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Workshop report: *Caenorhabditis* nematodes as model organisms to study trait variation and its evolution

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A fundamental problem in biology is to understand how genome expression translates into variation in molecular, cellular, developmental, physiological, behavioral, or life-history traits. During the summer of 2014, worm biologists with a keen interest in evolutionary biology and natural ecology met in Les Treilles (France) to define the problems of trait variation better and to discuss empirical approaches using *Caenorhabditis* species to address these problems. Compared with other model organisms, *Caenorhabditis* has several advantages, such as well-defined traits that can be subjected to highly controlled environmental and genetic manipulation and the possibility for long-term experimental evolution that can be coupled with genome-wide mapping of trait variation. The Les Treilles workshop brought together researchers studying the evolution of phenotypic plasticity, gene-networks, genome structure and population genetics, sex-determination and development in the laboratory, behavior and the life-history of natural *Caenorhabditis* populations. Here, we outline the key aims of this workshop and summarize the contributions of each participant.

Each individual results from the expression of a unique genome in a unique environment. For this reason, attempting to measure the contributions of genetics versus environment to trait etiology at the individual level is pointless.[1] At the population level, however, partitioning trait variation among individuals into differences between the environments and differences in their genetic constitutions has a long tradition in biology. The infinitesimal models of evolutionary quantitative genetics allow the estimation of trait heritability in single or multiple environments and responses to natural selection without the need to understand the interaction between genomes and environments. However, it is uncertain if the implicit assumptions of evolutionary quantitative genetics are met, specifically when non-linear relationships between genotypes and phenotypes are common. If the genotype-phenotype map is generally non-linear, then only an understanding of gene-network function in the context of natural ecologies will resolve the questions concerning trait variation.

In the summer of 2014, a workshop in Les Treilles focused on how studies in *Caenorhabditis* species integrate environmental and genetic manipulations, in the laboratory and in nature, to understand trait variation. H. Teotónio (École Normale Supérieure Paris, France) introduced the workshop with some of the concepts of genotype-by-environment interactions (GxE) and discussed several statistical alternatives to divide trait variation found among individuals in a given population in deterministic and stochastic components. One issue, also later brought up by other workshop participants, is that it is not known how stochastic variation at the individual level can have an independent genetic basis from among individual trait variation. Additionally, most studies tend to ignore or minimize the covariance structure between genetics and environments for analytical convenience but at an unknown expense of biological reality.

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Steve Proulx (University of California Santa Barbara, USA) reviewed the classic population genetics theory on adaptation to predictably fluctuating environments, emphasizing that in most cases for adaptation to occur the geometric mean fitness across environments should increase through the evolution of trans-generational traits.[2] He then showed experimental evolution results consistent with this hypothesis. In populations of *C. elegans* with standing genetic diversity, the evolution of maternal traits underlies adaptation to a predictably fluctuating larval anoxic environment. A recent study similarly showed that GxE can experimentally evolve though changes in trans-generational traits. In *C. remanei* populations evolving from standing genetic diversity, the offspring of parents exposed to high temperatures were less able to survive a heat shock than those of parents not exposed to high temperatures. However, selection on larval survival to an acute heat shock resulted in the evolution of insensitivity to high temperature in adults.[3,4]

Evolution experiments in *Caenorhabditis* have been used to study the properties of mutational distributions among environments. For example, Charles Baer (University of Florida Gainesville, USA) championed the notion that environmental stress elevates mutation rates. In one mutation accumulation experiment, temperature led to increased deleterious mutation rates in *C. elegans* but not in *C. briggsae*.[5] In another *C. elegans* mutation accumulation experiment, deleterious mutation rates were indistinguishable under artificially elevated endogenous oxidative stress and under natural oxidative stress.[6] In this last study, the environment did condition the appearance of novel genetic diversity. As the first study shows, however, the extent of environmental conditioning may depend on the genotype being studied.

