SHORT REVIEW

Ecological genomics in *Daphnia*: stress responses and environmental sex determinationBD Eads^{1,2}, J Andrews^{1,2} and JK Colbourne¹¹Center for Genomics and Bioinformatics, Indiana University Bloomington, Bloomington, IN, USA and ²Department of Biology, Indiana University Bloomington, Bloomington, IN, USA

Ecological genomics is the study of adaptation of natural populations to their environment, and therefore seeks to link organism and population level processes through an understanding of genome organization and function. The planktonic microcrustacean *Daphnia*, which has long been an important system for ecology, is now being used as a genomic model as well. Here we review recent progress in selected areas of *Daphnia* genomics research. Production of parthenogenetic male offspring occurs through environmental cues, which clearly involves endocrine regulation and has also been studied as a toxicological response to juvenoid hormone analog insecticides. Recent progress has uncov-

ered a putative juvenoid *cis*-response element, which together with microarray analysis will stimulate further research into nuclear hormone receptors and their associated transcriptional regulatory networks. Ecotoxicological studies indicate that mRNA profiling is a sensitive and specific research tool with promising applications in environmental monitoring and for uncovering conserved cellular processes. Rapid progress is expected to continue in these and other areas, as genomic tools for *Daphnia* become widely available to investigators.

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Introduction

A meaningful integration of ecology and genomics promises important new insights into the process by which populations respond and adapt to their environment. This research begins by identifying the genetic basis for ecologically relevant traits. Then, explicit and quantifiable associations are made between the heritable molecular variation within populations and individual fitness across varying ecological conditions (Gibson, 2002; Feder and Mitchell-Olds, 2003). In practice, two approaches are taken to achieve this level of integration; each presents a different set of advantages and challenges. First, ecological and evolutionary studies are pursued using traditional genetic model species that have well developed genomic tools, such as *Drosophila*, *Caenorhabditis*, *Arabidopsis* and *Mus* (cf. Landry *et al.*, 2006; Shimizu and Purugganan, 2006). Although these species offer a staggering amount of molecular biological knowledge, many have ill-defined population boundaries and ecologies. This situation is starting to change owing to increased ecological study of these model organisms. Second, molecular genetic and genomic studies are initiated using organisms that have well-understood ecologies and evolutionary histories (Crawford,

2001; Strauss and Martin, 2004; Lai *et al.*, 2006), of which the water flea, *Daphnia*, is one. Here, we outline the progress in the use of *Daphnia* as a model in evolutionary and ecological functional genomics.

Several attributes make *Daphnia* an especially tractable system for ecological genomics. In addition to their well-understood ecology and their sentinel roles within freshwater ecosystems, these microcrustaceans are easily collected in large numbers and reared in the lab with a 5- to 10-day generation time. They have a relatively small and sequenced genome (ca. 200 Mb) and perhaps most interesting, they reproduce by cyclical parthenogenesis. Females typically reproduce asexually, but environmental conditions can induce parthenogenetic production of males (genetically identical to their mothers) and haploid gametes that are fertilized and enter a state of extended metabolic dormancy called diapause. This life cycle allows the creation of inbred or outbred lines for mapping studies, and provides tremendous power for dissecting genetically based differences (among clones) from experimental or developmental noise by measuring replicates within a clone.

The ecology and evolution of *Daphnia* is well explored with respect to habitat differentiation (Fryer, 1991; Wellborn *et al.*, 1996; Colbourne *et al.*, 1997), but progress has been less in characterizing molecular mechanisms responsible for traits conferring a fitness advantage in defined environments. For instance, diurnal and seasonal variations in predation, parasitism, food availability and temperature interact in complex ways to produce phenotypes such as investment in sexual reproduction (Cáceres and Tessier, 2004). Although production of sexual eggs

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and males is expected to be coupled at some level as an adaptation to make diapausing embryos, mechanisms of this induction by environmental cues are not well understood. An important first step towards such an investigation is to examine the genetics of environmental sex determination. Similarly, *Daphnia* are often in temperature- and oxygen-stratified habitats, which has important ecological implications for predator avoidance and access to alternative grazing resources (Williamson *et al.*, 1996). Acclimation to hypoxia via hemoglobin (Hb) synthesis has been a subject of extensive study over the last decades. Here, we discuss new discoveries enabled by genomic approaches that provide a mechanistic linkage between two well-studied phenomena, male production and Hb induction in *Daphnia*.

