Hunt Institute for Botanical Documentation
5th Floor, Hunt Library
Carnegie Mellon University
4909 Frew Street
Pittsburgh, PA 15213-3890
Telephone: 412-268-2434
Email: huntinst@andrew.cmu.edu
Web site: www.huntbotanical.org

The Hunt Institute is committed to making its collections accessible for research. We are pleased to offer this digitized item.

Usage guidelines
We have provided this low-resolution, digitized version for research purposes. To inquire about publishing any images from this item, please contact the Institute.

Statement on harmful and offensive content
The Hunt Institute Archives contains hundreds of thousands of pages of historical content, writing and images, created by thousands of individuals connected to the botanical sciences. Due to the wide range of time and social context in which these materials were created, some of the collections contain material that reflect outdated, biased, offensive and possibly violent views, opinions and actions. The Hunt Institute for Botanical Documentation does not endorse the views expressed in these materials, which are inconsistent with our dedication to creating an inclusive, accessible and anti-discriminatory research environment. Archival records are historical documents, and the Hunt Institute keeps such records unaltered to maintain their integrity and to foster accountability for the actions and views of the collections' creators.

Many of the historical collections in the Hunt Institute Archives contain personal correspondence, notes, recollections and opinions, which may contain language, ideas or stereotypes that are offensive or harmful to others. These collections are maintained as records of the individuals involved and do not reflect the views or values of the Hunt Institute for Botanical Documentation or those of Carnegie Mellon University.

About the Institute
The Hunt Institute for Botanical Documentation, a research division of Carnegie Mellon University, specializes in the history of botany and all aspects of plant science and serves the international scientific community through research and documentation. To this end, the Institute acquires and maintains authoritative collections of books, plant images, manuscripts, portraits and data files, and provides publications and other modes of information service. The Institute meets the reference needs of botanists, biologists, historians, conservationists, librarians, bibliographers and the public at large, especially those concerned with any aspect of the North American flora.

Hunt Institute was dedicated in 1961 as the Rachel McMasters Miller Hunt Botanical Library, an international center for bibliographical research and service in the interests of botany and horticulture, as well as a center for the study of all aspects of the history of the plant sciences. By 1971 the Library's activities had so diversified that the name was changed to Hunt Institute for Botanical Documentation. Growth in collections and research projects led to the establishment of four programmatic departments: Archives, Art, Bibliography and the Library.

April 21, 1980

Dear Dave,

Thanks for your note which came a while ago. I (we) enjoyed having you here and certainly agree that the sense of forward direction on the book is the best ever. In fact I've kept at it and am now getting most of the revisions and additions in shape for my Section II chapter. You should have it by May 1 allright. It's taken me longer than I'd hoped but I've learned a few things and feel it's improved.

I thought the job hunting season was about closed but one more has come up and I must request another letter. I learned on Friday of an opening, which may not be filled yet, in the Math. Dept. at Smith College. I talked with the chairman by phone and he seems quite interested in someone with applied math. and interdisciplinary research interests. They want some one to teach statistics and introductory math. courses as well.

Since this is a Math. rather than a Biology department, the second paragraph in your letter to Barnard should probably be simply ommitted. The rest seems to me to be ok. The letter should go to:

4/28/80 ✓

Dr. James Callahan, Chairman
Dept. of Mathematics
105 McConnell, Smith College
Northampton, MA 01063

Thanks again, Dave, for your help on these things as well as many kindnesses in the past. I'll keep you posted.

All the best,

Lois

Letter for Dr. Lois Abbott to: Dr. James Callahan, Chairman (address attached)

I write to enthusiastically support Dr. Lois Abbott's application for a position ~~it~~ to teach statistics and introductory math courses. She is eminently well qualified for the position, both in mathematical and personal qualities.

SKIP THE SECOND PARAGRAPH

Then continue ~~it~~ with the 3rd paragraph of the copy of the letter attached.

DJR/ABBOTT
Letters of recommendation dated 18 March 1980:

Dr. Robert J. Gehrig
Acting Chairman, Biology Department
Russell Sage College
Troy, New York 12180

Dr. W. Kundig, Chairperson
Department of Biology
Hood College
Frederick, Maryland 21701

Dr. Marcia Ontell
Department of Anatomy and Cell Biology
University of Pittsburgh
School of Medicine
3550 Terrace St.
Pittsburgh, Pennsylvania 15261

Mar. 12, 1980

Dear Dave -

I've decided it would be a good idea for you to send your letter of reference to Russell Sage at this time. (They have all of your test that went to Math. Dept. a couple of summers ago.)

Anyhow, if you please, to:

a position at Russell Sage

Dr. Robert J. Gehrig
Acting Chmn., Biology Dept.
Russell Sage College
Troy, N.Y. 12180

Thanks & look forward to seeing you soon!

Lee

March 4, 1980

Dear Dave,

Thanks for your letter. We look forward to seeing you on the 22nd and will meet you at t he Albany Airport at 6PM! Frank will get in some time that night also. We can read each other's stuff that day and perhaps socialize a bit in the evening. Then Monday morning we should be all set to go at it hot and heavy. (I've spent the day reading two books today related to my math models chapter and think I see how to revamp and rewrite it rather thoroughly.)

Your letter to Barnard was very good and most generous and I thank you for it . The only change I can suggest is that the third sentence in the 2nd paragraph be changed to something like "Following her thesis work in modelling development of Drosophila imaginal wing discs, she learned electron microscopy and is applying it to the biological aspects of Drosophila wing growth and development." This is a moreaccurate statement of the chronology and can then be followed by your next statement.

I have heard no more so far from Barnard, so far. There is a possibility which may open up at Sage and I am following that one but there is nothing you need do just yet. I have decided to apply at two other places although they are a little far; they would require adjustments which I would be willing to make if they should prove to be interesting. I would appreciate your sending letters to:

✓Dr. W. Kundig, Chairperson
Dept. of Biology, Hood College
Frederick, Md. 21701 (By Mar. 15, 1980)

teaching position at Hood,

and

✓Dr. Marcia Ontell
Dept. of Anatomy and Cell Biology, Univ. of Pittsburgh School of Medicine
3550 Terrace St., Pittsburgh, PA 15261

post doctoral at Pittsburgh

The one at Hood is a teaching job in developmental biolpgy and genetics which are probably my best biological areas. Pittsburgh is the ideal post-doc since it combines electron microscopy and computer analysis on a problem in muscle development! (The ad was in Science of Feb. 1, 1980 as was the Hood one.)

See you soon,

Lois

14 February 1980

Dr. Patricia L. Dudley, Chairman
Department of Biological Sciences
Barnard College
Columbia University, New York 10027

Dear Dr. Dudley:

I write to enthusiastically support Dr. Lois Abbott's application for a position as Assistant Professor in embryology at Barnard. She is eminently well qualified for the position you describe, both in scientific and personal qualities.