The evolution of trait variation in different environments depends on the population structure created by geography and dispersal to novel environments or by whether individuals reproduce by self-fertilization or outcrossing.[7] These points were highlighted during the workshop by Matthew Rockman (New York University, USA), among other participants. He presented field data on the population structure of tropical *Caenorhabditis* species and discussed how to estimate fitness in these circumstances, stressing the crucial need to take into account the demography of the populations and individual reproduction mode. Ivo Chelo (Instituto Gulbenkian de Ciência Oeiras, Portugal) further discussed the need to characterize the effects of self-fertilization and outcrossing on individual fertility distributions, showing how the experimental evolution of inbreeding depression in novel environments depends on the reproduction modes.

Besides experimental evolution, Christian Braendle (Institute of Biology Valrose Nice, France) introduced the workshop during the first session by discussing some other advantages of using *Caenorhabditis* to study trait variation and its evolution. Because of a long history of intensive study, *C. elegans* is known to display ample natural genetic variation for molecular, cellular, developmental, physiological, behavioral, and life-history responses to varying environments.[8] More significantly, the availability of a large collection of natural inbred isolates, with well-known geographical origin, and several panels of recombinant inbred lines (RILs) has allowed great control of genetic background under several laboratory environments and the mapping of quantitative trait loci (QTL) involved in GxE.

As an example, Kammenga and colleagues identified several QTL impacting hermaphrodite fertility, age at maturity, growth rate, lifespan and body size across thermal gradients, using RILs between the reference strain N2 and the genetically divergent Hawaiian isolate CB4856. Of particular interest, a specific coding polymorphism in the *tra-3* gene modulates body size in response to temperature.[9] Despite this example, one frustrating limitation of QTL studies so far has been the inability to measure trait variation on a large scale. In this respect, standardized high-throughput approaches were discussed at the workshop by Erik Andersen (Northwestern University Evanston, USA) to measure worm fecundity and growth in liquid culture, a proxy of fitness. These approaches promise to greatly improve the power of genome-wide association analysis (GWAS), where several hundreds of environmental conditions can be tested simultaneously. Moreover, Erik Andersen presented preliminary results of a linkage mapping analysis using a panel of N2 x CB4856 RILs exposed to several toxic compounds for the first time allowing a glimpse at the extent of pleiotropy of gene action among environments and the degree of genetic interactions among QTL. Similarly, the feasibility of characterizing epistasis was illustrated by Patrick McGrath (Georgia Institute of Technology Atlanta, USA). Using QTL mapping based on long term lab adapted strains, he showed how to dissect such complex genetic interactions underlying behavioral traits.[10]

Recently, QTL mapping has been extended to *C. briggsae* using RILs derived from crosses between the isolates AF16 (from India) and HK104 (from Japan). In contrast to *C. elegans* and despite similar reproduction modes, *C. briggsae* presents higher levels of genetic diversity and wild isolates cluster into distinct geographic groups, such as temperate and tropical clades.[11] Asher Cutter (University of Toronto, Canada) presented data on *C. briggsae* genetic diversity using whole-genome sequencing and QTL mapping of life history and behavioral trait variation at different temperatures, also using newly developed high-throughput assays. *C. briggsae* may therefore be valuable model to investigate thermal acclimation and the genetic basis of adaptation to different temperatures.

A remarkable example of *Caenorhabditis* GxE is the formation of the dauer stage, presumably the dispersal stage, in response to certain environmental cues.[12] The propensity to form dauer shows considerable variation among natural isolates, and QTL mapping suggests an especially intricate architecture underlying intraspecific variation. For example, Simon Harvey (Canterbury Christ Church University, UK) presented on-going research extending the analysis of natural genetic variation of dauer formation in *C. elegans* [13] and *C. briggsae* using a combination of RILs, nearly-isogenic lines (NILs), and mutation accumulation lines to quantify the effects of candidate QTL. On the other hand,

Mark Viney (University of Bristol, UK) presented assays and selection experiments quantifying genetic variation in dauer formation in response to specific ascarosides [14] – the pheromones controlling dauer induction. Dauer induction in distinct *C. elegans* natural isolates may greatly differ in response to specific ascarosides, and their relative concentrations, as well as in the production of dauer-inducing ascarosides.

