Chapter 18
Ecological Genomics of Nematode Community Interactions: Model and Non-model Approaches

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Abstract The effects of human-induced environmental change are evident at multiple levels of biological organization. To date, most environmental change studies have focused on effects at the ecosystem, community and organismal levels. However, the ultimate controls of biological responses are located in the genome. Thus, genetic and genomic studies of organismal responses to environmental changes are necessary. Recent advances in genome analysis now make such analyses possible. In this chapter we describe a research approach and program that can begin to span this gap by using genome-enabled approaches to characterize organismal changes and then employing a genetically tractable model organism to identify genes involved in the response to environmental perturbations.

Abbreviations

GO Gene ontology
TD_{50} Time to death for 50% of a population

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P. Pontarotti (ed.), *Evolutionary Biology: Concept, Modeling, and Application*,
DOI: 10.1007/978-3-642-00952-5_18,

18.1 Introduction

18.1.1 Global Environmental Change

The world is changing around us at an unprecedented pace (Millennium Assessment, IPCC 2007). The role of human activities in these changes has been understood for some time. In fact, in 2000, the National Science Board in the United States issued a report that stated:

> *Human activities are transforming the planet in new ways and combinations at a faster rate and over broader scales than ever before in the history of humans on Earth. Accelerated efforts to understand Earth's ecosystems and how they interact with the numerous components of human-caused global changes are timely and wise.*

This was a challenge to scientists to study the effects of environmental change. Human-induced changes to the abiotic environment include climatic shifts in temperature and rainfall, effects of pollution and changes in land use, such as conversion of natural landscapes to agriculture (Hannah 1995; Dobson 1997). Of these, the latter appears to be making the greatest impact (Foley 2005). In order to gain the greatest understanding, it is important to study the effects of global environmental change at multiple levels of biological organization.

18.1.2 The Ecological Genomic Approach

The natural environments of organisms present a multitude of biotic and abiotic challenges that require both short-term ecological and long-term evolutionary responses. These responses have long been the subject of biological interest, yet their inherent complexity has made genetic and mechanistic dissection empirically difficult. However, recent technical advances in high-throughput sequencing, genotyping and genome-wide expression profiling, coupled with bioinformatics approaches for handling such data, hold great promise for dissecting these responses with unprecedented resolution. The implementation and application of new techniques requires a multidisciplinary approach, combining organismal analyses with molecular genetics and genomics, laboratory experiments with field studies and all within an ecologically relevant framework. The emerging field of ecological genomics seeks to understand genetic mechanisms underlying the responses of organisms to their natural environment by combining genomic and ecological approaches. These responses include modifications of biochemical, physiological, morphological, or behavioral traits of adaptive significance. Such an integration of fields faces challenges but will revolutionize our understanding of ecological responses at a genetic, genomic and eventually, a mechanistic level (Ungerer et al. 2008).

18.2 Evolutionary Framework for Ecological Genomic Studies

As changing environments are ubiquitous, one of the greatest challenges in biology is understanding and predicting effects of environmental changes on the ecology of the world's biota. Organisms respond to environmental changes on both ecological and evolutionary time scales. The magnitude and extent of human-induced changes to the environment create additional challenges for organisms, including changes to climate (e.g., global temperatures, rainfall patterns and insolation), landscape structure (e.g., urbanization, deforestation, fragmentation of the landscape) and communities (e.g., exotic species in new environments due to agriculture or global commerce/transportation). All of these changes lead to novel interactions among species to which, given the rapidity of human-induced change, organisms must adapt at an unprecedented pace. Recent and growing evidence suggests that organisms may adapt in a microevolutionary sense on decadal time scales to rapid environmental change, a process called contemporary evolution (reviewed in Stockwell et al. 2003; Carroll et al. 2007; Smith and Bernatchez 2008). Contemporary evolution due to human-caused selection is now well documented.

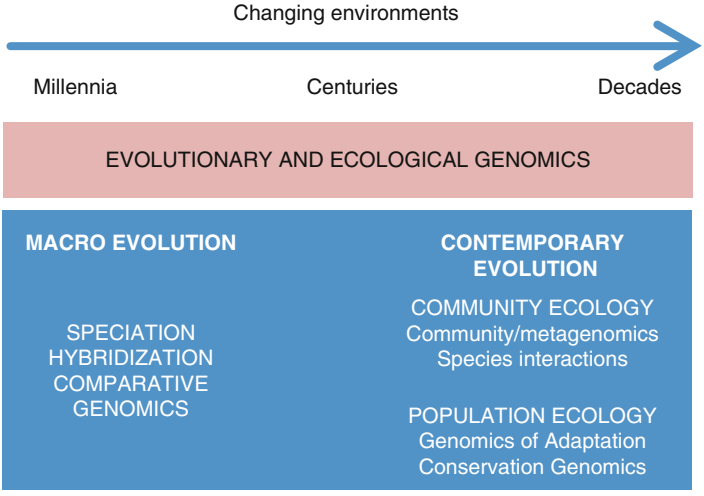

Fig. 18.1 Evolutionary framework for ecological genomics studies. Organisms respond to changing environments through long-term macro-evolutionary and short-term ecological time scales, as depicted by the arrow at the top of the figure. Recent evidence suggests that organisms can adapt to changes in the environment over decadal time scales in a process that has been termed "contemporary evolution." The mechanisms that organisms use to respond to these changes are lodged in the genome, whose discovery requires an evolutionary and ecological genomic approach. Just as organisms respond to environmental change on both ecological and evolutionary time scales, the research addressing these changes must focus on different time scales. Those disciplines and the types of genome-enable approaches they employ are indicated

Rapid adaptive evolution has been shown in flowering time (Franks et al. 2007); photoperiodism (Bradshaw and Holzapfel 2008); the sexual signal of invasive field crickets (Tinghitella 2008); and in response to changes in climate (Reusch and Wood 2007). Change, human-induced or not, elicits organism responses via mechanisms lodged in the genome; whose study requires an evolutionary and ecological genomic approach. Just as organisms respond to environmental change on both ecological and evolutionary time scales, the research addressing these changes must focus on different time scales (Fig. 18.1).

