Research Projects for Analysis of Stress in Biological Systems

The two summers and the intervening academic year will be spent working on research projects related to the response of biological systems to stress. Each entering student group will be working on a designated research project. They will either work in one project for their entire two-year experience or in two closely related projects under the guidance of one Mathematics faculty and one Biology faculty for each project. During this time they will gain an in-depth understanding of both the biology and mathematics that relate to their research. The research projects share similar mathematical and biological concepts, methodologies, and techniques, which allows all students to participate as one big group in the seminars and computer labs. Students will be strongly encouraged to work with other students outside their respective research groups, collaborate, and to provide assistance and peer mentoring. We will draw from the following projects over the three-year period of the *ASMS* project.

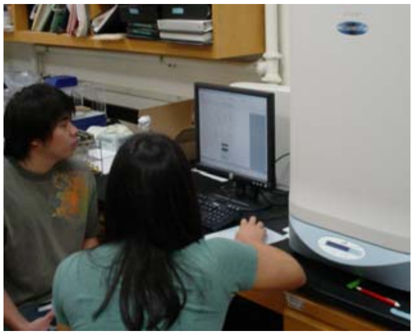
Project 1. The Functional Genomics of Adaptation to Heavy Metals in Wild Mustard Plants.

(G. Kuleck, K. Dahlquist, B. Fitzpatrick)

Understanding the genetic basis of physiological diversity remains among the major challenges of biology. Of special importance are those variations that allow plants to survive in harsh, polluted environments. Increasingly, plant biologists are turning to the tools of genomics and bioinformatics to unravel the regulatory mechanisms and transcriptional networks that govern plant responsiveness (Alba *et al*., 2004; Gibson *et al.*, 2002). While DNA microarrays can be used to measure changes in gene expression in the presence of stressors such as heavy metals, they are typically only available for model plants, e.g. *Arabidopsis thaliana*. However, it is becoming clear that *Arabidopsis thaliana* and its wild relatives (species in the mustard family) will play an important role in the emerging synthesis of genomics and ecology. (Mitchell-Olds, 2001). In this project, *DNA* microarrays of the model plant *Arabidopsis* thaliana [available from the Genome Consortium for Active Teaching (GCAT) for a nominal fee] will be used to explore the response of populations of wild radish plants (*Raphanus sativus)* to the Ballona Wetlands environment. Specifically, we will examine how the most abundant mustard family species in Ballona, *Raphanus sativus* (wild radish) responds to heavy metal stressors in microniches within Ballona by focusing on those genes in the *Arabidopsis DNA* microarrays which have been identified as responders to heavy metal stress.

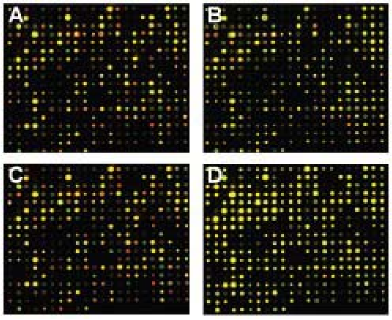
Technical challenges include determining which microniches to sample in the wetlands; other research efforts funded by a recent Merck/AAAS grant will provide evidence as to which areas show greatest differential in heavy metal contamination. In the first phase of the project, *Arabidopsis thaliana* and *Raphanus sativus* will be grown in the laboratory under identical conditions to establish a baseline from which to evaluate comparative genomic differences. Since the genome of *Raphanus sativus* has not been sequenced, we do not know how similar their DNA sequences will be to the *Arabidopsis* sequences. Some genes will be highly conserved while others will not. In order to accurately analyze the data we will need to estimate the level of similarity of the sequences between *Arabidopsis* and the wild plants. By focusing on a subset of well-characterized genes responding to heavy metal stress in *Arabidopsis* spp. (Becher *et al.*, 2004), it should be possible to estimate the level of similarity by DNA sequencing and EST analysis. An impressive array of information is available on *Arabidopsis* and its relatives which will aid in the analysis (Huala *et al.*, 2001, Rensink *et al.*, 2005). We will then construct a mathematical model to allow us to explore gene expression differences between wild radish plans growing in different microniches in the wetlands in the wetlands with the wild radish plants growing in different microniches in the second phase of the project. There is evidence that this cross-species transcript profiling and analysis can be successful (Becher *et al.,* 2004), but most studies to date have used laboratory-grown plants to compare to the *Arabidopsis* plant. Our work will expand the use of model organism *DNA* microarrays to an environmental setting, the next wave of advance in utilizing genomics to study ecological problems. (Zhou *et al.,* 2004)