Dr. Abbott, who did her Ph.D. work with me, is a biologist with several areas of knowledge and interest: cellular, developmental, theoretical and mathematical. In general, her skills are in the holistic framework rather than in the reductionist mode. [In the course of her thesis work, which was modelling of the developmental genetics in Drosophila, she used electron microscopy with outstanding results.] From this work, I am confident that she can fulfill the requirements you have.

[change this sentence.) over

Lois is an accomplished teacher with considerable experience in undergraduate and graduate teaching here at the University of Colorado, and more recently, at Russel Sage. She has a masterful ability to combine concepts of mathematics and biology, and is able to communicate her ideas and concepts clearly and with enthusiasm. She works well at various levels--in seminars, and in small, or medium-sized classes. I did not have an opportunity to observe her performance before large lecture sessions.

Lois works well with others in a variety of settings. While here, she worked with several professors on a professional level, both in biology and in mathematics. When working with the mathematicians, she frequently acted as the biological specialist, and with the biologists, as the mathematical specialist. Several papers, either published or in press, attest to these statements.

Dr. Abbott was one of the best students I have ever had. She has great width and depth of interest and knowledge in biology as well as in music. She also participates in extracurricular activities that contribute to the community. Her personality is exceptional: confident, but not

Insert this sentence in indicated place in 2nd paragraph.

Following her thesis work in modelling development of *Drosophila* imaginal wing discs, she learned electron microscopy and is applying it to the biological aspects of *Drosophila* wing growth and development.

aggressive, pleasant but not cloying. She is one of the most vigorous persons I have known, with the ability to keep many different activities going simultaneously, without seeming to be hurried or harried.

I am confident that Dr. Abbott would be an ideal faculty member at Barnard College. I recommend her at the highest level.

Sincerely yours,

David J. Rogers
Professor of Biology

DJR/pch

27 November 1979

Dr. Kathryn Eschenberg
Department of Biological Sciences
Mt. Holyoke College
South Hadley, Massachusetts 01075

Dear Dr. Eschenberg:

start 2nd Para to Dudley here

I write to support the application of Dr. Lois Abbott who has applied for the position in your Department to teach electron microscopy and other subjects of mutual interest. Dr. Abbott, who did her Ph.D. with me, is a biologist with several areas of knowledge and interest: cellular, developmental, theoretical and mathematical. In general, her skills are in the holistic framework rather than in the reductionist mode. In the course of her thesis work, which was modelling of the developmental genetics in Drosophila, she used electron microscopy with outstanding results. From this work, I am confident that she can fulfill the requirements you have.

Lois is an accomplished teacher with considerable experience in undergraduate and graduate teaching here at the University of Colorado, and more recently, at Russel Sage. She has a masterful ability to combine concepts of mathematics and biology, and is able to communicate her ideas and concepts clearly and with enthusiasm. She works well at various levels--in seminars, and in small, or medium-sized classes. I did not have an opportunity to observe her performance before large lecture sessions.

Lois works well with others in a variety of settings. While here, she worked with several professors on a professional level, both in biology and in mathematics. When working with the mathematicians, she frequently acted as the biological specialist, and with the biologists, as the mathematical specialist. Several papers, either published or in press, attest to these statements.

Dr. Abbott was one of the best students I have ever had. She has great width and depth of interest and knowledge in biology as well as in music. She also participates in extracurricular activities that contribute to the community. Her personality is exceptional: confident, but not aggressive, pleasant but not cloying. She is one

of the most vigorous persons I have known, with the ability to keep many different activities going simultaneously, without seeming to be hurried or harried.

I am confident that Dr. Abbott would be an ideal faculty member at Mt. Holyoke. I recommend her at the highest level.

Columbia University
Barnard College

Sincerely yours,

David J. Rogers
Professor of Biology

DJR/pch

Barnard College

DEPARTMENT OF BIOLOGICAL SCIENCES

COLUMBIA UNIVERSITY, NEW YORK 10027

30 January 1980

Dr. David Rogers
Department of Biology
Hale Hall
Boulder, Colorado 80302

Dear Dr. Rogers,

Dr. Lois Abbott has recently applied for a position as Assistant Professor in embryology at Barnard College and has given us your name as a referee.

We would greatly appreciate a letter of reference concerning her at your earliest convenience. We are particularly interested in her potential as a teacher-scholar functioning in a relatively small undergraduate college.

Thank you very much.

Sincerely,

Patricia L. Dudley

Patricia L. Dudley
Chairman
Professor of Biological Sciences

PLD:ark

Dr. Edward Flaccus, Secretary
Science Division
Bennington College
Bennington, Vt. 05201

Fill as copies of outgoing letters -

Dear Dr. Flaccus:

I write to support the application of Dr. Lois Abbott, who has applied for the position you announced in Science. Dr. Abbott, who did her PhD under my direction, is a biologist with several areas of knowledge and interest: cellular, developmental, theoretical and mathematical. In general, her skills are in the organismic xxxxxxxx; xxxxxxxxxxxxxxx and holistic framework rather than in the reductionist philosophy. She is accomplished as a teacher, with considerable experience in undergraduate and graduate teaching here at the University of Colorado, and as an inovative researcher. She has a masterful ability/ixxcombine to concepts of mathematics and biology, and is supplied with an abundant capability to communicate her ideas in different contexts--formally in a classroom, in seminars, and on an individual basis, mxxxxxxxxxxxxxxxxxxxxx/such as tutorials.

Dr. Abbott is one of the best all-around students I ever had. I know that she has great musical ability, and that she participates in other valuable extracurricular activities that contribute to the community. Her personality is exceptional--not overly distant, nor too friendly, but a proper x level to make people feal at ease with her. She does not overpower one with her intellect, but she is as intellectual as anyone I know.

During her tenure here, she cooperated with several professors in mathematicax and biology, on a level of professionalism seldom matched in students. She also participated in work at other institutions, such as the University of California at Irvine, in one of the country's leading developmental biology laboratories.

It is my personal feeling that Dr. Abbott would be an ideal faculty member at Bennington. I know that she will be a great contributor to your program.

UNIVERSITY OF COLORADO
AT
BOULDER, COLORADO 80309

Department of Environmental,
Population and Organismic Biology

Sept. 9, 1977

Dr. K. S. Kinerson
Russel Sage College
145 Ferry St.
Troy, N.Y. 12308

Dear Dr. Kinerson:

Dr. Lois Abbott has asked me to write a letter for her, and I am pleased to do so. Lois did her Ph.D. under my direction (although she really had 3 advisors, all with equal input) and I must say that she was the best student I have ever had. She feels as I do, that biologists are woefully short in their understanding of applied math, and that there is a great need to teach math to biologists at all levels, really from high school onwards. I feel that I have had some success in working with mathematical colleagues in the process of developing some very powerful analytic and synthetic methods because of our cooperation. Dr. Abbott falls into the same mold. Her skills mathematically were sufficiently great that she worked as a professional colleague with members of our math department, rather than as a strict student-teacher relationship.