18.3 Nematode Ecological Genomics: Model and Non-model Approaches

18.3.1 Global Environmental Change and the Grassland Ecosystem

Grasslands (Samson and Knopf 1994) perform many essential ecosystem services, such as supplying clean water, recycling essential nutrients and preserving biodiversity (Daily 1997) and are among the most endangered ecosystems on Earth, largely having been replaced by agricultural systems that alter both above- and belowground communities (Baer et al. 2002). In addition, grasslands are among the most sensitive to an array of global change phenomenon (Samson and Knopf 1994; Collins et al. 1998; Field and Chiariello 2000; Buckland et al. 2001; Knapp and Smith 2001; Reich et al. 2001; Briggs et al. 2005). For example, the structure and function of grasslands are determined by patterns of climatic variability and nutrient availability but altered precipitation patterns, enhanced nitrogen deposition and changes in land use (fire and grazing regimes, conversion to agriculture) have the potential to dramatically influence these relationships (Collins et al. 1998; Knapp and Smith 2001; Briggs et al. 2005). Patterns and controls of ecological processes in grasslands and the effects of natural and anthropogenic disturbances have been the focus of long-term research at the Konza Prairie Biological Station (near Manhattan, KS) for more than 25 years (Knapp et al. 1998).

18.3.2 The Importance of Nematode Ecology

We have focused on nematodes because they are among the most abundant invertebrates in soils and are an important component of the microfauna in grasslands (Curry 1994). Nematode species occurring in soils encompass a wide variety of feeding strategies (Freckman 1988), including many free-living species that feed on soil microbes (bacteria or fungi). Microbial-feeding nematodes may be the most important consumers of bacteria and fungi in many soil communities (Blair et al.

2000; Yeates 2003) and their interactions with microbial decomposers affect ecosystem processes including decomposition and nutrient cycling (Freckman 1988; Coleman et al. 1991). Nematodes are also known to be responsive to changing environmental conditions (Freckman and Ettema 1993; Todd 1996; Todd et al. 1999), making them ideal model organisms to assess the potential impacts of global change on soil communities. Several studies have demonstrated that the soil nematode community in tallgrass prairie responds strongly to perturbations, including nutrient enrichment through nitrogen addition, increased soil moisture and different experimental fire regimes (Seastedt et al. 1987; Blair et al. 2000; Todd 1996; Todd et al. 1999; Jones et al. 2006b).

18.3.3 The Nematode Ecological Genomic Approach

The disturbances caused by global environmental change are complex, involving changes in the biotic environment that include microbes, competitors and predators, as well as changes in the abiotic soil environment. To begin to sort out these interactions, we have focused on the responses of microbial-feeding nematodes to the microbial aspects of the grassland biotic environment. We have employed an interdisciplinary approach using high-throughput molecular techniques to first characterize shifts in the nematode community as well as the interacting bacterial community. Next, we have modeled these interactions using the genetic model organism *Caenorhabditis elegans* to begin to understand the interactions of genes with the environment in non-model systems such as the native grassland soil nematode community in grasslands at Konza Prairie. An understanding of the genetic mechanisms underlying ecological interactions should provide a predictive value previously not possible.

18.3.4 C. elegans as a Model Nematode

C. elegans is a free-living nematode found in enriched soils that has been used in genetic research for over 40 years. Its short generation time, small size and ease of maintenance have led to the development of sophisticated genetic tools as evidenced by the thousands of genes that have been isolated and analyzed. In addition, the use of RNA mediated interference (RNAi) induced by treating animals with double-stranded RNA (dsRNA) corresponding to a gene of interest allows one to quickly and easily see the effects of removing, or at least crippling, any gene to determine its function by examining the effects on the phenotype (Fire et al. 1998). Finally, genetic, molecular and sequence data are continually annotated and made available through Wormbase (http://www.wormbase.org). While the high degree of evolutionary conservation allows *C. elegans* to be a good model for the biology of

higher organisms, such as humans, it may be an even better model for understanding the responses of soil nematodes.

Model organism systems, such as *C. elegans*, have well developed genetic and genomic tools that allow for powerful analyses. However, they were chosen for characteristics (e.g., small size, small genomes and rapid life cycles) that facilitate genetic analysis but may not be typical of many organisms. While some researchers have chosen to study the ecology of selected model organisms (Roberts and Feder 2000; Weinig et al. 2002) others have chosen to develop genomic capabilities for more ecologically important taxa (Kessler et al. 2004). Both approaches have yielded interesting results. In fact, a combined approach as was done in the use of *Arabidopsis* to discover genes induced by flavinoid release by the invasive species spotted knapweed (Bais et al. 2003), promises to be extremely fruitful. We have chosen this latter approach for our nematode studies.

18.3.5 Non-model Approaches

18.3.5.1 Grassland Nematode Community Responses

To determine the effects on nematode communities, Jones et al. (2006b) used an ongoing long-term experiment at the Konza Prairie Biological Station established in 1986 to address belowground responses to fire, mowing and nutrient enrichment. An understanding of these effects on soil processes, including the soil food web and its invertebrate and microbial components, is integral to predicting the consequences of global change for both natural and managed ecosystems. Nematodes were sampled from four replicates of four treatment combinations (annually burned versus unburned and ammonium nitrate addition versus no addition). We focused specifically on microbial-feeding nematodes and used sequence differences in a 900 base pair (bp) fragment consisting in the 5' 500 bases of the 18S rRNA gene and the entire adjacent internally transcribed spacer region (ITS1) to develop dual-labeled fluorescent probes (e.g., Taqman probes), which were used for detection of 16 different nematode taxa from among 984 individual nematodes samples (Jones et al. 2006a). Sequencing nematodes that were not identified with existing probes identified an additional three taxa. The 19 identified taxa represent three taxonomic families and recent analyses indicate that each of these families belong to different phylogenetic clades (Blaxter 1998; Holterman et al. 2006).