In this project, student researchers will gain experience with genomics and bioinformatics by using *DNA* microarrays and other molecular techniques to collect data for analysis. Software for analyzing the microarray images will be used to extract large multivariate datasets. Our *UBM* students will then have to build statistical models to analyze these data. Statistical approaches here involve hidden state variables due to the differences between the *Arabidopsis* plant and the wild plants. To understand and model the processes, we will consider response surface construction (Box & Draper, 1987) developed within a Bayesian statistical framework to handle the hidden state variables. *UBM* students will test, improve, and validate Bayesian response surface models to explore data collected from *Raphanus sativus* grown in the Ballona wetlands ecosystem.



Project 2. Modeling Gene Expression Networks in *Saccharomyces cerevisiae.* (K. Dahlquist, E. Camacho)

Budding yeast, *Saccharomyces cerevisiae,* has been used extensively as a model organism because it is amenable to molecular genetics techniques, has a small genome of approximately 6000 genes, and yet is a single-celled eukaryote which performs many of the same basic functions that higher eukaryotic cells perform. Early on, DNA microarray technology for measuring the expression level of thousands of genes simultaneously was developed for yeast and extensive datasets that detail yeast's transcriptional response to environmental stresses are publicly available (Gasch *et al,* 2000; Causton *et al.,* 2001). Furthermore, genome-wide location experiments and bioinformatics approaches have determined the identity of gene regulatory modules by matching transcription factors with target genes (Lee *et al.,* 2001; Wang *et al*., 2005). These data have been valuable resources for taking a first look at the topology of gene expression networks in yeast, but they are only a beginning.



Study of time course of gene expression using yeast microarrays. Each box represents a different time point. Each spot represents a unique gene sequence; the color differences represent differential hybridization of competing RNA populations. Dr. Kam Dahlquist and Dr. Gary Kuleck utilize DNA microarrays in research and teaching.

In this project we will take a systems biology approach to understanding gene regulatory networks at both the transcriptional and translational levels. In particular we are interested in gaining insight into the behavior and properties of the gene regulatory network that controls the response to environmental stresses, such as changes in temperature, nutrient availability, osmolarity, and oxidative stress. Yeast responds to environmental stresses through characteristic programs of gene expression. Understanding the robustness to genetic perturbations of the network is also one of our objectives. In this project, the students will create a mathematical model of the gene regulatory network in yeast, and then make predictions based on the model. These predictions will then be tested in the laboratory by performing the appropriate DNA microarray experiment with a yeast strain deleted for a particular transcription factor. The new data will be compared to the model and the model will be refined. Dr. Dahlquist has particular expertise in analyzing DNA microarray data, having helped develop the GenMAPP (Gene Map Annotator and Pathway Profiler) software as a postdoctoral fellow. Dr. Dahlquist has successfully mentored undergraduate students performing research with DNA microarrays acquired from the Genome Consortium for Active Teaching (GCAT). A sophomore working with Dr. Dahlquist on DNA microarray research won an award for an outstanding poster presentation at the 2006 West Coast Biological Sciences Undergraduate Research Conference for his poster entitled "The Transcriptional Response of Baker's Yeast (*Saccharomyces cerevisiae*) to Cold Shock and Recovery".

A first natural step in modeling gene expression is to understand the interplay of the transcription factors, the associated mRNAs, and proteins. Dr. Camacho guided a group of undergraduates in AMSSI 2005 in developing a differential equations model that focused on three different classes of mRNAs and their corresponding proteins in a generic eukaryote (http://www.amssi.org/). Previous models have been successful in modeling the interactions involved in the transcription component of gene expression of various well-known organisms but not the translation component (Shea & Ackers, 1985). In this UBM project, we will use the previous work as a framework to model the transcription and translation that occurs in *Saccharomyces cerevisiae*. At each step of the project, the students will work with both the experimental and mathematical aspects of the model.