Dr. Abbott has sufficient knowledge and skills that I asked her to teach my course in multivariate methods for biologists during my leave of absence. She did an outstanding job, and the students were very appreciative of her teaching. She has also assisted in and taught our course in biometrics. It is thus obvious that Lois is an applied mathematician, and would, therefore, be admirably suited to teaching statistics.

I must say that her thesis was one of the most inovative that I have ever had the pleasure to direct (although I really did not have to do any directing -- merely making suggestions). Her biology was really new research, not as so many present-day Ph.D. theses, an extension of the professor's work; and the application of mathematics in the process also produced some new concepts. She worked in one of the most complex areas of biology -- developmental genetics, and she improved on mathematical models initially devised by Lindenmayer that have great power for fundamental improvements in our knowledge of organismic development. The introduction to her thesis will be required reading for all my students.

I am, therefore, pleased, and honored to recommend Dr. Lois Abbott for work in your department. She will, I know, be very successful.

Sincerely yours,

David J. Rogers
Professor of Biology

DJR:eo

Nov. 4, 1977

Dear Dave,

It was good to talk with you last night though too bad about Frank's schedule problems. However, we should be able to get things in good shape by then and perhaps be ready to do final editing and putting together for submission. I'll be sending you my first chapter next week and perhaps at Xmas we can figure out some sample chapters.

The enclosed is the copy of the RPI announcement that I mentioned to you. I am really more of a ~~dxx~~ geneticist than a developmental biologist which is what they think they want. However, I have had a good course in embryology in the first year of my graduate work (1971) and of course my research in insect development. What I think we should try to convince them is that my mathematical approaches to developmental problems are particularly relevant in a technical institute such as RPI. In other words, I think my background in this area is sufficient to teach it at the undergraduate level (with a little work which I am willing and interested in doing in this field) and that my research interests though not standard could be quite appropriate. Further, I think some statement from you regarding my ability to ~~xxx~~ work independently would be supportive.

All the best to you
and Connie,

Lars

spaces and characters each). Ads over 25 millimeters billed at $5.80 per millimeter. No charge for use of box number. Rates net. No agency commission for ads under 4 inches. No cash discount. Prepayment required for foreign advertising. Purchase orders and billing information required for all other ads.

Advertising closing is each Wednesday, 3½ weeks before Friday issue date requested. Send copy for all positions wanted ads and display ads under 5 inches to:

SCIENCE, Room 207
1515 Massachusetts Ave., NW
Washington, D.C. 20005
Telephone: 202-467-4456

Send copy for display ads, 1/6 page and larger, to:

Scherago Associates, Inc.
11 West 42nd Street
New York, N.Y. 10036
Telephone: 212-736-1858

Blind Ad replies should be addressed as follows:

Box (give number)
SCIENCE
1515 Massachusetts Ave., NW
Washington, D.C. 20005

POSITIONS WANTED

Biochemist: Ph.D., seeks faculty/research/administrative position. See Jourdikian ad in the 12 August 1977 issue of SCIENCE. 9/30; 10/7

Biomedical Communications. Periodicals editor seeks supervisory position in medical center, government, or industry. Experienced in organizing symposia for publication. Northeast preferred. Box 356, SCIENCE. X

EM Technician: 10 years of experience in biomedical TEM and 6 years in SEM; 5 years as lab supervisor. Write: L. R. Melsen, 2431 Gawain Drive, Birmingham, Alabama 35226. X

Environmental/Marine Chemist (M.S. in inorganic, 1973). Three years plus of experience in marine pollution work, flameless AA, nutrients, metal speciation; shipboard experience; computers; publications; bilingual. Seeks academic or government position. Box 357, SCIENCE. X

Neuropharmacologist/Electrophysiologist requires position in pharmacology, physiology, biology, zoology, or a marine station for teaching and research at the assistant professor level, or a pharmaceutical industry position. Box 358, SCIENCE. X

POSITIONS OPEN

ASSISTANT PROFESSOR IN DEVELOPMENTAL/CELLULAR BIOLOGY. The Biology Department of Rensselaer Polytechnic Institute is seeking applicants for a position as assistant professor to begin September 1978. Candidates must have a doctoral degree and background appropriate to teaching assignments which will include participation in undergraduate courses in general biology and embryology, and an advanced undergraduate or graduate course in cellular-level developmental biology. The successful applicant will be expected to vigorously pursue an independent program of research. Send curriculum vitae, current reprints, and three letters of reference to:

Dr. Joyce J. Diwan
Search Committee Chair
Biology Department
Rensselaer Polytechnic Institute
Troy, New York 12181

Equal Opportunity/Affirmative Action Employer

ment of Graduate Medical Education. Applicants should have a Ph.D. degree with background in administration and education. Saint Barnabas Medical Center is located in a beautiful suburban community, 45 minutes from New York City. For further information, submit curriculum vitae to Abdol H. Islami, M.D.

Saint Barnabas Medical Center
94 Old Short Hills Road
Livingston, N.J. 07039
(201) 533-5500

Saint Barnabas Medical Center is An Equal Opportunity Employer

ASSISTANT PROFESSOR OF BIOLOGY
EMORY UNIVERSITY

The Department of Biology of Emory University is seeking a **comparative invertebrate physiologist** at the assistant professor level starting September 1978. A strong commitment to research and teaching is essential. Postdoctoral experience is preferred. Undergraduate teaching will be primarily in introductory and invertebrate biology courses, with teaching and supervision of M.S.-Ph.D. students in the applicant's specialty.

Send curriculum vitae and summary of research interests by 1 November and arrange for three letters of reference to be sent to:

Dr. William A. Elmer
C.I.P. Search Committee
Department of Biology
Emory University
Atlanta, Georgia 30322

An Affirmative Action/Equal Opportunity Employer

PLANT ECOLOGIST
EMORY UNIVERSITY

The Department of Biology of Emory University is seeking a **population/community-oriented, terrestrial plant ecologist** at the assistant professor level for the fall 1978. A strong commitment to research and teaching is essential. Undergraduate teaching will be in ecological and botanical courses, with graduate teaching and supervision of M.S.-Ph.D. students in the applicant's specialty. Send curriculum vitae and course résumé by 1 November and arrange for three letters of reference to be sent to:

Dr. Harvey L. Ragsdale, Chairman
Ecology Search Committee
Department of Biology
Emory University
Atlanta, Georgia 30322

An Affirmative Action/Equal Opportunity Employer

ASSOCIATE SCIENTIST

Position available for Ph.D. in biochemistry and/or immunology with graduate training in immunochemistry and cellular immunology and postdoctoral experience in tumor immunology, chromosome biology, somatic cell genetics, hybridization techniques, protein chemistry, with strong interest in cancer research. Applicant should intend to develop his/her own ongoing research projects. Salary range: $10,000 to $13,000. Submit detailed résumé to:

Sandy D'Ambrosio
The Wistar Institute
36th and Spruce Streets
Philadelphia, Pa. 19104

sion expects to make a
ant professor level for
neurobiology. Applica
Preference will be given
experience, records of
and postdoctoral trainin
*The University is an E
women and members of
cally encouraged to app
sional persons who are
work send letters of rec
Applications should inc
your most significant pu
your research goals, an
**Chairman, S
Departm
16 Di
Cambridg**
prior to 1 November 19