A consortium of investigators is progressing rapidly in creating tools for *Daphnia* genomics. First, the genome sequencing and assembly of a *D. pulex* genome by the Department of Energy's Joint Genome Institute is complete, and its annotation has begun. The annotation goals of identifying genes and their structures are being guided by a growing number of cDNA libraries and sequences for both *D. pulex* (Colbourne *et al.*, submitted) and *D. magna* (Watanabe *et al.*, 2005). Second, microsatellite DNA markers are deployed for population surveys (Fox, 2004) and quantitative trait locus (QTL) mapping experiments (Colbourne *et al.*, 2004). Many of these markers form the basis of a genetic map (Cristescu *et al.*, 2006) that is being integrated along with large insert BAC libraries to identify chromosomal segments under high and low rates of recombination. Third, both cDNA and oligonucleotide microarrays are being used for gene expression analysis in *D. magna* and *D. pulex*. Finally, bioinformatic efforts have resulted in the creation of wFleabase (Colbourne *et al.*, 2005), an online repository of genetic, molecular and genomic data for *Daphnia*, while laboratory projects to create cell lines and virally induced transgenic lines are also ongoing (Robinson *et al.*, 2006).

These resources are opening the way for the systematic investigation of the genes and pathways underlying ecologically relevant traits. Presentations at a recent meeting of the *Daphnia* Genomics Consortium summarized progress in the areas of gametogenesis, embryonic patterning, parasitology, toxicology and molecular evolution (<http://conferences.cgb.indiana.edu/daphnia2006/>). To date, much of the data obtained about molecular mechanisms in the area of ecological genomics stem from physiological rather than evolutionary perspectives, which is reflected in our choice of literature to review. Specifically, we focus on progress in understanding sex determination and environmental stress responses.

Male production pathway as an entry into ecological genomics

The ecological basis of sex determination in *Daphnia* has long been appreciated (Banta and Brown, 1939) and genetic variation for this trait is substantial (Yampolsky, 1992). Environmental factors such as temperature, photoperiod and crowding stimulate male production by parthenogenesis (Hobæk and Larsson, 1990) and, although varying investments into sexual reproduction have important ecological and evolutionary consequences (Tessier and Cáceres, 2004), molecular studies

have only begun. Male production is a promising research avenue for several reasons. First, it provides an opportunity to study the molecular basis of environmental sex determination. Second, it is a basis for broader exploration of invertebrate endocrine function and disruption in general, which has lagged far behind our understanding of vertebrate systems (Oetken *et al.*, 2004). Third, as a classic example of phenotypic plasticity and epigenetic regulation, it offers the possibility of uncovering molecular mechanisms responsible for these ecologically and evolutionarily central mechanisms. Fourth, evidence indicates important couplings between juvenile hormone (JH) pathways and ecdysone pathways in *Daphnia* (Mu and LeBlanc, 2004), which are likely to be useful for genetic and genomic analyses of these pathways across the Ecdysozoa.

Olmstead and LeBlanc (2002) were first to demonstrate production of male broods of *D. magna* in response to the sesquiterpenoid hormone methyl farnesoate (MF), the unepoxidated form of insect JH III. Discovery of MF in crustaceans prompted extensive efforts to characterize the functions of this hormone, which largely appears to mirror the effects of JH in insects (Laufer and Biggers, 2001). Other JH analogs (JHAs) in addition to MF have been shown to induce male production in *Daphnia*. The insecticides pyriproxyfen, fenoxycarb and methoprene cause male broods (Tatarazako *et al.*, 2003), with orders of magnitude greater potency than MF for pyriproxyfen and fenoxycarb. Interestingly, all of these compounds also induce Hb production in *Daphnia* (Rider *et al.*, 2005). Therefore, male production and Hb induction appear to be coupled at some level by JHA signaling (see below).