The hierarchical structure needed for the modeling effort relates to different levels of detail (see Stelling, 2004) in approaching cellular networks. Modeling often takes place at one of three levels of resolution: interaction-based models that identify connections between genes, constraint-based models that attempt to quantify cell behavior in terms of optimization (typically of microbial growth), and kinetic modeling (as detailed above) in which rate constants are required and dynamic predictions of reactions are obtained. The first level is very much a descriptive approach, yielding interactions without dynamics or directionality. The third level is the most detailed but requires a large number of parameters to be inferred. Moreover, at the systems level, this type of model is very complex. The constraint-based approach offers an interesting compromise, allowing dynamic models at a coarser level of measurement and reaction. Beal *et al.* (2005) have applied Bayesian estimation techniques to infer a linear model of the hidden state variable dynamics. Student researchers will need to extend this approach in order to integrate the microarray data and to conduct some basic exploratory data analysis to determine appropriate likelihood models for the inference.

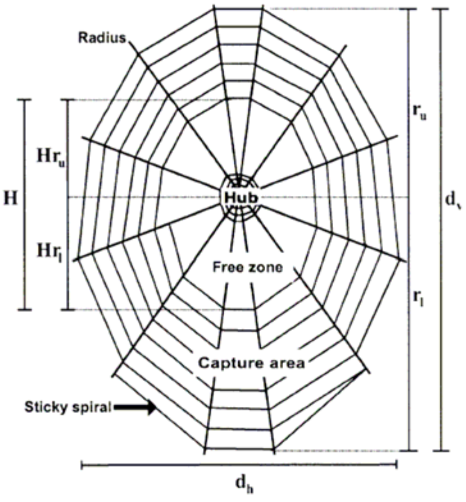
In order to develop and analyze the constraint-based functional models, we will iterate between experiment and modeling, using microarray data to investigate gene expression. Dynamic data can be obtained to see cascading and interacting reactions in gene expression. Coupling these data with a dynamic (differential equation) model and a Bayesian statistical structure for parameter estimation, our students will see how the model evolves, predicting future experimental outcomes, designing additional experiments to test the model, and refining the model (and hence our theoretical understanding of the processes) as more data is collected.

Project 3. Biological Consequences of Heavy Metal Concentration in a Wetlands Spider. (M. Ramirez, B. Fitzpatrick)


Argiope trifasciata

The transfer of heavy metal pollutants from insects to spiders has long been known (e.g., Hopkin & Martin, 1985). However, much less is known about the effects of heavy metal concentrations on the natural history parameters of spiders, although such effects have been documented for many other invertebrates (e.g., Spurgeon *et al.,* 2000; Baatrup *et al.,* 2001). In the present study, we propose to document life-cycle traits, web characteristics, and metal loads of individual garden spiders (*Argiope trifasciata*) which reside at the Ballona Wetlands, a metal-contaminated salt marsh on the western edge of Los Angeles (Cohen *et al.*, 2001).