**ASSISTANT PROFESS
OMY)** beginning Septe
if possible. Specialty is
fector or sensory ph
vertebrates. Applicant
ground, and experien
anatomy/physiology to
quired. Appointee will
per-level and graduate
gram in specialty and
activity involving ele
Relevant facilities incl
center, minicomputer
er. Annual (three-quar
depending on experienc
present academic year.
meeting graduate and
temporary appointmen
ology in winter and spr
riculum vitae and name
to: **Chairperson, Physio
ogy Department, North
Mass. 02115.** *Northeas
Opportunity/Affirmative*

The Department of M
cine, University of So
tions from qualified in
**ASSISTANT PROFESS
didates must posses
completed postdoctor
terest in teaching me
health students. Deve
imaginative research pr
ence will be given to ca
training in immunologic
Interested individuals
vitae and names of th
**Smith, Ph.D., Professor
of Microbiology, Schoo
South Dakota, Vermillio**
ing date for application
Opportunity/Affirmative

**STATE UNIVER
A
GEOPHYSIC**

The Department of Ge
plications for a position
fessor in the field of so
in fall 1978. We seek ca
interests and a strong b
as geophysics. Applica
lum vitae, including na
**Kevin Burke, Departm
State University of New
ington Avenue, Albany,**
*of New York is an E
Action Employer.*

THE GRADUATE SCHOOL

of

THE UNIVERSITY OF COLORADO

FINAL EXAMINATION

of

LOIS ANN BERGEN ABBOTT

FOR THE DEGREE

DOCTOR OF PHILOSOPHY

Oral Examination July 6, 1977

10:30 a.m. Hale - Room #108

Examining Committee:

David J. Rogers, Chairman

Linda K. Dixon Co-chairman

Jerome I. Malitz

David W. Crumpacker

Jeffry B. Mitton

OUTLINE OF STUDIES

Major Field: Biology

BIOGRAPHICAL NOTES

B.A., Cornell University, 1949

M.A.T., Harvard Graduate School of Education, 1950

M.A., University of Colorado, 1973

THESIS

Complete title of thesis A Biological and Mathematical Analysis of Wing Morphogenesis in Drosophila

Prepared under the direction of Dr. David J. Rogers

SUMMARY

Including statement of the problem, methods of attack, results or conclusions and their significance.

Mathematical thinking can be usefully applied to biological problems. The case chosen to demonstrate this is wing morphogenesis in Drosophila. The relation of cellular organization, in two dimensions, to the development of morphological structure is investigated. A reciprocating combination of biological and mathematical methods is used. The chief biological one is clonal analysis in which spatial position of cell lineages is visualized at various stages in wing development. Geometry, probability, and formal language theory provide the major mathematical tools.

Recent work in Drosophila wing morphogenesis has indicated the existence of particular restrictions in the spatial distribution of groups of cell lineages. The resulting compartments are predictable areas with geometrically straight boundaries which arise sequentially. They may be the building blocks in development of wing structure. The first result in this study is that compartments are not bounded hy any kind of physical barrier separating one population from another. Instead cell populations are differentiated and the boundary between them is formed by interaction at their interface.

Such interaction must be determined by the growth pattern of the cells themselves. Cells in Drosophila wing are known to be regular hexagons. Mathematical requirements for binary division of cells in hexagonal arrays were investigated to model cell growth in Drosophila wing. Testing

several models led to the conclusion that the primary growth program utilizes random direction of division of pairs of cells. The displacement of cells required predicts some clone splitting. This was shown to exist early in clone growth. It was also shown that single-cell clones isolated from their kin usually fail to grow up. Cell death is implicated. The result is the over-uniform boundary and clonal integrity which is observed. It suggests that compartment boundaries are no more than clone boundaries "writ large".

THE GRADUATE SCHOOL

UNIVERSITY OF COLORADO

Report on Final Examination for the Ph.D. Degree

The committee appointed to examine Lois Ann Bergen Abbott for the Ph.D. Degree reports as follows: (Delete word which does not apply.)

Final examination. July 6, 1977 (date) Satisfactory: unsatisfactory.

Examining committee:	Satisfactory Signed:	Unsatisfactory Signed:
David J. Rogers, Chairman	David J. Rogers	
Linda K. Dixon, Co-Chairman	Linda Dixon	
Jerome I. Malitz	Jerome Malitz	
David W. Crumpacker	David W. Crumpacker	
Jeffry B. Mitton	Jeffry B. Mitton	

University of Colorado — **FINAL GRADE CARD** — Graduate School

000 39 7391
Matric. No.

B DC CSC MC
Campus: circle one

Abbott, Lois Ann Bergen
(Last Name) (First Name) (Middle Name)

Dept. and Course No. EPOB 800

Title of Course Doctor's Thesis

For the 1st Summer semester 1977 to count 3 semester hours / quarter hours

Thesis Defense 7/6/77
Date

Final Grade A

David J. Rogers
Instructor's Signature

Return to Graduate School, 914 Broadway

Form No. GS-4-73 Form No. GS-168-76

A BIOLOGICAL AND MATHEMATICAL ANALYSIS OF WING MORPHOGENESIS IN *DROSOPHILA*

by

Lois Ann Bergen Abbott

B.A., Cornell University, 1949

M.A., University of Colorado, 1973

A thesis submitted to the Faculty of the Graduate School of the University of Colorado in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Environmental, Population and Organismic Biology

1977

November 24, 1976

Dr. B.C. Clark, Chairperson
Eloise Gerry Fellowship Committee
2051 Highlandview Dr.
Powell, Ohio 43065

Dear Dr. Clark:

I write to support the application of Ms Lois A. Abbott for one of your fellowships. I am Ms Abbott's committee chairman for the PhD, and she is one of my outstanding students. She has served as teaching assistant and instructor in my courses, and has, as well, worked as a teaching assistant in our general biology course. I have known her since 1972, when I was a member of her Master's Degree committee, and Dr. Linda Dixon, at Denver, was her chairperson.

None of the usual superlatives applied to one's own students are really adequate to describe Lois Abbott, because she does not fit into the normal graduate student mold. She is a mature woman with a grown family of five very fine children, the wife of the Director of the Colorado Commission on Higher Education, an accomplished musician, and an interdisciplinary student of mathematics and biology of the highest order. I frankly consider her more as a colleague of equal standing than I think of her as a student. About my only role is to provide her with the necessary administrative channels to pursue a unique degree, which has a basic concept combining biological and mathematical theory in a unique way.

Basically both she and I are concerned with biological pattern recognition and analysis, and the application of models to the complex processes involved. Her mix of biology and math is a very well-balanced approach, and whereas she could be considered either as a mathematician or as a biologist of professional standing, she prefers to say that she applies mathematics to biological problems. Not only does she have a thorough grasp of the principles involved, but she has demonstrated that she can put together an imaginative program of research that is within the realms of practicality. This capacity is unusual in the regular run of graduate students, and only develops with experience and maturity. Lois has presented her proposal for her thesis to the PhD committee, which consists of four biologists and one mathematician at the University, and an additional biologist from California (Irvine), and we are all agreed with her work, her concepts, and her approach.