18.3.5.2 Differential Nematode Response

Statistical analyses of relative nematode abundances in each plot revealed that season, nitrogen addition and burning were shown to affect nematode abundance in multiple taxa, with nitrogen addition and season having the most pronounced effects. In addition to these main effects, nematode taxa were differentially affected

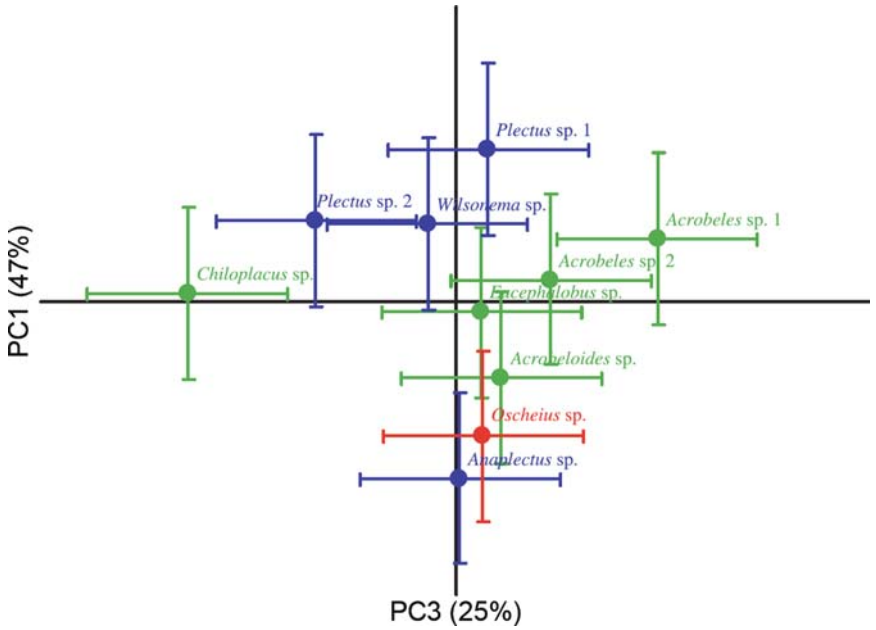

Fig. 18.2 Canonical plot of the first and third principal components of mean adjusted response of the nematode community. Members within taxonomic families are designated by color (Cephalobidae, green; Plectidae, blue; Rhabditidae, red). Data are means ± standard error of the difference (Printed with permission of Molecular Ecology)

by interactions between the burning and nitrogen addition treatments. A principal components analysis illustrating the variation due to burning in the presence of nitrogen (PC1) versus that of the variation due to nitrogen in the presence of burning (PC3) is shown in Fig. 18.2. On the whole, taxon responses were similar within members of a family. However, for each family there was a taxon (*Chiloplacus* sp., *Anaplectus* sp., and *Oscheius* sp.) that responded differently than others within their family. Additionally, although nematodes from different taxonomic groups on average respond differently, similar responses were seen in nematode taxa that span three taxonomic families (e.g., *Acrobeloides* sp., *Oscheius* sp., and *Anaplectus* sp.). What drivers might account for these differential nematode responses? They must involve a combination of indirect and direct effects of the biotic and abiotic environment, respectively. Indirectly, the nematodes may be responding to changes in the community structure (i.e., food resources, parasites/pathogens, competition, or predation). Alternatively, as nematodes live in a film of water and are in direct contact with their environment, sensitivities to soil chemistry may influence the observed responses. As the genetic responses to the biotic and abiotic aspects of the environment are complex, they will need to be dissected separately. However, one must be careful as changes in the abiotic environment may have indirect effects on the nematode's biotic environment. Furthermore, as

the biotic interactions affecting the nematode community are highly complex, we first have characterized the nematode response to the bacterial aspects of their biotic interactions.

18.3.5.3 Microbial Community Response to Nitrogen Addition and Burning

One force shaping the bacterial-feeding nematode community could be the response to changes in the microbial community. Thus it might be that nitrogen addition and burning treatments alter the microbial communities, which, in turn, might play a role in structuring nematode communities by altering food resources and pathogens. To demonstrate whether this is possible, we adopted a mass parallel sequencing technique ("454 sequencing") that generated >200,000 short sequences (about 100 bp). To amplify the soil bacteria signal, we used PCR primers that flank the hypervariable V3 region in the 5'-end of the 16S rRNA gene (Baker et al. 2003) on DNA that was extracted directly from the soil. While we were able to derive bacterial sequences for four separate projects (Jones, Coolon, Todd and Herman, unpublished observations), here we only consider the results obtained from the plots in which we previously measured nematode community responses.