In a prior investigation (Ramirez *et al.,* 2003), *A. trifasciata* were found to be abundant in the dune area on the western edge of Ballona, so this will be a major area of activity in this study, although spiders from other parts of the system will be included if feasible. For any spider selected for monitoring, a support structure connected to its web (e.g. a branch) will be marked with colored tape and the web's GPS coordinates will be recorded. Web site fidelity in *A. trifasciata* is quite high, so once located, individual spiders will likely be findable throughout the study. *A. trifasciata* constructs a standard orb web, composed of a central hub, radii, sticky spirals, supporting frames, and sometimes, a vertical silken zigzag (stabilimentum) which functions as an insect attractant (Tso, 1996). Following Tso (1996) and Blackledge & Gillespie (2002), we will record the following web characteristics on the initial visit and daily thereafter: stabilimentum length; hub diameter; number of sticky spirals; radius of the upper and lower halves of the web; and horizontal and vertical web diameters. These values will be used to calculate mesh size (average distance between two consecutive sticky spirals); catching area (web area covered by sticky spirals); hub asymmetry (displacement of the hub from geometric web center); and web asymmetry (web area above the hub compared to that below the hub), using formulas presented in Tso (1996) and Blackledge & Gillespie (2002). Once a week, the resident spider will be temporarily removed for measurement [size (carapace width, mm), weight (mg)] to assess growth rate and overall body condition (Jakob *et al.,* 1996), prior to return to its web. Since *A. trifasciata* has an annual life-cycle, with spiderlings emerging from over-wintering egg sacs in spring and adulthood being attained from late summer to fall, all spiders in this study will be collected following their attainment of adulthood and stored at –85°C pending analysis of their metal content. Heavy metal levels (Cu^{+2}, Cd^{+2}, Cr^{+2}, Ni^{+2}, Pb^{+2}, Zn^{+2}) will be determined following published protocols for spiders (e.g., Laing *et al.*, 2002). Basic statistical analysis will focus on establishing the relationships between spider metal load, growth rate, body condition, and web parameters.



The advanced mathematical aspects of this problem are many, but of primary importance are statistical techniques for extracting information from our data. A great deal of work exists on quantifying the structure of spider webs, and many different measures of size, capture area, and asymmetry can be used to assess health. However, it has been observed that simple models based on a few measures (e.g., vertical radii-to-hub or ellipse-to-hub) become poor predictors of capture area in the presence of web asymmetry [see Blackledge & Gillespie (2002) for details]. With the aid of high-resolution digital photography equipment in the Mathematics Department's Computer Vision Lab, coupled with Scion Image and Matlab Image Processing software, we will capture more detailed pictures and measurements of spider webs in the field and in the lab. Beginning with hub radius and outer radius parameterized by angle, students will build and analyze a multivariate statistical model that will allow analysis of capture area to be determined more accurately from the higher dimensional measurement of the web.

A fascinating mathematical problem that relates to the statistics and web measurement problems is that of the effect of increasing resolution in the measurement and parameterization process. Bayesian statistical techniques provide a direct means to understand this process: one can construct prior distributions with respect to functional parameters, thus maintaining the structure of the inference independent of the resolution level of the measurement (e.g., Fitzpatrick 1991). The Bayesian approach to statistical analysis will allow us to examine the data we collect in new ways. Moreover, environmental variation can be included in a straightforward manner. Model building will take after the traditional response surface methodology (Box & Draper, 1987), but with Bayesian extensions to handle the high dimensional web shape data. The resulting model will be compared to additional data in order to validate the fit and explore predictive power. Overall, this study of the biological responses of a common spider to heavy metals will help us better understand the biomagnification of contaminants in the Ballona Wetlands and their impact on wildlife.

Project 4. Morphological asymmetry as an indicator of stress for plant and vertebrate populations in an urban coastal wetland.

(P. Drennan, W. Binder, E. Camacho, B. Fitzpatrick)

The issue of physical and developmental stability ("health") in relation to the environment is particularly important for species in environmentally impacted areas, exemplified by urban coastal wetlands such as Ballona in Los Angeles County. Abiotic stresses to organisms include organic and heavy metal pollutants associated with urban runoff, e.g., PCBs, lead, and zinc. Morphological and anatomical measures of organismal health are amenable to collection and analysis and allow for an interesting interface between ecology, functional anatomy, and mathematics. In the proposed project we will look at indicators of health by characterizing the patterns of morphological asymmetry in three plant and two animal species occurring in the wetlands and relating it to their heavy metal content.