Lois is, of course, a very energetic person. She has outstanding drive, both physically and mentally. She is well organized, and does a fine job of presenting herself both in writing and orally. She is unpretentious, friendly, and sure of herself as a human being. She is one of the most refreshing individual I have the good fortune to know, and I am honored to recommend her.

Sincerely yours,

David J. Rogers
Professor

DJR:js

TO: Drs. Rogers, Dixon, Crumpacker, Mitten, and Malitz

FROM: Lois Abbott

RE: Ph.D. Committee and plans for comprehensives

I have recently completed the selection of my committee and come to tentative agreement with the members individually on the four fields of examination for the comprehensives. I am sending this memo so that everyone is aware of the overall picture which is the following:

Dr. Rogers - quantitative methods and models in biology
Dr. Dixon - developmental genetics
Dr. Crumpacker - evolutionary theory
Dr. Mitten - biometrics and multivariate methods
Dr. Malitz - outside examiner (mathematics)

I would appreciate any comments you may have on these fields before we consider them final. I am currently planning to take the comprehensive exams in early February, 1976. Since I am working under the "old" plan this would necessitate my having the questions just before Xmas vacation. I will then have several weeks when I don't have teaching responsibilities to work on them.

My thesis project is not totally defined as yet but it will be, generally, to develop the application of a mathematical model to the wing development system in Drosophila. In the spring when my plans are formulated, I would appreciate the opportunity of at least an informal meeting with the committee - in the manner of the new Ph.D. comps. - to review my proposal.

Preface

Morphological shape is clearly of fundamental interest to biologists of many diverse disciplines. Measurements of external form are the basis of much of classical taxonomy. Heritable variations in the normal morphology of organisms are manifestations of genetic mutations and thus form the operational definitions of genetic studies. From the very beginning of biology, the development of morphological structures over time has been studied and described. It is clear that the form of living organisms is produced according to the "program" provided by the DNA of that organism. Yet the exact mechanisms and procedures by which genes mediate the particular organizations of cells which characterize a given organism are only beginning to be explored.

The overall problem has real applicability to practical needs of mankind that is beyond its appeal to scientific curiosity about a fundamental life process. Presumably, deeper understandings of the genetic action at the secondary level; i.e., beyond that defined by biochemical gene products, could apply to practical problems ranging from plant breeding to human birth defects. Evolutionary studies too might be revolutionized if we knew more exactly how the allelic heterozygosity measureable by electrophoresis was expressed in the external morphological characteristics that are exposed to natural selection.

Ultimate understanding of development must involve description of the organization at all levels beginning with the fundamental biochemistry. However, as Lindenmayer (1975) puts it, "This goal is not only unattatinable but is actually undesireble since such a description would be so complex that nothing could be done with it. . . Since in higher organisms the cells appear to be the only functionally autonomous units with genetic continuity, we assume them to be units in our developmental description." I, too, propose to work at this level where the cell is the "black box" possessing such properties as the ability to enlarge, differentiate and change shape, divide, and die.

The intriguing possiblities of mathematical constraints on the form of cell aggregates or tissues was pointed out in 1917 in the classical work of D'Arcy Thompson. More recently the development of mathematical work on cellular automata and formal language theory has been exploited for its application to the growth of aggregates of cells in one and two dimensions by Ulam (1970) and Lindenmayer (1968, 1975) and others. It appears that the growth of cell populations to form particular forms must be subject to rules deducible from the mathematical logic of the system.

The investigation of the mechanisms for biological regulation and organization at the inter-cellular level could benefit from a reciprocating combination of mathematical analysis and biological experimentation. Models and simulations of the growth of groups of cells can help to define viable hypotheses. However, the fact that a particular mechanism is theoretically possible does not make it biologically real. Thus the proposed growth rules must be continually tested in biological systems. This, to me, is the case for applying both mathematical and biological methods in the same study of mechanisms for the development of morphological form.

Proposal

My overall interest is in investigating mechanisms for the formation of wing shape in Drosophila melanogaster. Drosophila has the obvious advantage of being well known genetically and having many morphological mutants available and its wing is a "simple", observable, external organ only 2 cell layers thick. The Drosophila system itself is particularly well suited to developmental analyses of morphogenesis. This is due both to its natural developmental features and to the highly refined techniques which are available for studying insect development. (See Background A) The morphogenetic processes of wing development have been described in considerable detail already and a number of growth parameters are known. A fate map of the wing disc has been published and cell lineage relationships have been investigated. (See Background B.) In addition a number of single gene mutants are known which change the shape of the external wing margin of the adult fly. Several of these cause "scalloping" in that pieces appear to have been cut out of the wing margin leaving the rest of the margin and the veins in tact. Cell death has been shown to be the mechanism of alteration of wing shape in several of these mutants. (See Background C.)

Of the many mutants which result in abnormal wing margins, I propose to use one called Lyra (Ly). It results in the absence of virtually all of the normal anterior and posterior wing margin bristles; that is, the triple row of heavy bristles anteriorly and single row of long, thin bristles posteriorly. The usual rounded edges of the wing appears to have been sheared off except at the distal end. Genetically Ly is a dominant lethal and is maintained in a balanced sytem with Dichaete or

Stubble . It is known to be a chromosomal deficiency at 40.5 on the third chromosome. My reasons for this choice are two. Its expression is extremely regular and unlike most other wing scalloping mutants does not vary in position along the margin from one individual to another. (Even when I crossed it with cut which causes a pointed wing tip, its expression continued in an autonomous fashion.) The second reason for choosing Ly is that its development has not been studied except in the early work of Waddington (1940) so that the developmental parameters themselves will be original research.

Given then , a biological system in which there is genetic alteration of shape, what possible mechanisms for the control of this shape can we investigate? Several interesting problems suggest themselves.

Insect systems of the holometabolus type are known to be highly autonomous in the sense that cells are determined early in the developmental process and retain their "fate" even when transplanted into foreign environments or mixed with cells different from themselves. Thus discs implanted into the abdomens of larval hosts continue to develop into tissues characteristic of the original determination appropriate to that disc. It is presumed given this high degree of autonomy (Bryant, 1976) that discs of mutants such as Lyra would produce mutant wing tissue upon implantation in wild type hosts as well as in Ly hosts. However no implant experiments using mutant wing discs or mutant hosts have yet been reported.

Autonomy of development can be investigated on a gross level using implants. If the disc's determination is undisturbed by transplantation, this is evidence that control of disc development occurs on a more local level that that of its overall position in the larva. Morphogenetic mosaics provide a more refined method of investigating the interactions among cells and their immediate neighbors. (See Background A.) Such mosaics consist of a clone of cells affected by a morphogenetic mutant allele in a wild type background or vice-versa. Expression of the mutant may or may not occur depending on the site and/or position of the clone. Clearly wing scalloping mutants express differentially in the margin as compared to the interior of the wing. Santamaria (1976) found this to be the case in ct^6 and Bx^J and further that mutant expression even in the margins tended to be supressed in small clones. This suggests incomplete autonomy with respect to individual cells and indicates at least neighborhood interaction among cells. No such experiments have been done using Lyra.

