södertörns
högskola

Dr Yves Brun
Systems Biology/Microbiology Faculty Search
Department of Biology
Indiana University
Jordan Hall 142
1001 E 3rd Street
Bloomington, IN 47405-7005 U.S.A.

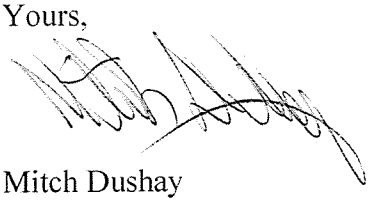
9 September, 2005

Dear Dr Brun

This is my application for the Assistant Professorship in Systems Biology at IU. My laboratory works on innate immunity and the nuclear lamina in the fruit fly, *Drosophila melanogaster*. Coagulation is a first response to injury and is important for preventing infection as well as for hemostasis and wound healing. Our work on the clot and its function incorporates genomics and proteomics, blood cell differentiation, cell signaling, and gene regulation, and forces a new, whole system view of immunity in the fly, and of innate immunity in general. Our work on lamins is at an earlier stage, but we have confirmed and extended published observations that *lam* mutant adults can't fly, walk unsteadily, and are unable to right themselves after flipping over. These phenotypes suggest muscle defects, but they are also similar to old wild type flies. This puzzle is interesting because a form of muscular dystrophy and progeria are both caused by mutations in the human lamin A gene. Learning how changes in nuclear lamin proteins in different cells causes these phenotypes will improve our understanding of the nuclear lamina's functions in gene regulation and its roles in development and senescence.

At Södertörn I have attracted students and built a lab group, developed projects, gotten grant support, and been promoted to docent. In other circumstances I would be hired permanently, but a Swedish government move to downsize Södertörn forces me to leave. IU is known to Drosophilists world over as the home of the Bloomington Stock Center of course. I have also lived in IN, and would look forward to coming back. I am enclosing my CV, statements of research and teaching interests, and I have asked that letters of recommendation be sent. Please contact me if I can provide any additional information.

Yours,



Mitch Dushay

Institute for Insect and Innate Immunity, Department of Life Sciences
Visitng address:
Södertörns högskola
Alfred Nobels allé 7
141 89 Huddinge

Delivery adress:
Varumottagningen
Afred Nobels allé 3
141 52 Huddinge

Invoice adress:
Ekonomiservice
Box 4102
141 04 Huddinge

Organisationsnummer:
SE202100489601
Telefon: 08-6084000
Fax: 08-6084010

Summary of Past Research

After my doctoral training with Jeff Hall in behavior genetics of circadian rhythms, I've worked on different aspects of innate immunity in insects. I studied developmental effects in the fascinating three-way interaction between parasitoid wasp, associated polydnavirus, and caterpillar host (Dushay and Beckage 1993). Then I returned to *Drosophila* and discovered *Relish*, a central regulatory protein in *Drosophila* immunity in Dan Hultmark's lab at Stockholm University (Dushay et al. 1996). I continued working on the humoral response at the University of Notre Dame (Dushay and Eldon 1998; Dushay et al. 2000). From there I went to the University of Maryland to learn mosquito transformation and study the spreading behavior of the *Hermes* transposable element. Then I shifted focus to coagulation; one of the first responses to injury and infection. Because the clot is an insoluble matrix that forms so rapidly, and *Drosophila* larvae are so small, study of clotting has lagged behind work on humoral responses. It took years, but my laboratory and Dr. Uli Theopold's developed methods to study clotting (Karlsson et al. 2004; Scherfer et al. 2004) and identified a salivary glue protein as a key clot constituent (Korayem et al. 2004). We described the appearance and properties of the *Drosophila* clot and demonstrated that while primary soft clot formation in *Drosophila* does not require phenoloxidase, this enzyme later crosslinks the clot to produce a stronger, more brittle matrix (Bidla et al. 2005).

I became interested in lamins as a potential way to study the development of immune tissues and regulation of immune genes in the fly. Lamin proteins form a matrix - the nuclear lamina - that underlies the nuclear membrane and gives the nucleus shape. Chromosomal attachment to the lamina is necessary for proper chromosomal function. Mammalian studies suggest that the lamina also affects cell differentiation and gene expression, and I thought if this is true in *Drosophila*, it could provide a new means of studying immune development in the fly. We began by characterizing in detail the phenotypes of *lam* mutants. *lam* mutations are lethal, but some adults survive a few days. These adults walk unsteadily, and cannot fly or turn over, and mutant larvae also do not crawl as high to pupate. These behavioral phenotypes could be similar to muscular dystrophy (some forms of which are caused by mutations in a human lamin gene), or to accelerated aging (Huntington-Guilford Progeria Syndrome is also caused by a mutation in a human lamin gene (Eriksson et al. 2003)).

Present work

• *Drosophila* clot - We continue our study of coagulation *Drosophila* larvae with functional genomic analyses of the roles of selected candidate clotting factors. Having demonstrated that

the *Drosophila* clot binds bacteria (Korayem et al. 2004; Bidla et al. 2005), we are testing the relative importance of coagulation and humoral immunity in larval resistance to infection. We are also studying the regulation of phenoloxidase activity in the clot, and we have submitted a manuscript describing the rapid local activation of this enzyme, independent of infection and of the major known transcriptional activation pathways, and the involvement of apoptosis.