These results will be described in detail elsewhere, briefly we developed bioinformatic methods that enabled Operational Taxonomic Unit (OTU) designation across sampled plots. OTUs were generated at each of 18 sequence identity levels (80–98%). At each level of sequence identity, sequences were parsed by plot and used to calculate the frequency of occurrence of all OTUs for each of the plots. The number of OTUs increased as the per cent sequence identity increased from 80% to 98%, following expectations of biological complexity, with OTUs generated at different levels of sequence identity being of different taxonomic resolutions. Using replicated field plots and statistical analysis, we showed reproducible treatment responses within the microbial community. Overall taxonomic richness, dominance and diversity were calculated for each plot and analyzed across treatments by analysis of variance (ANOVA). In order to determine not only whether the community responded but also to infer which level of biological organization (phylum, order, family, etc.) responded, we plotted these community measures at each of the 18 sequence identity levels (80–98%). These analyses demonstrated that richness and diversity increased with sequence identity level while dominance decreased, indicating the levels of biological organization that respond to added nitrogen. For example, treatment elicited differences in richness were consistently significant across all levels of sequence identity, suggesting high order changes in the bacterial community in response to nitrogen addition. These results confirm that bacterial populations, similar to nematodes, are highly responsive, with the magnitude and direction of the changes being different even across taxa of similar taxonomy. Thus, it is plausible that in response to changing environments, such as nitrogen addition, bacterial-feeding nematode communities may be shaped, in part, by responses to changes in the bacterial community.

18.3.6 Model Approaches

18.3.6.1 Use of *C. elegans* to Model Ecological Interactions

So far we have described experiments that documented responses of the soil nematode community to changes in the environment and identifying potential drivers, such as changes in the bacterial community. Next, we modeled these interactions using *C. elegans* in the laboratory to investigate the mechanisms underlying the native nematode responses observed on Konza Prairie. One aim of these studies was to use *C. elegans* as a gene discovery tool to examine gene expression in response to environmental change. Although *C. elegans* has not been found in our experimental plots, other related Rhabditid taxa, specifically *Mesorhabditis* sp., *Oscheius* sp. and *Pellioditis* sp., do occur there. Further, we know from EST databases that *C. elegans* is likely to share 50–80% of gene sequences with most nematode taxa (Parkinson et al. 2004), thus we expect the native Konza taxa more closely related to *C. elegans* (i.e., Rhabdtids) to share more genes than those that are less related. Ultimately we will test the homologs of the candidate genes identified in *C. elegans* for their function in the native soil nematodes.

18.3.6.2 *C. elegans* Genes Involved in Response to Changes in Bacterial Environment

To model naturally occurring nematode–bacterial interactions, as well as to use new environments for gene discovery in the laboratory, we isolated bacteria from grassland prairie soils at the Konza Prairie Biological Station. We isolated three bacteria from Konza soils: *Micrococcus luteus*, *Bacillus megaterium* and *Pseudomonas* sp., of which the latter two were isolated in association with bacterial-feeding nematodes (*Oscheius* sp. and *Pellioditis* sp., respectively). *Pseudomonas fluorescens* was the closest match (98% sequence identity) in the Ribosomal Database Project to the 16S rDNA sequence of the isolated *Pseudomonas* sp.

We used oligonucleotide microarrays to identify *C. elegans* genes that were differentially expressed in response to altered bacterial environments. We compared expression patterns of *wild-type C. elegans*, fed each of these soil bacteria as well as its traditional laboratory food, *Escherichia coli* and all pair-wise comparisons were performed (Coolon et al. 2009). We identified 204 unique genes whose expression was significantly changed in response to bacterial environment. These results indicated that nematode populations express different suites of genes when raised in different bacterial environments.

Within the *C. elegans* genes identified as differentially expressed in response to bacterial environment, metabolism genes were highly represented (9.3%) as expected. Interestingly, genes previously implicated in innate immunity (9.8%) and cuticle biosynthesis or collagens (8.8%) were also found to be highly abundant

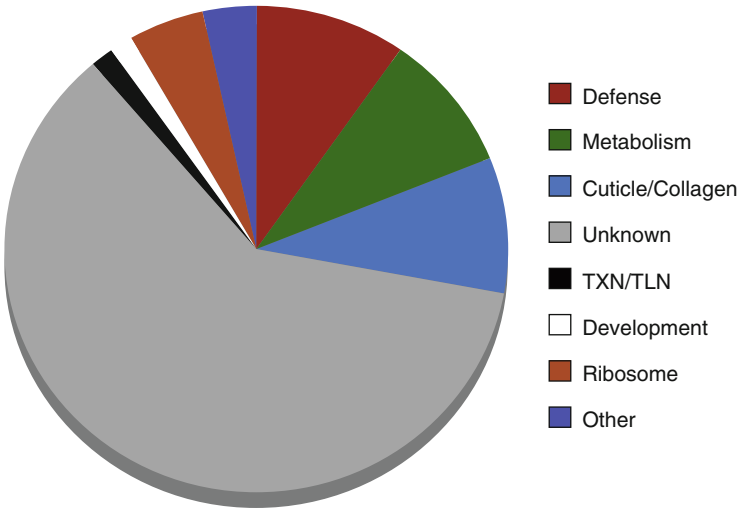

Fig. 18.3 Gene ontology (GO) terms for identified differentially expressed genes. Gene ontology (GO) terms were amended with recently published information and used to categorize the identified differentially expressed genes. Clustering was done manually by grouping GO terms of similar function (Coolon et al. 2009)

within the genes identified. Finally, genes of unknown function made up the largest portion (61% of the total, Fig. 18.3), also as expected since one aim of the work was to determine functions for such genes helping to further characterize the major proportion of the *C. elegans* genome that remains unknown after four decades of genetic dissection. However, ultimately, functional data obtained by interfering with gene function are needed to determine which genes really matter for a particular interaction. To this end, we obtained all available viable non-sterile mutations for the 204 differentially expressed genes in our study (21/204, ~10% of the total genes identified) from the *Caenorhabditis* Genetics Center (CGC) and used them for biological validation of the microarray results (Table 18.1). Functional tests measuring multiple aspects of life history were used to calculate absolute fitness by life table analysis and lifespan was measured with pathogenicity assays in all four bacterial environments. Specifically, age-specific reproduction (m_x) and survival (l_x) were used to calculate intrinsic growth rate ($Ro = \Sigma l_x m_x$), generation time ($\Sigma l_x m_x)/(\Sigma x l_x m_x$) and Lambda ($\lambda = e^{(\ln Ro/T)}$), which was used as a measure of absolute fitness. Lifespan was measured as time to death for 50% of a population (TD_{50}) (Tan and Ausubel 2000) using survivorship curves and is indicative of the pathogenicity of *C. elegans* food sources. We found that many of the mutations had effects on life history traits that differed significantly from wild type in a given bacterial environment, demonstrating that many of the genes specifically induced in response to different bacteria function to contribute to nematode fitness and longevity in different bacterial environments (Coolon et al. 2009; Table 18.2).