Studies with MF demonstrate that *D. magna* and *D. pulex* produce male broods in a dose-dependent manner, with concentrations above 300 nM resulting in all male broods (Olmstead and LeBlanc, 2002; personal observation). Furthermore, the sensitive period for MF induction of male development is 1–2 days before egg laying during the vitellogenic period of oogenesis. We have studied the transcriptional effects on genes from developmentally staged females exposed to MF, by using a microarray platform described elsewhere (Colbourne *et al.*, submitted). Briefly, females from a single mother were kept in a common garden until their first broods were released. Then, 20 individuals were randomly selected for a 3-day exposure to either 400 nM MF (Echelon Biosciences, Salt Lake City, UT, USA, changed daily) or to methanol carrier (0.004%). Three replicates of each condition were used for RNA isolation, cDNA labeling and hybridization, and analysis according to standard protocols (<http://dgrc.cgb.indiana.edu/microarrays/support/protocols.html>).

Compared to controls, MF-treated animals showed higher steady-state levels of 39 unique transcripts and lower levels of 16 transcripts (Table 1). Twenty-two differentially regulated genes shared significant sequence homologies with proteins archived in the NCBI non-redundant database. In particular, Hb and neuronal acetylcholine receptor transcripts were elevated under MF treatment, in addition to arginine kinase, amylase, cytochrome *c* oxidase and cytochrome *b*, several cuticle proteins, actin and a putative ribosomal biogenesis regulatory protein. Control animals had higher levels of glucosamine-6-phosphate deaminase, glucose-6-phosphatase, several proteases, an actin de-polymerizing

Table 1 Transcript levels of genes increased or decreased after 3-day incubation in 400 nM methyl farnesoate

GenBank accession	Blast <i>e</i> -value	Gene ID	Fold change
BAC36909.1	1e ⁻³⁹	Glucosamine-6-phosphate deaminase	-1.3
BAC65417.1	1e ⁻¹¹	Cathepsin-L protease	-1.7
AAU84921.1	1e ⁻²⁶	Profilin	-1.5
AAF56122.1	1e ⁻³⁹	Zn-peptidase	-1.3
AF194426.1	5e ⁻³⁰	Cysteine protease	-1.7
XP_392564.1	5e ⁻⁷⁷	Rheb G-protein receptor	-1.8
AAH62466.1	3e ⁻⁶²	Ribosomal protein S9	-1.4
AAH60331.1	5e ⁻³⁷	Glucose-6-phosphatase	-2.8
AAV84227.1	0.006	Putative receptor	-1.4
AAB08704.1	7e ⁻¹³	Ecdysteroid-regulated 16 kDa protein	-1.3
NP_600374.1	0.006	ABC transporter	-1.4
AAH55615.1	1e ⁻⁵⁵	Ribosome biogenesis regulatory protein	+1.7
CAB99474.1	1e ⁻¹¹⁶	Actin	+1.4
BAB62537.1	1e ⁻¹⁷	Hemoglobin	+1.5
AAL25092.1	1e ⁻¹⁰²	Arginine kinase	+1.3
AAL68188.2	1e ⁻¹¹	Serine peptidase	+1.3
AAH95821.1	0.0002	Monooxygenase, DBH	+1.4
AAN71654.1	1e ⁻²⁹	Fatty-acid binding	+2.5
P82119	5e ⁻¹⁷	Cuticle protein	+1.8
AAH09121.1	5e ⁻⁵⁸	Amylase	+1.6
XP_313747.1	1e ⁻⁷⁴	Gram-negative Bacterial binding protein	+1.5
P23414	1e ⁻²⁸	Acetylcholine receptor	+1.7

Fold increase or decrease is calculated relative to controls incubated in 0.004% methanol, using three biological replicates analyzed with the Bioconductor packages Olin and Limma (<http://www.bioconductor.org>). Putative identifications were assigned by finding best Blast (Altschul *et al.*, 1990) matches in the non-redundant protein database of GenBank. Genes with no putative homolog (e-values > 0.01) or no functional annotation are not shown, and fold changes are calculated on the arithmetic (not logarithmic) scale.