Variation in dauer formation along with the production of, and sensitivity to, ascarosides has an obvious relevance for the evolution of dispersal in nature. Many other traits show variation that may be important for the evolution of viability and survival from embryo to reproductive maturity. For example, Marie Delattre (École Normale Supérieure Lyon, France) presented research focusing on the first asymmetric cell division of embryogenesis, a conserved developmental event, which underlies both inter- and intraspecific variation in cellular mechanisms.[15] Given the comprehensive understanding of cellular and developmental biology in *C. elegans*, describing trait variation at these levels of organization in other species and clades is a promising venue of research.

Stochastic trait variation in constant environments can be manifested as variation among individuals of the same genotype or within individuals. The recent development of techniques allowing quantification of individual RNA transcripts have advanced our understanding of the causes for stochastic trait variation. Using single molecule FISH in *C. elegans*, Scott Rifkin (University of California San Diego, USA) showed results suggesting that the stochastic expression of genes responsible for intestinal specification underlies the incomplete penetrance of some mutations.[16] Scott Rifkin further provided an in-depth overview of the historical development of the notions of incomplete penetrance and thresholds of gene action, which were already proposed in the early days of genetics by Richard Goldschmidt.[17] Marie-Anne Félix (École Normale Supérieure Paris, France) addressed the significance of such non-linear properties for robustness of biological systems when facing perturbations and explained the need for clearer definitions of the term robustness, which so far has remained vague or imprecise. Marie-Anne Félix then presented empirical research focusing on the characterization of developmental system robustness through exact molecular quantification of dose-response curves and stochastic variation of signaling pathway activities during *Caenorhabditis* vulval induction.[18] Taken together, these studies illustrate that central, long-standing questions in developmental and evolutionary biology can be experimentally addressed through highly quantitative analyses of well-defined genetic networks and their phenotypic outputs.

Our current understanding of *Caenorhabditis* biology is greatly hampered by the poor knowledge of the ecology and life history of these nematodes in the wild. Mitigating this point, Karin Kiontke (New York University, USA) provided an analysis of morphological trait diversity and evolution in the rapidly expanding *Caenorhabditis* phylogeny (over 25 species are currently cultured in the laboratory; http://www.cgc.cbs.umn.edu).[19] Significantly, in contrast to *C. elegans*, certain species display highly specialized interactions with invertebrate carriers.[20] Likewise, Eric Haag (University of Maryland College Park, USA) presented analyses on several *Caenorhabditis* genome structures and transcriptomes, identifying candidate loci modulating specific mating behaviors.[21]

Caenorhabditis are microbivores because they feed on a wide range of bacteria and fungi. These microbes, however, may also be potential pathogens.[22] The study of natural host-pathogen interactions has allowed the first identification of natural viruses in *C. elegans* and *C. briggsae* [23] and some of the molecular polymorphisms underlying natural variation in *C. elegans* viral sensitivity.[24] Evolution experiments in *C. elegans* have indicated that host-pathogen interactions are complex but may be key to maintaining outcrossing in the wild.[25,26]

The availability of new gene transformation technologies for species other than *C. elegans*, such as the CRISPR-Cas9 system, now allows for comparative analysis of gene function in a phylogenetic context. The presentation of Ronald Ellis (Rowan University SOM Stratford, USA) illustrated the use of such gene editing methods in hermaphroditic *Caenorhabditis* species to study the evolution of self-sperm activation. Although all 3 hermaphroditic species, *C. elegans, C. briggsae*, and *C. tropicalis*, have retained 2 redundant sets of sperm activation genes for use in males, Ronald Ellis could show that each hermaphroditic species has co-opted only one of these sets to activate their self-sperm.

Although the study of natural *Caenorhabditis* populations has greatly increased over the past decade, basic information on the demography, dispersal rates, and population genetic structure continues to be scarce. Finding the ecologically relevant environmental parameters to study trait variation and its evolution thus remains difficult for the community at large. For this reason, at the end of the workshop, participants reached the consensus that prioritizing efforts to study *Caenorhabditis* natural ecology is a major task for the future. It is hoped that integrating studies of *Caenorhabditis* species other than *C. elegans* will be particularly useful by allowing quantitative comparative analysis of trait variation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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