Table 18.1 Genes and alleles used for functional tests

Gene	Allele	Predicted molecular function
acdh-1	ok1489	Acyl-CoA dehydrogenase
C23H5.8	ok651	Unknown function
cey-2	ok902	Cold-shock/Y-box domain containing
cey-4	ok858	Unknown function
cpi-1	ok1213	Homolog of cysteine protease inhibitors (cystatins)
ctl-1	ok1242	Cytosolic catalase
dhs-28	ok450	17-Beta-hydroxysteroid dehydrogenase 4
dpy-14	e188	Type III (alpha 1) collagen
dpy-17	e1295	Cuticle collagen
elo-5	gk182	PUFA elongase
cyp-37A1	ok673	Unknown function
F55F3.3	ok1758	Unknown function
fat-2	ok873	Delta-12 fatty acyl desaturase
gei-7	ok531	Predicted isocitrate lyase/malate synthase
gld-1	op236	Meiotic cell cycle/oogenesis
hsp-12.6	gk156	Predicted heat-shock protein
mtl-2	gk125	Metallothionein
pab-2	ok1851	Polyadenylate-binding protein
rol-6	e187	Cuticle collagen
sqt-2	sc108	Cuticle collagen
Y57A10C.6	ok693	Predicted thiolase

List of 21 mutants used for functional tests, predicted molecular functions are indicated.

18.3.6.3 Specificity of the *C. elegans* Functional Response

In order to compare across bacterial environments we investigated genotype-by-environment interactions (GEI) and examined mutant norms of reaction across bacterial environments (Fig. 18.4). GEI exists when there is re-ranking of the phenotypic responses of genotypes across environments, or genotypes may have more similar phenotypes in one environment than in another, therefore differences in the magnitude of effects exist between different environments (Falconer and Mackay 1996). Reaction norms of fitness (Fig. 18.4a) and lifespan (Fig. 18.4b), revealed differential effects of the bacterial environments on the different mutant genotypes demonstrating the specificity and complexity of mutational effects on these complex traits.

How can we infer whether a particular gene is truly important for a given environmental interaction? A simple assumption that a gene upregulated in an environment positively regulates a particular life history trait predicts that loss of that gene function would cause a reduction in fitness in that environment. One such example is *hsp-12.6* that encodes a heat-shock protein (Hsu et al. 2003) and was found to be upregulated when wild-type *C. elegans* was grown on *E. coli* compared to growth on *B. megaterium*. We found that *hsp-12.6* mutants have a significant reduction in fitness as compared to wild type when the mutant is grown on *E. coli* from that observed on *B. megaterium*. Not only is this difference significant but fitness of *hsp-12.6* mutants was significantly increased relative to wild type when