enzyme (profilin), a putative receptor of unknown function and a homolog of the Rheb G-protein-coupled receptor. Another transcript with lower levels during treatment had putative homology to an ecdysteroid-regulated transcript from *Manduca Sexta* (GenBank accession AAB08704.1). Several other genes differed in response to MF, but whose putative homologs in arthropods (such as the *Drosophila melanogaster* genes CG6770 and CG31997) have no known function. Subsequent study of these genes may reveal whether they have conserved roles in JH response. Several dozen genes with no sequence similarity to entries in the non-redundant protein database showed differential expression as well.

Taken together, results from this microarray experiment are consistent with current data for MF activity in crustaceans and also offer new avenues for exploration (Figure 1). First, higher steady-state levels of Hb are consistent with transcriptional control of this protein, an area of active investigation explored further below. Second, changes in polysaccharide metabolism-related genes, cuticle genes and cytoskeletal protein genes imply a coordinated shift in carbohydrate usage away from ATP production and towards chitin metabolism. Physiological and biochemical studies would be useful to uncover such systemic changes in metabolism in response to JHA treatment. Furthermore, alteration of chitin metabolism underscores connections between MF and ecdysis due to genetic crosstalk with ecdysone pathways (Mu and LeBlanc, 2004). Third, involvement of the rheb receptor in response to MF is, to our knowledge, a novel observation linking this protein to a crustacean hormonal response. In *D. melanogaster*, Rheb participates in cell growth and proliferation via the S/G1 mitotic transition, in addition to starvation response (Patel *et al.*, 2003). These biological functions in flies are consistent

Figure 1 Schematic depicting the methyl farnesoate pathway in male production and hemoglobin induction. For further discussion, see Laufer and Biggers (2001) and Mu and LeBlanc (2004). Arrows (induction or production) and blocked lines (repression) may be direct or indirect effects (possibly both, indicated by dashed lines). Sequence and expression data have been obtained from cDNAs encoding farnesoic acid methyltransferase, two CHH peptides and homologs of *D. melanogaster* sex determination genes sex lethal (*Sxl*) and transformer-2 (*Tra-2*) (data not shown).

with a role for Rheb in *Daphnia* related to molting. Fourth, an intriguing and under-explored connection between secretion of JHA and cholinergic regulation is highlighted by increased acetylcholine receptor mRNA levels under MF treatment. Research into molecular mechanisms of cholinergic hormonal control is only beginning to be investigated in arthropods (Kuo, 2002). Genomic tools available in *Daphnia* may help to inform

research in suitable biochemical and physiological crustacean models of both rheb and cholinergic pathway interactions with JHA. Finally, genes of unknown function that are expressed constitutively in response to MF may be members of the MF pathway, and could be interesting targets of subsequent studies such as time course, dose-response and exposure to other JHA.

The MF pathway is an early example of genomic studies investigating the mechanism for environmental sex determination in natural settings. Current work in our laboratories includes additional microarray experiments using both wild-type and naturally occurring isolates that never produce males and are thus unresponsive to MF. Mapping panels from crosses between male and non-male producers will help identify QTL for this trait, which could subsequently be used to study ecological correlates of genetic polymorphisms.

Genomics of environmental stress responses

Research in *Daphnia* has long focused on the environmental stresses they encounter and mechanisms they use to counter or mitigate them. Prominent among these areas are toxicology, hypoxia, temperature, parasites, predators and ultraviolet radiation. Responses to these challenges have been documented at the level of physiology, genetics, maternal effects, morphology, behavior and life history. Adequately addressing these areas is beyond the scope of this review; our aim instead is to highlight recent progress in uncovering molecular mechanisms underlying these changes.