As mentioned above the mechanism for several wing mutants is increased cell death. It is likely though not yet established that Ly is a cell death mutant. How wide spread the cell death phenomenon is as an instrument of morphogenetic "sculpturing" is an interesting problem. Saunders (1966) has shown that it operates in the formation of the chick wing axilla. Its rôle in normal wing development in Drosophila is disputed. (See Background C.) Certainly cell death should be considered as a possible canalizing agent in cases of homeostatic regulation toward the normal morphological form.

These are all problems that I would find interesting. However, the most general approach to a mechanism for the development of wing shape, and the one in which I propose to concentrate my efforts in this current study, is the analysis of cell growth via the study of clones. (See p. 13 (A)) The two fundamental processes which interact to give the basic wing shape are growth via cell division and the stretching and folding of the disc which is essentially a single layered shell of cells. The problem sifts down to the arrangement of clones of contiguous cells (cell lineages) in a tube closed at one end.

It is at this point that it first becomes useful to analyse the observed biological facts mathematically. The question is what mathematically minimal hypothesis can account for these observations in clonal studies:

(1) the failure of clones to cross-over certain lines of demarcation after particular times in development;

(2) the appearance of these boundaries as "straighter" than those of ordinary clones;

(3) the regular appearance of straight line veins;

(4) the regulation of mosaic wings containing fast growing cells so that normally shaped wings are still produced as is reported (Garcia-Bellido, 1976).

We (J. Malitz, A. Ehrenfeucht and I) have reviewed the current proposals of workers in the field and added another, simpler hypothesis to them. In summary the three major hypotheses are these:

(1) simple geometric growth occuring over the whole surface of the developing wing given the possibility of oriented cell division. (Malitz and Ehrenfeucht, 1976).

(2) similar growth properties with the addition of cell recognition properties so that subgroups of cells associate preferentially with their own kind and antagonistically with cells from other populations. (Bryant, 1976),

(3) the formation of "fences" or actual physical separators between cell populations which become increasingly restrictive as development proceeds. (Kauffman, 1976).

Note that any of these models can include modification by cell death and by local differences in cell size and shape. Also the observations on clones and the latter two hypotheses are important factors in the current discussions on "compartments" and their role in insect development which is the hot issue in the field at present. See the Background D for definitions of the proposed compartments and a review of the literature on them.

Going back to the hypotheses in relation to the observations, the simplest one can account for the first three observations. Kauffman's was devised specifically as a way to deal with the fourth. The problem now goes back to seeking biological evidence before further refinements on any one mathematical model are made. In summary the specific experiments that I propose are these: (details of these experiments are in the next section.)

(1) Clonal Analysis of Lyra

In the Lyra mutant the normal bristle cells of the wing margin are missing - presumably due to cell death which occurs during the third instar (72 to 120 hours). (See Background C.) According to the compartment theory (Background D) dorso-ventral developmental restrictions are established by 30 hours. If these restrictions take the form of an actual, physical barrier between cell populations which is then destroyed by cell death during the third instar, one would expect the following clonal manifestations. Clones in Ly would develop as in non-mutants until shortly after 72 hours; that is, there would be no clones which would continue on both dorsal and ventral wing surfaces. During the third instar period cells die off along the margins. Subsequent growth of marked cells in clones along the border should result in some clones which continue on both sides if their growth had been restricted by the marginal cells. If clones do not cross the de facto wing boundary remaining after cell death, there are two possibilities - either there never was a physical barrier or it has been rapidly re-established. (See Background E on the Kauffman hypothesis for details on these possibilities.)

(2) Study of non-Minute clones in Lyra wings

The question we are focusing on in this study is that of the control of shape despite the overgrowth of non-Minute clones. Does this regulation require the physical presence of the margin? Since the margin of the wing is identical with a portion of the dorso-ventral boundary in the proposed compartment hypothesis, this question is directly relevant to testing that theory. Also particular attention will be paid to observations on the effect of overgrowing clones on vein development. So far nothing has been reported in the literature on this.

(3) Cell death in Lyra

Histological means will be used to determine whether Lyra, too, is a cell death mutant. Modification of wing shape after it basic formation by cell death is a mechanism that is compatible with all of these models. However, if there is no cell death involved, then other models would have to be devised to account more specifically for the Lyra wing shape.

The last step would be redefine and refine the appropriate mathematical model based on the results of these experiments. If evidence for physical boundaries is found, then some mechanism for their formation must be suggested. If it is not, then refinements of the simpler cell growth models would seem in order. Obviously either case will lead to new questions that will define further biolgical experiments. It is clear, then, that my current proposal is only one step in a series of problems that I find of special interest because they require both mathematical and biological methods.

Proposed Experiments in Detail

(1) Clonal Analysis of Lyra

Objectives: The major objective is to look for evidence of physical compartment boundaries as explained above. In addition it would be useful to make a preliminary comparison of the shapes of clones in Ly wing with those previously reported (Bryant, 1970) for normal wings. (The Ly mutant could act by changing the proportion of oriented cell divisions so that the clones as well as the wings become narrower.) Also observations on the relation between clones and veins will be recorded since they have not yet been reported in the literature.

Procedure

Crosses: Basically one must prepare a heterozygote for the markers in such a way that cross-over in a single cell produces a line of marked (i.e., homozygous) cells. In our case one of the third chromosomes must also carry Ly.

The simplest way to construct a suitable hybrid is to cross multiple wing hair (mwh; 3- 0.0) or mwh and ebony (e; 3- 70.7) with Ly (3-40.5) Half of the offspring will have the third chromosome shown in Fig. 1a and will be of Lyra phenotype.

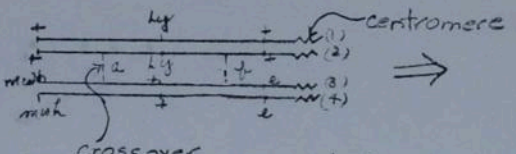


Fig. 1 a

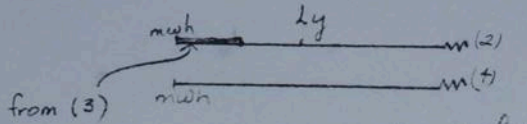


Fig. 1 b

A second cross can be used which produces mwh clones which are also yellow (y; 1-0.0) making use of the deficiency-duplication mutant, sc^{J1}. The tip of the third chromosome carries a translocation from the X containing y^+. The other third chromosome carries mwh and Ly combined by meiotic cross-over. One X carries yellow and the other a deficiency for the yellow locus ($Df(1)sc^8, w^a$). The matings are: mwh; y ♀ ⊗ Dp(1:3) sc^{J+}; Df(1) $sc^8 w^a$ ♂ (These males are the standard sc^{J1} stock.)