Table 18.2 Biological validation of identified *Caenorhabditis elegans* genes

Gene	*Escherichia coli* (OP50)		*Micrococcus luteus*		*Pseudomonas* sp.		*Bacillus megaterium*	
	λ	TD_{50}	λ	TD_{50}	λ	TD_{50}	λ	TD_{50}
wt	3.60(0.19)	5.6(0.22)	2.63(0.18)	4.1(0.22)	3.99(0.25)	8.7(0.27)	2.81(0.16)	12.3(0.27)
acdh-1	2.99(0.03)$^{-}$	5.0(0.35)$^{-}$	2.54(0.25)	5.0(0.35)$^{+}$	3.78(0.74)	5.5(0.79)$^{-}$	3.01(0.37)	10.4(0.42)$^{-}$
C23H5.8	2.72(0.03)$^{-}$	7.8(0.57)$^{+}$	2.42(0.04)$^{-}$	3.6(0.42)$^{-}$	3.07(0.02)$^{-}$	6.0(0.79)$^{-}$	3.30(0.04)$^{+}$	8.9(0.74)$^{-}$
cey-2	3.08(0.04)$^{-}$	6.1(0.42)	2.11(0.06)$^{-}$	3.5(0.35)$^{-}$	2.83(0.03)$^{-}$	7.5(0.61)$^{-}$	2.79(0.01)	7.0(0.35)$^{-}$
cey-4	3.51(0.13)	5.6(0.42)	2.84(0.06)$^{+}$	3.6(0.42)$^{-}$	3.57(0.07)$^{-}$	5.9(0.22)$^{-}$	2.95(0.02)	3.7(0.27)$^{-}$
cpi-1	3.25(0.15)$^{-}$	7.6(0.22)$^{+}$	3.01(1.17)	4.4(0.22)	3.65(0.43)	6.6(0.42)$^{-}$	3.19(0.41)	12.4(0.42)
ctl-1	2.91(0.07)$^{-}$	6.2(0.84)	2.53(0.07)	4.8(0.29)$^{+}$	2.77(0.18)$^{-}$	3.9(0.42)	2.29(0.07)$^{-}$	8.5(0.35)$^{-}$
cyp-37A1	3.59(0.08)	8.0(0.50)$^{+}$	2.37(0.06)$^{-}$	4.4(0.42)	3.64(0.03)$^{-}$	8.5(0.35)$^{-}$	2.85(0.04)	9.5(0.50)$^{-}$
dhs-28	2.23(0.18)$^{-}$	6.7(0.27)$^{+}$	2.01(0.21)$^{-}$	3.6(0.22)$^{-}$	2.43(0.14)$^{-}$	7.3(0.27)$^{-}$	1.86(0.27)$^{-}$	10.2(0.76)$^{-}$
dpy-14	1.89(0.44)$^{-}$	2.4(0.22)$^{-}$	1.60(0.07)$^{-}$	2.1(0.22)$^{-}$	1.85(0.17)$^{-}$	3.1(0.42)$^{-}$	0.96(0.02)$^{-}$	4.1(0.42)$^{-}$
dpy-17	2.84(0.52)$^{-}$	4.0(0.35)$^{-}$	2.70(0.34)	3.1(0.42)$^{-}$	3.20(0.45)$^{-}$	3.0(0.35)$^{-}$	2.69(0.80)	12.3(0.57)
elo-5	4.11(0.07)$^{+}$	5.5(0.35)	3.02(0.10)$^{+}$	2.6(0.42)$^{-}$	4.07(0.12)	5.0(0.50)$^{-}$	4.18(0.05)$^{+}$	9.5(0.35)$^{-}$
F55F3.3	3.53(0.15)	3.1(0.55)$^{-}$	2.25(0.14)$^{-}$	2.6(0.55)$^{-}$	2.24(0.07)$^{-}$	5.0(0.35)$^{-}$	2.06(0.07)$^{-}$	5.5(0.35)$^{-}$
fat-2	3.27(0.13)$^{-}$	9.9(0.82)$^{+}$	2.97(0.04)$^{+}$	8.5(0.35)$^{+}$	4.23(0.04)	11.4(0.74)$^{+}$	3.18(0.09)$^{+}$	13.7(1.15)$^{+}$
gei-7	3.52(0.25)	5.7(0.27)	2.73(0.12)	4.5(0.00)$^{+}$	3.77(0.26)	7.6(0.22)$^{-}$	3.27(0.48)	14.3(0.27)$^{+}$
gld-1	3.15(0.13)$^{-}$	5.6(0.22)	2.51(0.28)	3.5(0.35)$^{-}$	3.53(0.06)$^{-}$	4.3(0.57)$^{-}$	2.78(0.04)	5.5(0.35)$^{-}$
hsp-12.6	3.10(0.08)$^{-}$	5.7(0.45)	2.50(0.18)	3.7(0.27)$^{-}$	3.72(0.14)	6.6(1.29)$^{-}$	3.00(0.08)$^{+}$	9.5(1.00)$^{-}$
mtl-2	3.77(0.17)	6.1(0.22)$^{+}$	3.02(0.23)$^{+}$	5.2(0.27)$^{+}$	4.09(0.28)	8.0(0.35)$^{-}$	3.75(0.40)$^{+}$	13.8(0.27)$^{+}$
pab-2	4.14(0.06)$^{+}$	6.6(0.42)$^{+}$	2.72(0.47)	5.4(0.42)$^{+}$	4.29(0.24)	7.7(0.57)$^{-}$	3.20(0.11)$^{+}$	8.9(0.74)$^{-}$
rol-6	2.82(0.22)$^{-}$	3.1(0.82)$^{-}$	2.28(0.11)$^{-}$	2.9(0.22)$^{-}$	3.11(0.09)$^{-}$	7.7(0.45)$^{-}$	2.56(0.04)$^{-}$	10.2(0.76)$^{-}$
sqt-2	2.97(0.01)$^{-}$	6.9(0.42)$^{+}$	2.69(0.06)	3.7(0.57)	3.72(0.06)$^{-}$	4.2(0.57)$^{-}$	3.39(0.47)$^{+}$	7.2(1.35)$^{-}$
Y57A10C.6	3.37(0.18)	6.3(0.45)$^{+}$	2.09(0.09)$^{-}$	4.5(0.00)$^{+}$	3.41(0.33)$^{-}$	8.2(0.57)	2.62(0.23)	15.0(0.35)$^{+}$

Wild-type (N2) and mutant *C. elegans* strains were grown on the four bacterial isolates and absolute fitness (λ) and time to death for 50% of the individuals in a population (TD_{50} in days) were measured. *P*-values are shown for contrasts between environments within strain for fitness and TD_{50}. Standard error (SEM) is given in parenthesis. Additionally, + indicates a significant ($P < 0.05$) increase relative to wild type and – indicates a significant ($P < 0.05$) decrease of the mutant relative to wild type (Coolon et al. 2009).