Over the past 20 years, measures of bilateral asymmetry (deviation from perfect symmetry between the right and left side of an organism) have been used to measure developmental instability in a number of species of plants and animals. Numerous studies have found a significant association between asymmetry and developmental instability, some attributable to genetic effects and some due to environmental effects (Kark, 2001; Möller & Shykoff, 1999; Moller & Thornhill, 1998). Of particular interest is the observation that asymmetry may increase in the presence of heavy metal stress for both plants (Kozlov *et al.* , 1996) and animals (Marques *et al.*, 2005; Clarke, 1995). Asymmetry in *Lathrum salicaria* (purple loosestrife), an invasive species of wetlands, can possibly be used as an ecological indicator for heavy metal stress (Mal, *et al.*, 2002). Similarly, developmental instability in a riparian population of *Mus spretus* (Algerian mice) suggests this vertebrate provides a means of monitoring environmental levels of heavy metals (Nunes *et al.*, 2001).

However, while the use of asymmetry as an indicator of health is widespread, no single type of asymmetry or data analysis has been established as the most effective for such studies. Thus this area of study, which involves mathematical understanding and analysis that go well beyond simple statistical methods, is ideal for the *UBM* teams. Three types of bilateral asymmetry have been defined, including directional asymmetry, antisymmetry, and fluctuating asymmetry (Kark, 2001; Palmer and Strobeck, 1986; Van Valen, 1962)


Jaumea carnosa

We propose to look at all three types of asymmetries, and compare the presence and importance of each. In addition to the types of asymmetry, there are multiple methods of measuring relative differences in side asymmetry. Standard methods, historically applied to animals, include simply measuring the lengths of structures on either side of the body, but newer methods include looking at shapes of structures on either side of a body and using more complex shape analysis [such as procrustes methods (Klingenberg & McIntyre, 1998)] to compare them. This has the dual benefit of comparing a more traditional method with a newer methodology, and of introducing students to geometric morphometric analyses. For plants, asymmetry may be measured for the bilateral leaves; however, indeterminate growth and modular organization create different types of asymmetries (Freeman *et al.*, 1993) for which various methods of analysis may be developed. The analysis of asymmetry data has been recently criticized as statistically ineffective and newer Bayesian methods applied with some success (Van Dongen, 2001). We plan to use Bayesian statistical analysis, applying techniques closely related to those in the spider project, reinforcing the methods and concepts and showing their breadth of biological applicability.

Measurement of asymmetries will be conducted for three native plant species, each associated with habitats in the wetlands that are likely to receive water, and hence pollutant loading, from different sources. The extent of pollutant presence in the Ballona wetlands is currently being researched as part of project funded by Merck/AAAS grant. *Jaumea carnosa* occurs adjacent to tidal channels and is directly exposed to pollutants from surface storm water runoff. The sand-bar willow, *Salix exigua*, grows in areas of subterranean freshwater input, and *Rhus integrifolia* (lemonade berry) receives predominantly aerial input. Species in the genera *Salix* and *Rhus* show increased asymmetry under stress (Freeman *et al.*, 2004; Zvereva *et al.*, 1997) and *J. carnosa* exhibits morphometric plasticity in response to changes in soil conditions (Seliskar, 1985).

We will be measuring and analyzing asymmetries in two vertebrates; a local mammal, *Mus musculus* (house mouse), and a local reptile *Sceloporus occidentalis* (western fence lizard), each commonly found in Ballona (from a 2005 survey of Ballona wetlands). Using these groups is particularly interesting as it has been noted that reptiles have higher concentrations of heavy metals than mammals, and they may be able to tolerate these levels better than do mammals (Wren, 1986). Data such as body lengths and toe lengths are easy to obtain directly from trapped animals, while radiographs will allow for skull measures and more accurate limb measures. Our portable radiograph can be used onsite to minimize the stress on the animals and the resulting images can be scanned and analyzed computationally. Parameterizations of the shapes (and morphs) come from spline-based representations. Again, the Bayesian approach to inference permits multiple scales of measurement to be analyzed within the same basic framework, and the resulting models from the different resolutions are directly related through the prior and posterior distributions. Thus, the inference can be made robust to changes in the number of marker points in the shape measurement, and increasing the number of parameters does not necessarily make for an over fitting situation.