Mitotic crossover in the left arm of the third chromosome eliminates y^+ ~~as well as mwh~~ from one daughter and thus patches are marked with yellow as well as multiple wing hairs. It is also possible to use first chromosome crossover with markers such as yellow and forked. Currently I am trying out these crosses. Ebony has not been used to date in this work; the sc^{J1} method was used by Bryant .

Irradiation: The second step is to produce somatic crossovers. This is done by irradiating the heterozygous larvae from crosses above with X-rays. Both medical X-ray machines and gamma ray sources have been used. The dosages required range from 1000 to 1700 r. The dosage rate does not appear to be critical - 100 to 1000 r per minute have been reported. The source I am currently trying is a gamma ray source inthe CU Chemistry Dept. which produces 100 r / second.

Cross-over in the mwh, e system produces clones as shown in Fig. 1b. Note that crosses at site (b) in that figure will produce patches marked with both e and mwh but these cells no longer are Lyra. (In this case true xx genetic mosaics for Ly are assured.)

Scoring: The third step in the procedure is to dissect out the thoraces with attached wings, mount between cover slips in Euparal and examine for clones.

Recording: Fourth, the clone shape in general and location of clones with respect to veins must be recorded in addition to looking for the presence of clones continuing over the wing margins. I will probably record shape by tracing on standard wing diagrams though I have given a little thought to digitizing them for computer analysis if there seems to be any need for it.

Experimental Design

In this experiment we would irradiate 72 hour larvae in order to produce reasonably large clones with the fairly high frequency - about 30%. We have a particular interest in clones bordering the anterior and posterior margins, though records will be make of all clones, marginal and interior. It is hard to say how many wings with clones will have to be examined to get a sample of 20 to 25 marginal clones to check for clone crossings. Perhaps 150 to 200 wings with clones will supply them which means mounting and examining three times that many.

(2) Study of non-Minute clones in Lyra wings

Objectives: In Garcia-Bellido's work on non-Minute clones growing in Minute wings, the wing shape is reported not to be altered despite the fact that the non-Minute clones account for 30 to 90 % of the dorsal or ventral surface due to their comparatively rapid growth rate. The interpretation of this in compartment theory, especially as presented by Lawrence and Crick , is that the compartment boundaries act somehow so as to physically contain the clones and delimit their growth.

The objectives of this experiment are two: to determine whether overgrowth of clones in Lyra result in the usual wing shape despite lack of the margins and to repeat the Garcia-Bellido observations on wild types since they will be used as controls. In addition it is planned to make specific observations on vein development in these mosaics.

Expected results of the experiment are hard to write about specifically in the absence of any preliminary work as yet. If Lyra wing shape should be changed much more radically than is the wild type by overgrowing clones in the absence of the bounding margins, it suggest confinement as by a fence - albeit an elastic or growing fence. If both Ly and wild types shape are little altered by non-Minute clone, control of shape must be vested in the growth patterns of the cell population(s) as a whole with or without separation into compartments. (See Background E.)

Procedure:

Crosses: Combine a Ly/ mwh e third chromosome with a first chromosome of genotype y f^{36a} / M (1) o^{sp}. The latter is a first chromosome Minute which has the cell autonomous effect of reducing the growth rate. Flies attain normal size but their overall developmental time is increased by 36 to 40 hours. Forked (f; 1- 56.7) causes short, bent bristles and trichomes (hairs). The recombinations in the first chromosome marked by yellow forked phenotype are non-Minute clones in a Minute background which is also Lyra. Clones of mwh etc. can occur also due to third chromosome crossover; they serve as internal controls since they remain Minute clones in the overall Minute phenotype. Similar procedures are used for the experimental control observations on non-Minute clones in Minute background omitting the Ly on the third chromosome.

Irradiation, scoring and recording techniques would be the same as in the clonal analysis of Lyra alone.

Experimental Design:

Preliminary observations will first have to be made on the overall developmental rate of flies carrying both Minute and Lyra with two particular questions in mind. (1) Is the regular Lyra phenotype expression affected by changes in growth rate due to Minute? (2) Is the usual developmental rate of Minute changed?

Allowing for modification according to these results as necessary, the experimental plan is the following. Minute for unknown reasons increaseses the rate of mitotic crossover .

Since it also radically increases clone size, as few as 200 wings may be sufficient to produce a sample of 25 to 30 clones impinging on border areas of the wings. The fact that Minute adds 36 to 40 hours to the overall time to pupation necessitates later irradiation times. I plan to use 96 hour larvae for this reason since Garcia-Bellido's data shows that by this age a high proportion of the clones produced affect only one wing surface in the normal fly.

(3) Cell Death Observations

Objectives: The purpose is to establish whether or not Ly does in fact belong to the group of scalloping mutants caused by increased cell death.

Procedures: (1) Stain Ly whole discs with acridine orange and examine under the fluorescence microscope (available at UCD) (Spreij, 1971) Degenerating cells show up as a bright orange yellow.

(2) Section discs and stain with Feulgen to discern pycnotic (necrotic) cells. (Suggested by Bryant, 1976, and also described in Spreij, 1970). Or discs can be fixed, embedded, sectioned, and stained with methylene blue as described in Fristrom, D., 1968. Also see Wehman, (1969), for further histological methods.

(3) Comparison of wing size with that of the normal wing (Santamaria, 1976) and calculation of comparative cell numbers using wing size parameters and trichome density as measured according to the method of Dobzhansky, (1929).

Background A:

Appendix A ◂ Developmental Analyses in Drosophila

The Drosophila system is a particularly useful one for studying the development of morphogenetic form. Developmentally the individual represents a mosaic of relatively autonomous cells. Flies are holometabolus insects in which "nests" of cells called imaginal discs are set aside in the embryo. Though the discs grow during the larval stage they do not participate in its functioning. When pupation occurs the cells of the larva are histolysed and the entire external body of the adult fly develops from the discs. The discs have acquired a high degree of determination at an early stage and a particular disc normally makes only one external organ such as a leg or a wing or an eye. There is relatively little epigenetic regulation and very little morphogenetic movement.

Besides these natural features several techniques for studying development in flies are available. Though the major ones were first suggested in the thirties, they have been highly developed in the past ten years and are the chief experimental procedures for the work of the laboratories of Garcia-Bellido in Madrid, Spain and Schneiderman and Bryant in Irvine, California.

The first method is in vivo culture of discs by implanting them by micro-injection into either the abdomens of adult flies for growth or into larvae for metamorphosis in the differentiated tissues characteristic of that disc. The tissue when removed from the host adult does not display the exact size and shape of the adult organ but many cuticular landmarks

are recognizable and much of their spatial ordering is maintained. Pieces of discs may be continually reimplanted in adult hosts for several years before being assayed by transfer to larvae and allowed to differentiate. For the most part they will maintain their original determination to form a particular organ. However, certain transdeterminations occur with measurable probabilities resulting, for example, in wing tissue from leg disc (Kauffman, 1973). Interestingly enough the effect is paralleled by that of the so-called homeotic mutants. The injection technique was first described by Ephrussi and Beadle (1936) and later used extensively by Hadorn (1963) and Ursprung (1967) to study determination and cell autonomy. It is technically challenging but not impossible and is one of the techniques I was able to learn during my recent visit to Peter Bryant's laboratory in Irvine.