• *Anopheles* clot - After *Drosophila*, we can begin to study coagulation in other insects. The mosquito *Anopheles gambiae* is a first choice for several reasons. i) *Anopheles* is distantly related to *Drosophila,* separated by about 250 million years of evolution. ii) Mosquito larvae are aquatic, while *Drosophila* larvae live on rotting fruit. This imposes different physiochemical constraints on coagulation. iii) *Anopheles gambiae* is the most important vector for the transmission of malaria, making its immune system the subject of world-wide study. Its genome has been published, making it possible to use proteomics to analyze coagulation in *Anopheles* as we have done for *Drosophila*. Early this year I obtained a short-term EMBO fellowship to apply the techniques we've developed in *Drosophila* to the study of coagulation in *Anopheles* larvae in the Kafatos laboratory at EMBL Heidelberg. My preliminary results show that although *Anopheles gambiae* larvae recover from injury, seal wounds, and produce melanotic scars like *Drosophila*, coagulation appears very different in these two species.

• Lamins - We will soon finish a manuscript describing the behavioral and histological phenotypes of *Drosophila lam* mutant adults and larvae. We have generated flies bearing cDNA constructs of *Drosophila lam*, and both wild type and progerid forms of lamin A (collaborating with Dr Maria Eriksson). To our surprise, broad overexpression of all of these constructs is lethal to flies. We are studying this by driving expression in specific tissues, and we will also generate somatic null *lam* clones to test the focus of *lam* mutation pathology. For example, we will generate flies that lack *lam* genes in muscles but are otherwise normal to study how *lam* mutations cause the behavior defects we've observed and to learn more about lamin function.

Future plans

• Coagulation - We will continue characterizing the candidate clotting factors we've isolated. Experiments on the roles of these factors will greatly improve our understanding of coagulation and the clot's function in wound sealing, healing, and immune defense. We will also continue our work on phenoloxidase activation and apoptosis of hemocytes. A goal is to learn about its initiation; whether oxygen pressure, fluid sheer forces, or factors released by tissue damage are critical for clotting. Study of the *Anopheles* clot in turn will show how coagulation evolved

differently in different environments and may lead to new approaches to control insect pests and human disease transmission.

• Lamins - At the heart of this project are questions about how changes in the nuclear lamina affect cell function and differentially affect tissues, and what is the lamina's role in development, cellular differentiation, and aging/senescence. The results of our experiments on the cellular focus for *lam* mutation effects and critical developmental periods for gene action will address whether changes in the lamina affect differentiation or postmitotic cell function. A yeast two-hybrid project will identify candidate lamin-binding proteins, and we will confirm these and assay the importance of these interactions in the fly. Functional genomics will further clarify these processes in epigenetic gene control and cellular function. It is my hope that this will lead back to the development of immune tissues and complete the circle.

References

Bidla, G., M. P. M. Lindgren, et al. (2005). "Hemolymph coagulation in insect larvae and the role of phenoloxidase in *Drosophila.*" Developmental and Comparative Immunology **29**: 669 - 679.

Dushay, M. S. and N. E. Beckage (1993). "Dose-dependent separation of *Cotesia congregata*-associated polydnavirus effects on *Manduca sexta* larval development and immunity." Journal of Insect Physiology **39**: 1029 - 1040.

Dushay, M. S. and E. D. Eldon (1998). "*Drosophila* immune responses as models for Human immunity." American Journal of Human Genetics **62**: 10 - 14.

Dushay, M. S., J. B. Roethele, et al. (2000). "Two *attacin* antibacterial genes of *Drosophila melanogaster.*" Gene **246**: 49 - 57.

Dushay, M. S., B. Åsling, et al. (1996). "Origins of immunity: *Relish*, a compound Rel-like gene in the antibacterial defense of *Drosophila.*" Proceedings of the National Academy of Sciences USA **93**: 10343 - 10347.

Eriksson, M., W. Brown, et al. (2003). "Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome." Nature **423**: 293 - 298.

Karlsson, C., A. M. Korayem, et al. (2004). "Proteomic analysis of the *Drosphila* larval hemolymph clot." Journal of Biological Chemistry **279**: 52033 - 52041.

Korayem, A., M. Fabbri, et al. (2004). "A glue-like *Drosophila* salivary protein is expressed in hemocytes: evidence for a function in hemolymph coagulation." Insect Biochemistry and Molecular Biology **34**: 1297 - 1304.

Scherfer, C., C. Karlsson, et al. (2004). "Isolation and characterization of hemolymph clotting factors in *Drosophila melanogaster* by a novel pull-out assay." Current Biology **14**: 625 - 629.

Teaching

I have extensive teaching experience at both graduate and undergraduate levels. I gave lectures in the advanced Molecular Biology course at Stockholm University when I was a postdoctoral fellow, and at the University of Notre Dame I designed and taught an undergraduate course in behavior genetics for nonbiology majors. Here at Södertörn, I've given lectures in undergraduate genetics, development, molecular biology, and immunology courses. I also designed and was responsible for a upper level course in gene expression (Cell Biology/ Molecular Biology). The extent and quality of my teaching were recognized when I was promoted to docent. I am comfortable teaching a range of subjects to graduate or undergraduate students, alone or as a part of a team. I would also be interested in leading an advanced seminar in nuclear structure/function, innate immunity, or a more specialized *Drosophila* topic.