Acute hypoxia in *Daphnia* leads to compensation via increased heart rate or ventilation rate (Pirow *et al.*, 1999), but chronic hypoxia leads to induction of Hb synthesis. This synthesis is subunit-specific (Kimura *et al.*, 1999), with changes over time occurring at subunit-specific rates (Zeis *et al.*, 2003). Levels of mRNA for subunits *hb2* and *hb3* are rapidly and highly induced during hypoxia, while *hb1* is unaffected (Zeis *et al.*, 2003). These results, plus the identification of putative binding sites for the mammalian hypoxia-inducible factor 1 (HIF-1; Kimura *et al.*, 1999), led to a detailed investigation of Hb gene promoters. Induction of Hb gene expression during hypoxia is dependent on the binding of HIF to particular hypoxia response elements (HRE) in promoter regions of the four known *D. magna* Hb genes (Gorr *et al.*, 2004). Three HREs upstream of the globin-2 gene were found using heterologous transfection of HIF-expressing human and *D. melanogaster* cells, showing specific binding of HIF complexes at two sites *in vitro* and binding of an unknown constitutive transcription factor at the third site (Gorr *et al.*, 2004). A recent report (Gorr *et al.*, 2006) has identified a putative juvenoid response element (JRE) in the promoter of *hb2* in *D. magna* that binds an activated factor in response to JHA. This development is exciting for several reasons. Despite decades of intense interest and research into mechanisms of JHA action in arthropods, a nuclear hormone receptor for JHA has not been identified with certainty. The JRE is a powerful tool to establish the identity of the JHA receptor, which will undoubtedly stimulate molecular genetic research into pathways of JHA activity. These findings also link mechanisms for the ecologically relevant traits of male production and Hb induction for the first time.

The recent discovery of two additional members of the *D. magna* globin family using Southern blotting (Nunes *et al.*, 2005) indicates that many more discoveries will emerge from functional studies of these molecules. Studies of *Daphnia* populations that are locally adapted to oxygen-rich or oxygen-starved habitats may help elucidate the ecological implications of lower mass-specific oxygen consumption rates in hypoxia-acclimated animals (Seidl *et al.*, 2005) for community structure and trophic interactions. Furthermore, increased O₂ delivery due to higher Hb content incurs physiological and fitness costs via increased protein synthesis and predation (Pirow *et al.*, 2001), providing an opportunity to link physiological ecology with molecular mechanisms such as HIF binding.

In toxicogenomics, research into endocrine disrupting chemicals such as the JHA compounds discussed above continues to be a high priority (Oetken *et al.*, 2004). In this regard, *Daphnia* will be useful for elucidating gene expression patterns and associated ecological changes in response to these challenges. The difficulty and potential of combining a genomics approach with environmental monitoring has been a subject of intense interest (for example, Lettieri, 2006). Some conceptual issues are as follows: (1) How specific are mechanisms of toxicity or clearance, as revealed by the genes and pathways responding to toxicants? (2) How sensitive and useful is expression profiling at uncovering those genes or pathways? (3) Are traditional toxicological approaches (for example, end points, LC₅₀s, dose-response curves) optimal for genomic assays? (4) Are genomic responses to toxicants indicative of changes within a population and ultimately the state of its ecosystem? Data from groups performing toxicological genomics in *Daphnia*, although preliminary, bear directly on some of these questions. Future studies using more complete microarray platforms with tens of thousands instead of thousands of probes will provide interesting insight into variation among toxicological conditions. Yet, the trends described here are universal enough to provide a meaningful picture.

First, while 'stress response' genes or pathways tend to be induced consistently across physiological challenges as diverse as metal toxicity, ordinance (explosive) contamination or nanoparticle exposure, each challenge also provides a unique signature. These observations imply that certain types of molecular response, such as induction of a particular class of chaperones, may be diagnostic of organisms under stress. Also, condition-specific responses are likely, depending on experimental protocols (for example, exposure times and dosages) and the life stages of the animals. Second, low (sub-lethal and environmentally relevant) chronic exposures generally can induce measurable changes in gene expression. Low-dose exposures, along with exposure to simultaneous challenges, are often used as experimental tools for discerning potential mechanisms of toxicity (Lettieri, 2006). Therefore, transcriptional profiling should be useful, in combination with data from other sources, to uncover how toxins work. Beyond that, how genetic background of particular clones might affect outcomes (see Lopes *et al.*, 2006), the importance of trans-generational (maternal or grand-maternal) effects, and how sequence, gene expression and/or physiological response vary are unanswered questions.