As an indicator of health associated with environmental contamination, the *in vivo* heavy metal (Cu^{+2}, Cd^{+2}, Cr^{+2}, Ni^{+2}, Pb^{+2}, Zn^{+2}) concentrations will be measured for all plant and animal species, using atomic absorption spectrophotometry. Plant leaves will be sampled for heavy metals. The use of hair samples for mammals (Rashed & Soltan, 2005) and blood for reptiles (Kenyon, *et al.*, 2001) will allow us to perform non-lethal monitoring of our animals. We will compare measures of heavy metal contamination with asymmetry results to determine if these pollutants correlate with levels of asymmetry, and thus a negative trend of developmental health.

References

Mathematical Analysis and Modeling (B. Fitzpatrick, E. Camacho)

Fitzpatrick, B. G. (1991) “Bayesian Analysis in Inverse Problems” by Ben G. Fitzpatrick, Inverse Problems, **7**: 675-702.

Banks, H. T., and B. Fitzpatrick (1990) “Estimation of Growth Rate Distributions in Size Structured Population Modeling”. Quart. of Applied Math., **49**: 215-235.

Fitzpatrick, B. (1993) “Modeling and Estimation Problems for Structured Heterogeneous Populations”. J. Math. Anal. Appl. **172**: 73-91.

Berger, J. O. (1993). Statistical Decision Theory and Bayesian Analysis, Springer-Verlag, New York.

Box, G. E. P., and N. Draper (1987) Empirical Model-Building and Response Surfaces Wiley, New York.

Beal, M. J., F. Falciani, Z. Ghahramani, C. Rangel, and D. Wild. (2005) A Bayesian Approach to Reconstructing Genetic Regulatory Networks with Hidden Factors. Bioinformatics **21**: 349-356.

Topic 1. The Functional Genomics of Adaptation to Heavy Metals in Wild Mustard Plants. (G. Kuleck, K. Dahlquist, B. Fitzpatrick)

Alba, R., Fei Z., Payton P., Liu Y., Moore, S L., Debbie, P., Cohn, J., D'Ascenzo, M., Gordon, J.S., Rose, J.K.C., Martin, G., Tanksley, S., Bouzayen, M., Jahn, M.M., and Giovannoni, J. (2004) ESTs, cDNA microarrays, and gene expression profiling: tools for dissecting plant physiology and development. The Plant Journal, **39**: 697-714.

Becher, M., Talke, I.N. , Krall, L., Kramer, U. (2004) Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator *Arabidopsis haleri*. The Plant Journal **37**: 251-268.

Gibson, G. (2002) Microarrays in ecology and evolution: a preview. Molecular Ecology **11**; 17-24.

Huala, E., Dickerman, A. W., Garcia-Hernandez, M., Weems, D., Reiser, L., LaFond, F., Hanley D., Kiphart, D., Zhuang, M., Huang, W., Mueller, L.A., Bhattacharyya, D., Bhaya, D., Sobral, B.W., Beavis, W., Meinke, D.W., Town, C.D., Somerville, C. and Rhee, S.Y. (2001) The *Arabidopsis* Information Resource (TAIR): a comprehensive database and web-based information retrieval, analysis, and visualization system for a model plant. Nucleic Acids Research **29**: 102-105.

Mitchell-Olds, T. (2001) *Arabidopsis thaliana* and its wild relatives: a model system for ecology and evolution. Trends in Ecology and Evolution **16**: 693-700.

Rensink, W.A, and Buell, C.R. (2005) Microarray expression profiling resources for plant genomics. Trends in Plant Science **10**; 23-30.

Zhou, X.J., and Gibson, G. (2004) Cross-species comparison of genome-wide expression patterns. Genome Biology 2004 **5**: A232.1-5.

Topic 2. Modeling Gene Expression Networks in Saccharomyces cerevisiae. (K. Dahlquist, E. Camacho)

Causton, H.C., Ren, B., Koh, S.S., Harbison, C.T., Kanin, E., Jennings, E.G., Lee, T.I., True, H.L., Lander, E.S., and Young, R.A. (2001) Remodeling of Yeast Genome Expression in Response to Environmental Changes. Molecular Biology of the Cell **12**: 323–337.

Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., Storz, G., Botstein, D., and Brown, P.O. (2000) Genomic Expression Programs in the Response of Yeast Cells to Environmental Changes. Molecular Biology of the Cell **11**: 4241–4257.

Griffin, T.J., Gygi, S.P., Ideker, T., Rist, B., Eng, J, Hood, L., and Aebersold, R. (2002) Complementary Profiling of Gene Expression at the Transcriptome and Proteome Levels in *Saccharomyces cerevisiae*. Molecular and Cellular Proteomics **1**: 323–333.

Lee, T.I., Rinaldi, N.J., Robert, F., Odom, D.T., Bar-Joseph, Z., Gerber, G.K., Hannett, N.M., Harbison, C.T., Thompson, C.M., Simon, I., Zeitlinger, J., Jennings, E.G., Murray, H.L., Gordon, D.B., Ren, B., Wyrick, J.J., Tagne, J.B., Volkert, T.L., Fraenkel, E., Gifford, D.K., Young, R.A. (2001) Transcriptional regulatory networks in *Saccharomyces cerevisiae*. Science **298**: 799-804.

Shea, M.A., Ackers, G.K. (1985) The OR control system of bacteriophage lambda. A physical-chemical model for gene regulation. J Mol Biol. **181**: 211-30.

Stelling, J. Mathematical models in microbial systems biology. (2004) Curr. Opin. Microbiol. **7**: 513-518.

Wang W., Cherry J.M., Nochomovitz Y., Jolly, E., Botstein, D., Li, H. (2005) Inference of combinatorial regulation in yeast transcriptional networks: a case study of sporulation. Proc. Natl. Acad. Sci. U.S.A. **102**:1998-2003.

Saccharomyces Genome Deletion Project http://sequence-www.stanford.edu/group/yeast_deletion_project/

Genome Consortium for Active Teaching (GCAT). http://www.bio.davidson.edu/projects/gcat/gcat.html

Topic 3. Biological Consequences of Heavy Metal Concentration in a Wetlands Spider. (M. Ramirez, B. Fitzpatrick)

Baatrup, E., M. Bayley, F. F. Sørensen and Toft, G. (2001) Can animal behaviour predict population level effects? In: Forecasting the Environmental Fate and Effects of Chemicals (P. S. Rainbow, S. P. Hopkin & M. Crane, eds.). John Wiley & Sons Ltd., New York. Pp. 139-150.

Blackledge, T. A. & R. G. Gillespie. (2002). Estimation of capture areas of spider orb webs in relation to asymmetry. Journal of Arachnology **30**: 70-77.

Cohen, T., Que Hee, S. S., and Ambrose R. F. (2001) Trace metals in fish and invertebrates of three California coastal wetlands. Marine Pollution Bulletin **42**: 224-232.

Hopkin, S. P. and Martin, M. H. (1985) Assimilation of zinc, cadmium, lead, copper, and iron by the spider *Dysdera crocata*, a predator of woodlice. Bulletin of Environmental Contamination and Toxicology **34**: 183-187.

Jakob, E. M., S. D. Marshall & Uetz, G. W. (1996) Estimating fitness: A comparison of body condition indices. Oikos **77**: 61-67.

Laing, G. D., Bogaert, N., Tack, F. M. G., Verloo, M. G. & Hendrickx, F. (2002) Heavy metal contents (Cd^{+2}, Cu^{+2}, Zn^{+2}) in spiders (*Pirata piraticus*) living in intertidal sediments of the river Scheldt estuary (Belgium) as affected by substrate characteristics. The Science of the Total Environment **289**: 71-81.

Ramirez, M. G., Wall, E. A. and Medina, M. (2003) Web orientation of the banded garden spider *Argiope trifasciata (Araneae, Araneidae*) in a California coastal population. Journal of Arachnology **31**: 405-411.

Spurgeon, D. J., Svendsen, C., Rimmer, V. R., Hopkin, S. P. and Weeks, J. M. (2000) Relative sensitivity of life-cycle and biomarker responses in four earthworm species exposed to zinc. Environmental Toxicology and Chemistry **19**: 1800-1808.

