene expression. *Nature* **7**, 862–872.

Rockman, M. V. & Kruglyak, L. (2008). Breeding designs for recombinant inbred advanced intercross lines. *Genetics* **179**, 1069–1078.

Rockman, M. V. & Kruglyak, L. (2009). Recombinational landscape and population genomics of *Caenorhabditis elegans*. *PLoS Genetics* **5**, e1000419.

Rockman, M. V., Skrovanek, S. S. & Kruglak, L. (2010). Selection at linked sites shapes heritable phenotype variation in *C. elegans*. *Science* **15**, 372–376.

Rohde, C. B., Zeng, F., Gonzalez-Rubio, R., Angel, M. & Yanik, M. F. (2007). Microfluidic system for on-chip high-throughput whole-animal sorting and screening at subcellular resolution. *Proceedings of the National Academy of Sciences of the USA* **104**, 13891–13895.

Rual, J. F., Ceron, J., Koreth, J., Hao, T., Nicot, A. S., Hirozane-Kishikawa, T., Vandenhaute, J., Orkin, S. H., Hill, D. E., van den Heuvel, S. & Vidal, M. (2004). Toward improving *Caenorhabditis elegans* phenome mapping with an ORFeome-based RNAi library. *Genome Research* **14**, 2162–2168.

Salomon, M. P., Ostrow, D., Phillips, N., Blanton, D., Bour, W., Kepper, T., Levy, L., Sylvestre, T., Upadhyay, A. & Baer, C. F. (2009). Comparing mutational and standing genetic variability for fitness and size in *Caenorhabditis briggsae* and *C. elegans*. *Genetics* **183**, 1–8.

Schadt, E. E., Monks, S. A., Drake, T. A., Lusis, A. J., Che, N., Colinayo, V., Ruff, T. G., Milligan, S. B., Lamb, J. R., Cavet, G., Linsley, P. S., Mao, M., Stoghton, R. B. & Friend, S. H. (2003). Genetics of gene expression surveyed in maize, mouse and man. *Nature* **422**, 297–302.

Shmookler-Reis, R. J., Kang, P. & Ayyadevara, S. (2006). Quantitative trait loci define genes and pathways underlying genetic variation in longevity. *Experimental Gerontology* **41**, 1046–1054.

Shook, D. R., Brooks, A. & Johnson, T. E. (1996). Mapping quantitative trait loci affecting life history traits in the nematode *Caenorhabditis elegans*. *Genetics* **142**, 801–817.

Shook, D. R. & Johnson, T. E. (1999). Quantitative trait loci affecting survival and fertility-related traits in *Caenorhbaditis elegans* show genotype–environment interaction, pleiotropy and epistasis. *Genetics* **153**, 1233–1243.

Siedel, H. S., Rockman, M. V. & Kruglyak, L. (2008). Widespread genetic incompatibility in *C. elegans* maintained by balancing selection. *Science* **319**, 589–594.

Steiner, C. C., Weber, J. N. & Hoekstra, H. E. (2007). Adaptive variation in beach mice produced by two interacting pigment genes. *PLoS Biology* **5**, e219.

Teotónio, H., Manoel, D. & Phillips, P. C. (2006). Genetic variation for outcrossing among *Caenorhabditis elegans* isolates. *Evolution* **60**, 1300–1305.

Thomas, J. H. (1998). Social life and the single nucleotide: foraging behavior in *C. elegans*. *Cell* **94**, 549–550.

Toth, A. L. & Robinson, G. E. (2007). Evo-devo and the evolution of social behavior. *Trends in Genetics* **23**, 334–341.

van Swinderen, B., Galifianakis, A. & Crowder, C. M. (1998*a*). A quantitative genetic approach toward volatile anesthetic mechanisms in *C. elegans*. *Toxicology Letters* **100–101**, 309–307.

van Swinderen, B., Metz, L. B., Shebester, L. D., Mendel, J. E., Sternberg, P. W. & Crowder, C. M. (2001). Goα regulates volatile anesthetic action in *Caenorhabditis elegans*. *Genetics* **158**, 643–655.

van Swinderen, B., Shook, D. R., Ebert, R. H., Cherkasova, V. A., Johnson, T. E., Shmookler-Reis, R. J. & Crowder, C. M. (1997). Quantitative trait loci controlling halothane sensitivity in *Caenorhabditis elegans*. *MNAS Pharmacology* **94**, 8232–8237.

van Swinderen, B. B. A., Galifianakis, A. & Crowder, C. M. (1998*b*). Common genetic determinants of halothane and isoflurane potencies in *Caenorhabditis elegans*. *Anesthesiology* **89**, 1509–1517.

Vassilieva, L. L., Hook, A. M. & Lynch, M. (2007). The fitness effects of spontaneous mutations in *Caenorhabditis elegans*. *Evolution* **5**, 1234–1246.

Vassilieva, L. L. & Lynch, M. (1999). The rate of spontaneous mutation for life-history traits in *Caenorhabditis elegans*. *Genetics* **151**, 119–129.

Vieira, C., Pasyukova, E. G., Zeng, Z. B., Hackett, J. B., Lyman, F. R. & Mackay, T. F. C. (2000). Genotype-environment interaction for quantitative trait loci affecting life span in *Drosophila melanogaster*. *Genetics* **154**, 213–227.

Viney, M. E., Gardner, M. P. & Jackson, J. A. (2003). Variation in *Caenorhabditis elegans* dauer larva formation. *Development, Growth and Differentiation* **45**, 389–396.

Viñuela, A., Snoek, L. B., Riksen, J. A. G. & Kammenga, J. E. (2010). Genome-wide gene expression regulation as a function of genotype and age in *C. elegans*. *Genome Research* **20**, 929–937.

Wang, G.-X., Ren, S., Ren, Y., Ai, H. & Cutter, A. D. (2010). Extremely high molecular diversity within East Asian nematode *Caenorhabditis* sp. 5. *Molecular Ecology* **19**(22), 5022–5029.

Wang, Z. W., Saifee, O., Nonet, M. L. & Salkoff, L. (2001). SLO-1 potassium channels control quantal content of neurotransmitter release at the *C. elegans* neuromuscular junction. *Neuron* **32**, 867–881.

Wicks, S. R., Yeh, R. T., Gish, W. R., Waterson, R. H. & Plasterk, R. H. A. (2001). Rapid gene mapping in *Caenorhabditis elegans* using a high density polymorphism map. *Nature Genetics* **28**, 160–164.

Williams, B. D., Schrank, B., Huynh, C., Shownkeen, R. & Waterston, R. H. (1992). A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. *Genetics* **131**, 609–624.