A recent study of the effect of the fungicide propiconazole on *D. magna* (Soetaert *et al.*, 2006) illustrates the challenges and potential of environmental monitoring using microarrays. Propiconazole resembles other chemicals studied for toxicity and their effects on development (Kast-Hutcheson *et al.*, 2001), such as diethylstilbestrol (Baldwin *et al.*, 1995) and nonylphenol (Shurin and Dodson, 1997), which kill or deform embryos, often at concentrations similar to those in the environment. Transcriptional profiling of propiconazole treatment of 4- and 8-day-old animals using microarrays constructed from age-specific cDNA libraries established differential expression of over 10% of the arrayed genes (Soetaert *et al.*, 2006). Gene expression varied with both time and dose, which underscores the difficulty of choosing appropriate end points for such studies. However, results are encouraging for a research community poised to apply microarrays to discover the condition dependency of responses to toxicants. For example, transcripts related to development and reproduction were differentially expressed, such as an eightfold decrease in transcript levels of the primary yolk protein vitellogenin. Also, some transcripts either had homologs of unknown function in other organisms or appeared unique to *Daphnia*. Profiling gene expression across a range of environmentally relevant conditions should elucidate cellular and organism level processes that are vital within natural settings, but that are not typically elicited in laboratory studies of traditional model organisms.

It would be interesting to extend the above observations by profiling offspring from mothers given a propiconazole exposure, which affects embryonic development in *Daphnia* (Kast-Hutcheson *et al.*, 2001). The quality of the maternal environment influences traits such as size at birth and number of offspring (Sakwinska, 2004), strain-specific immunity to bacterial parasites (Little *et al.*, 2003) and energy allocation to offspring (LaMontagne and McCauley, 2001). Interestingly, mothers reproducing in poor environments produced offspring more than twice as resistant to bacterial infection as mothers in favorable environments (Mitchell and Read, 2005). These findings underscore an important requirement for systematic approaches to quantify sources and consequences of both genetic and environmental variability in ecological studies (Forbes, 1998).

Maternal effects have been experimentally addressed during exposure to predator kairomones, some effects of which occur across generations. The formation of defensive structures like helmets and neckteeth in response to kairomones is a classic example of cyclo-morphosis, and depends upon maternal environment to an even greater extent than in the late embryonic environment (Agrawal *et al.*, 1999). A study of heat-shock protein (HSP) induction and the actin and tubulin cytoskeleton in predator-exposed *Daphnia* (Pijanowska and Kloc, 2004) demonstrated particular maternal effects from kairomone exposure. Upon exposure to the invertebrate predator *Chaoborus* or to fish kairomones, HSP40, HSP60 and HSP70 all decreased in expression. Using anti-phosphohistone 3 as a control also showed that α -tubulin and actin protein levels decreased in exposed animals, and that tubulin expression decreased in F1 progeny of exposed animals compared to F1 from control animals. Interestingly, Cu and Zn both reduced neckteeth induction in *Daphnia* neonates exposed to

predator kairomones (Hunter and Pyle, 2004), indicating interference by metals in the cyclo-morphosis signaling pathway. Employing combinations of different environmental stressors has considerable promise for dissecting signaling pathways and molecular responses to these challenges.