Tso, I.-M. (1996) Stabilimentum of the garden spider *Argiope trifasciata*: A possible prey attractant. Animal Behaviour **52**: 183-191.

Topic 4. Morphological asymmetry as an indicator of stress for plant and vertebrate populations in an urban coastal wetland. (P. Drennan, W. Binder, B. Fitzpatrick. E. Camacho)

Clarke, Geoffrey M. (1995) Relationships between developmental stability and fitness: Application for conservation biology. Conservation Biology, **9**: 18-24.

Freeman, D.C., Graham, J.H., and Emlen, J.M. (1993) Developmental stability in plants: Symmetries, stress and epigenesist. Genetica **89**: 97-119.

Freeman, D.C., Brown, M.L., Duda, J.J., Graham, J.H., Emlen, J.M., Krzysik, A.J., Balbach, H., and Kovacic, D.A. (2004) Developmental instability in *Rhus copallinum* L.: Multiple stressors, years, and responses. International Journal of Plant Sciences **165**: 53-63.

Kark, S. (2001) shifts in bilateral asymmetry within a distribution range: the case of the Chukar partridge. Evolution **55**: 2088-2096.

Kenyon, L.O., Landry, A.M., and Gill, G.A. (2001) Trace metl concentrations in blood of the Kemp’s Ridley sea turtle (*Lepidochelys kempii*). Chelonian Conservation and Biology. **4**: 128-135.

Klingenberg, C. P. and McIntyre, G S. (1998) Geometric morphometrics of developmental instability: Analyzing patterns of fluctuating asymmetry with procrustes methods. Evolution **52**: 1363-1375.

Kozlov, M.V., Wilsey, B.J., Koricheva, J., and Haukioja, E. (1996) Fluctuating asymmetry of birch leaves increases under pollution impact. Journal of Applied Ecology **33**: 1489-1495.

Mal, T.K., Uveges, J.L., and Turk, K.W. (2002) Fluctuating asymmetry asan indicator of heavy metal stress in Lythrum salicaria. Ecological Indicators **1**: 189-195.

Marques, J.F., Costa, J.L., and Cabral, H.N. (2005) Variation in bilateral asymmetry of the Lusitanian toadfish along the Portugese coast. Journal of Applied Icthyology **21**: 205-209.

Möller, A.P., and Shykoff, J.A. (1999) Morphological developmental stability in plants: patterns and causes. International Journal of Plant Sciences **160**: S135-S146.

Møller, A.P., and Thornhill, R. (1998) Bilateral symmetry and sexual selection: a meta-analysis. American Naturalist **151**: 174-192.

Nunes, A.C., Auffray, J-C, and Mathias, M.L. (2001) Developmental instability in a riparian population of the Algerian mouse (*Mus spretus*) associated with a heavy metal-polluted area in central Portugal. Archives of Environmental Contamination and Toxicology **41**: 515-521.

Palmer, A. R., and C. Strobeck. (1986) Fluctuating asymmetry: measurement, analysis, patterns. Annu. Rev. Ecol. Syst. **17**: 391–421.

Rashed, M.N. and Soltan, M.E. (2005) Animal hair as biological indicator for heavy metal pollution in urban and rural areas. Environmental Monitoring and Assessment, **110:** 41–53.

Seliskar, D.M. (1985) Effect of reciprocal transplanting between extremes of plant zones on morphometric plasticity of five plant species in an Oregon USA salt marsh. Canadian Journal of Botany **63**: 2254-2262.

Van Dongen, S. (2001) Modeling developmental instability in relation to individual fitness: A fully Bayesian latent variable model approach. Journal of Evolutionary Biology **14**: 552-563.

Van Valen, L. (1962) A study of fluctuating asymmetry. Evolution **16**: 125–142.

Wren, C. (1986) Mammals as biological monitors of environmental metal levels. Environ. Monitoring and Assessment **6**: 127-144.

Zvereva, E., Kozlov, M.V., and Haukioja, E. (1997) Stress responses of *Salix borealis* to pollution and defoliation. Journal of Applied Ecology **34**: 1387-1396.