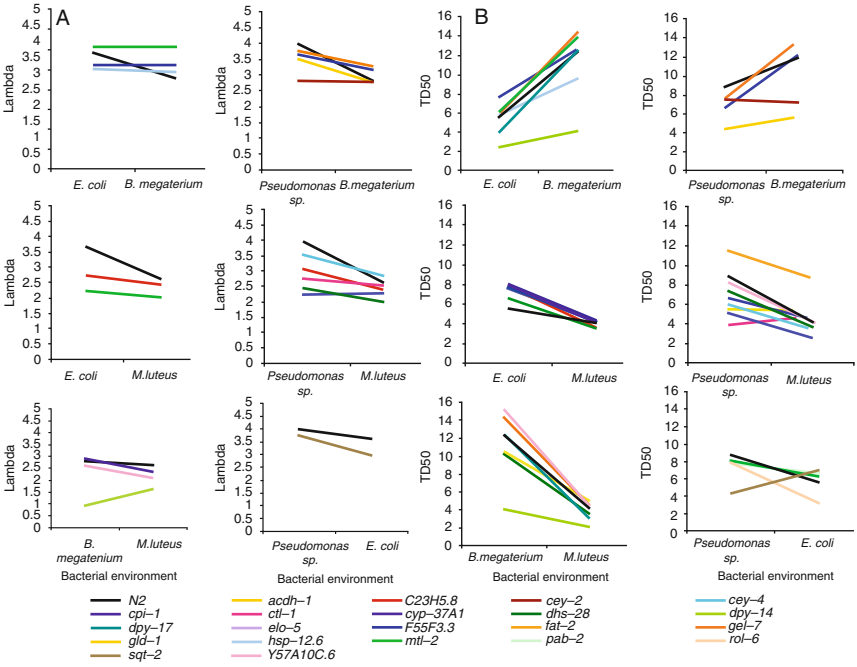

Fig. 18.4 Life history reaction norms with significant gene by environment interactions. Significant gene by environment interactions with Lambda (**a**) and lifespan as measured by TD_{50} (**b**) are illustrated by reaction norms. All pair-wise bacterial comparisons are shown. *B* = *Bacillus megaterium*, *M* = *Micrococcus luteus*, *E* = *Escherichia coli*, *P* = *Pseudomonas* sp.

grown on *B. megaterium* (Fig. 18.4a). This suggests that there was a cost associated with the expression of *hsp-12.6* in an environment in which it was not needed and a detriment to loss of function in an environment in which it was needed. Thus, the *hsp-12.6* allele had an antagonistic pleiotropic effect on fitness in these environments. We observed three other instances of antagonistic pleiotropy (Fig. 18.4a): *cpi-1* that encodes a cysteine protease inhibitor, also in the *E. coli* versus *B. megaterium*, as well as in the *Pseudomonas* sp. versus B. *megatarium* comparisons and *gei-7*, which encodes a isocitrate lyase/malate synthase that has been shown to function in lifespan extension (Tsuboi et al. 2002) also in the *Pseudomonas* sp. versus B. *megatarium* comparison. These observations suggest that these genes are likely under strong stabilizing selection in wild populations, with fitness trade-offs in different environments.

Although we observed examples that met the expectations of the simple prediction that genes positively impact particular life history traits, in many cases the underlying gene regulation may be more complex, involving positive and negative regulation and in some cases in a manner not yet elucidated. Thus in most cases we do not expect to be able to predict the directional effect of a particular mutation on the trait. Instead we predict that we would observe GEI between the environments in which differential expression was found. There were 37 instances of differential

expression among the 21 genes tested. ANOVA was used to determine that 49% (18/37) of the contrasts of mutant fitness in the six bacterial comparisons had significant gene by environment interactions (Fig. 18.4a) and that 35/37 (95%) of tests showed significant TD_{50} GEI (Fig. 18.4b). Thus, it appears that the majority of differentially expressed genes are functionally important in the specific environments in which they were regulated illustrating that gene by environment interaction is likely a common feature to genes that are regulated in response to different bacterial environments (Coolon et al. 2009).

18.3.6.4 Do Nematodes "Know" What Is Good for Them?

The *C. elegans* experiments described above were conducted using one environment at a time. However, in the wild, bacterial-feeding nematodes must be faced with many bacterial types as potential food sources, which also may expose them to risks of infection among other interactions. To begin to dissect these more complex interactions, we conducted food preference tests on wild-type *C. elegans* in response to the bacterial isolates and *E. coli*. Using a biased choice assay (Shtonda and Avery 2006) (Fig. 18.5a, upper) we determined food preference for all pairwise combinations of bacterial isolates (Fig. 18.5a, lower). Comparisons of the pair-wise measures of preferences revealed a hierarchy of food preferences: *Pseudomonas sp* was most preferred, followed by *E. coli*, which were both much more preferred than *B. megaterium*, which was slightly more preferable than *M. luteus*. Interestingly, this hierarchy mirrored the observed trend for fitness in the different bacterial environments (Fig. 18.5b, c), with *C. elegans* preferring *Pseudomonas* sp. on which it was most fit, followed by *E. coli*, *B. megaterium* and *M. luteus*, respectively. Thus *C. elegans* food preference appears to correlate with fitness, with bacterial environments on which worms were most fit being preferred (Coolon et al. 2009).

18.4 Conclusions

One aim of the research program described here was to learn what genetic mechanisms function to allow organisms to respond to the rapid changes to their environment as occurs as a consequence of human activities. This is indeed a great challenge and one biologists are now beginning to tackle using interdisciplinary approaches (Reusch and Wood 2007). What relevance does an understanding of the genetic basis of nematode community responses in the grassland ecosystem have on the larger questions of organismal response to environmental change? We chose to study processes in the grassland ecosystem as it is quite sensitive to global change phenomena (Samson and Knopf 1994; Collins et al. 1998; Field and Chiariello 2000; Buckland et al. 2001; Knapp and Smith 2001; Reich et al. 2001; Briggs et al. 2005). Within that ecosystem, the nematode community has been