A powerful tool for microevolutionary studies is the hatching of dormant diapause embryos from sediment core samples of ponds and lakes, dubbed 'resurrection ecology' (Kerfoot and Weider, 2004). Implications of migration through time, or the effects of overlapping generations on population dynamics, are an active research area (for example, Cáceres and Tessier, 2004). In addition, hatching dormant propagules may compare ancestral populations and their descendants, for example before and after introduction of a novel predator or toxicant, to measure genetic differences related to the environmental change (Weider *et al.*, 1997; Hairston *et al.*, 1999). For instance, a *Daphnia* population exposed to variable and well-documented fish predation over 30 years was examined for changes in an adaptive quantitative trait (phototactic behavior, related to diel vertical migration and thus predator avoidance) and in neutral genetic markers (Cousyn *et al.*, 2001). Results demonstrated increased behavioral plasticity in response to predator kairomones, and order of magnitude higher genetic differentiation for the trait than for neutral markers, consistent with a strong role for selection as the driver of rapid, directional changes. Another example demonstrated evolution of resistance to toxic food sources (cyanobacteria) through a reduction in phenotypic plasticity over time (Hairston *et al.*, 2001). In a similar way, combining molecular biology and genomics with paleolimnology may test hypotheses about adaptation in populations exposed to severe metal contamination (Pollard *et al.*, 2003). Monitoring changes in allele frequencies within naive and post-exposed populations is possible even with unhatched or gravely degraded embryos (Limburg and Weider, 2002); the greatest limitation, until now, was determining the sequence identity of candidate genes that have possibly evolved structurally in response to the documented changes in the environment.

Understanding genome structure in light of the stress of ecological competition (for example, for limited carbon or phosphorus) is a primary motivation for research into the functional consequences of rDNA length and copy number, termed biological stoichiometry (Weider *et al.*, 2005). Interest in rDNA *per se* stems from mechanistic links between copy number and intergenic spacer (IGS) length, and their effects on organismal growth rate and phosphate requirements (Weider *et al.*, 2005). The growth rate hypothesis (GRH) posits that N:P and C:P ratios reflect allocation of P to ribosomal RNA, with higher rates demanding more P for rRNA production and protein synthesis (Elser *et al.*, 2000). Predictions based on the GRH have been supported by numerous studies (Weider *et al.*, 2005), and environmental P concentrations are frequently low enough to limit growth of *Daphnia* (Anderson and Hessen, 2005). It is not clear whether stressful conditions due to poor food quality (low P content) are sufficient to drive the evolution of rDNA repeat length or copy number. However, evidence from laboratory experiments indicates that this may be the case. For example,

by selecting for divergent weight-specific fecundity in *D. pulex*, Gorokhova *et al.* (2002) were able to demonstrate significant changes in age of first reproduction, size of first clutch, RNA and phosphorus content and IGS length after only five generations.

Conclusion

The growing genomics toolbox for *Daphnia* is providing opportunities to extend laboratory research from the molecular basis of morphological, physiological and behavioral differences to questions addressing the degree and importance of genetic variation within natural populations. Although *Daphnia* genomics research is still nascent, understanding the function of key genetic networks modulating traits with clear ecological relevance is advancing. For instance, microarray experiments and sequence analysis are uncovering members of these networks with characterized functions, plus genes that are apparently conserved across arthropods, but have no known function. Other genes are presumably unique in *Daphnia*. Indeed, at the recent *Daphnia* Genomics Consortium meeting, we presented data suggesting that at least 57% of over 12 600 cDNA sequences for *D. pulex* showed no similarity to protein sequences archived within the NCBI non-redundant database.

The structure and function of a genome is a product of the genome's environment, with interactions occurring at multiple scales. Current work – on genes linked within asexual genomes, on genes locked within a co-evolutionary arms race against parasites, and on multiple gene families whose copy numbers may be tied to ecological processes – is paving the way for genome-wide searches for signatures of selection on genomic elements. For example, the attributes of species are important in the macro-evolutionary processes acting on genome composition. These signatures range from adaptive amino-acid substitutions in genes associated with differences between humans and chimpanzees (Clark *et al.*, 2003) to the distribution of elements such as introns and transposable elements as a function of long-term effective population size differences among some major divisions of life (Lynch and Conery, 2003). In a complementary way, research on populations of *Daphnia* will provide an important micro-evolutionary perspective. This view comes from studying genomic variation at local geographic scales and during recent demographic history, such as the latest demonstration that obligately asexual *Daphnia* lineages suffer increased deleterious mutations compared to sexual lineages (Paland and Lynch, 2006), which has clear evolutionary implications.

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