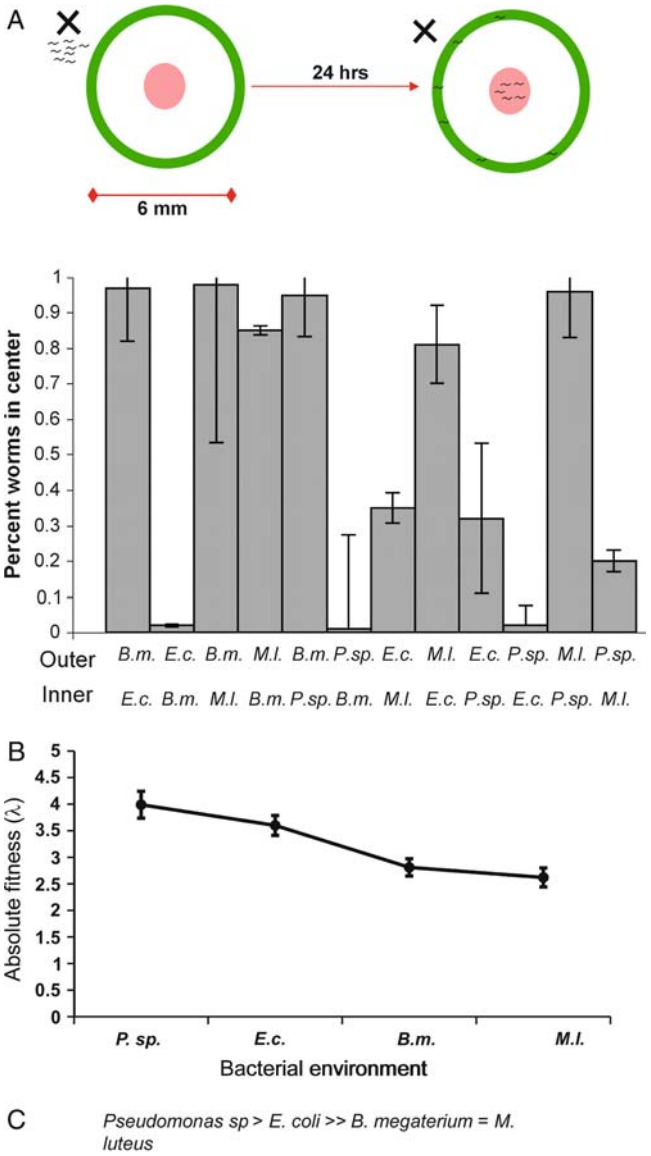

Fig. 18.5 Food preference correlates with fitness. (**a**) Food preferences of wild-type animals were measured in a biased choice assay modified from Shtonda and Avery (2006). (*Upper)* Bacteria were arrayed on an agar plate as shown. Synchronized L1 larvae were placed outside the outer circle (indicated by the X) and the fraction in the center bacterial type was determined after 24 h. (*Lower)* Fraction of nematodes in the center bacterial type is shown for all pair-wise comparisons and reciprocal comparisons were used for *Caenorhabditis elegans* food preference. Standard error for each mean is indicated with error bars. The bacteria listed under each bar were compared and are either outer (outer ring) or inner (inner circle) and B.m. = *Bacillus megaterium*, M.l. = *Micrococcus luteus*, E.c. = *Escherichia coli*, P.sp. = *Pseudomonas* sp. (**b**) Fitness (λ) of *wild-type* animals in the four bacterial environments. Error bars are SEM. (**c**) Hierarchy of food preferences and fitness are correlated

shown to be exquisitely sensitive to the relevant environmental changes and nematodes are good bioindicators of soil health (Bongers and Ferris 1999). Thus, it seems an understanding of the genetic basis of the nematode community response to environmental changes in the grasslands could be important to help us understand and predict the organismal response to global change. Indeed, we have been able to apply high-throughput molecular techniques to document changes in both the nematode and bacterial communities in response to changes in nutrient availability.

The main challenge in identifying the gene functions responsible for these changes in the native nematodes is the lack of available genetic tools. The approach we have taken is to model aspects of changes in the biotic environment using a genetically tractable laboratory nematode, *C. elegans*. To this end, we identified candidate genes that are differentially expressed in response to changes in the bacterial environment and biologically validated our approach by determining gene functions that affect fitness, lifespan and innate immunity. We also found that the hierarchy of food preference for the four bacterial isolates mirrored the trend observed for fitness in the different bacterial environments. This suggests that *C. elegans* prefers the environment in which it will be most fit. It will be interesting to see how *C. elegans* makes this choice and ultimately maximizes fitness. As we have observed that native soil nematodes differ in their susceptibility to the different bacteria in terms of infection/colonization (Coolon and Herman, unpublished data), pathogenicity might also contribute to soil nematode community structure. Taken together we suggest that the expression of metabolism and defense functions may in part drive nematode community dynamics in grassland soil systems.

The next challenge is to determine which gene functions are used in the native soil nematodes to respond to changes in the biotic environment. Since we have discovered several candidate genes in *C. elegans*, one approach is to identify homologs of these genes in the native nematodes and test their functions. While this is feasible, the major impediment to these studies is that the genomes of the relevant nematodes have not been characterized. However, the application of new sequencing methods will allow us to more readily obtain genome sequences for ecological relevant organisms. This promises to begin to close the tractability gap between model versus non-model organisms. An important aspect of this approach will be to be able to test gene function in the native nematodes. While RNA interference (RNAi) works well in *C. elegans* and some other nematode species, it does not work in all and one cannot predict its efficacy based upon phylogenetic relationships (Felix 2008). Thus in cases in which RNAi does not work, other methods will have to be employed.

Another aim of the ecological genomic approach is to better understand genome function in a well-studied genetic organism, which despite decades of research remains largely uncharacterized. The examination of *C. elegans* genome function in new environments uncovered new roles for previously studied genes as well as genes that had not been shown to have a function under standard laboratory conditions. We suggest that only through use of alternate environments does the detailed dissection of genomes become possible. Thus, it is clear that we are already reaping the benefits of the ecological genomic approach by further characterizing

genome function of well characterized models. However, work still needs to be done for our ecological genomic approach to identify gene functions that can predict the responses of native organisms to environmental changes. While the challenge is great, we are confident the application of ecological genomic approaches will produce major contributions to understanding organismal responses to global environmental change.

Acknowledgements Thanks to the members of the Ecological Genomics Institute at Kansas State University for discussions and helpful comments. The project was supported by grant number P20RR016475 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), NSF grant number 0723862 and KSU Targeted Excellence.

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