The second technique came from the "twin-spot" method used by Stern (1936) and Becker (1957). It was discovered that mitotic (or somatic) crossover could be produced in flies by X-ray irradiation. Heterozygotes of marker mutants when irradiated will thus form marked clones from any cell in which a mitotic recombination has occurred. Such events are relatively rare averaging 1 per 600 cells irradiated. Naturally due to increase in the number of target cells over time, early irradiation results in a few, large clones while later treatment produces more frequent but smaller ones. Analyses of these clones has been used extensively beginning with Gehring (1967) to study cell lineage relationships.

The third technique also makes use of mitotic cross-over induced by X-rays to produce genetic mosaics. Chromosomes can be arranged so as to produce marked clones of morphogenetic mutant cells in wild type backgrounds or vice versa. One example is the study of non-Minute clones in Minute individuals (Garcia-Bellido et. al., 1976) which has led to the current "hot issue"in insect development regarding the possibly fundamental role of the so-called compartments as independently bounded units of development. (See Appendix E).

Morphogenetic mosaics are particularly useful for studies of cell autonomy. Santamaria (1976) used them to determine autonomy of expression of the scalloping mutants, $\underline{ct}^6$ and $\underline{Bx}^J$ in relation to the position of the clones in the wing. Besides observing that increased cell death occurs in marginal rather than internal clones, he found that cell death in the scalloping mutant $\underline{ct}^6$ clones in wild type backgrounds tended to be spppressed especially in small clones suggesting incomplete autonomy and neighborhood interaction among cells. He also found a peculair non-autonomy between apposing dorsal and ventral cells in the margins only. His observation was that there were no cases of gaps caused by mutant clones in the margin area involving one wing surface alone. This was true despite the fact that clones themselves are restricted to one surface.

Operationally the difference between clonal analysis and morphogenetic mosaicism has to do with the relation between the marker genes, the morphogenetic gene and the location of the cross-over event. Clonal analysis requires only a marker gene and a cross-over between it and the centromere. (See Fig. 3a .)

Morphogenetic mosaic experiments aim to examine mutant but marked clones in a wild background or vice versa. Thus the marker must be on the same chromosome and very close to or surrounding the morphogenetic mutant. The cross-over must occur between that marker nearest the centromere and the centromere. (See Fig. 3b) In contrast to meiotic recombination, mitotic cross-over has been shown to occur with uniform frequency along the chromosome including in the region of the centromere (Becker, 1969, 1974). Kauffman (1976) is using this technique to time gene expression since it can effectively "knock out" a gene in a clone from the time of irradiation.

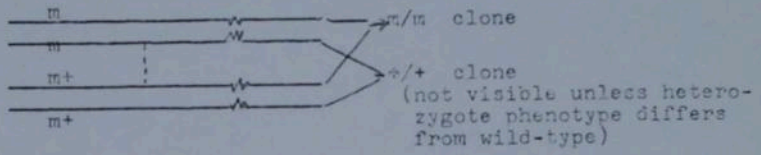
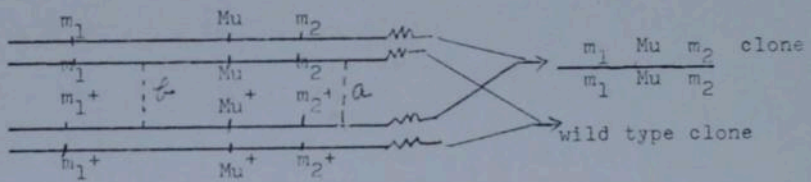


Fig. 3a



Note: If homozygous Mu is lethal only wild clones will be observed in the heterozygous background. If it is not then twin spots will result. Note also that if a cross-over occurs at b then a simple m_1 marked clone will result and the morphogenetic mutant status will not be changed in the clone.

Fig. 3 b

A fourth technique that has been used for mapping the embryonic organization of the wing disc (Ripoll, 1972) utilizes gynandromorphs (i.e., male-female mosaics) produced by the loss (with probability of 25%) of the unstable Catchside ring X chromosome which usually occurs in the first zygotic division. Loss of the ring results in male tissue in one daughter cell while the other retains the ring chromosome material. Thus the result is a 50-50 male-female mosaic. However, the axis of division in the first divisions are oriented at random. Therefore the frequency with which two adult cuticular landmarks are of the opposite sex is a measure of the separation of their presumptive cells in the embryo. The "distances" between all the possible pairwise combinations of cuticular landmarks are then mapped. The general results are that the relative positions of presumptive cells correspond with their adult positions; for instance, all the corresponding dorsal and ventral elements map close to each other. This fact is cited as evidence for the necessity of separation between dorsal and ventral cell populations (Garcia-Bellido et.al., 1973) though I doubt that the more parsimonious explanation of topological constraints has been rigorously applied.

All of these factors thus contribute to making Drosophila extraordinarily well suited to studying pattern formation and morphogenesis - a fact which was pointed out long ago by Curt Stern (1940)!

Background B: Physical Development of *Drosophila* Wing

1. General Description

The wing of *Drosophila* develops from the dorsal mesothoracic disc which also forms the dorsal portion of the thorax called the notum and the pleural structures which form the side of the thorax. The discs are, essentially, rounded sheets of epithelium , one cell layer thick and exhibiting fold patterns peculiar to each disc type. Disc tissue is closely associated with the larval nervous system and its tracheae. Wing disc develops by folding and eversion forming a recognizable pouch very early in the pupal stage. The pouch enlarges during two successions of cell growth and division followed by stretching and inflation into a balloon like structure that is subsequently flattened into a blade (Waddington, 1940). The wing blade preserves as its border the fold line that divides the dorsal and ventral cell groups. Thus the adult wing is a structure two cell layers thick. Its anterior, distal, and posterior margins are distinguishable by triple rows, double rows, and a single posterior row respectively of specialized bristles or macrochaetae (Bryant, 1975). The wing surface is covered with a regular pattern of hairs or trichomes each of which represents a single cell (Dobzhansky, 1929). A characteristic adult pattern of wing veins occurs upon deflation of the wing in the late pupa which results in apposition of the dorsal and ventral surfaces of the wing. The veins, which carry nerves and trachae, are thus hollow tubes representing areas of non-contact. Thus vein and border formation have not been shown to be functionally related although it is possible that the fold lines and veins are demarcated by the same mechanism.

2. Review of the known parameters of wing development

Several detailed studies on growth parameters of the wing have been made recently. Cell lineage studies, using the mitotic cross-over technique mentioned above have been made by Bryant (1970) and Garcia-Bellido and Merriam (1971). Both conclude that clonally related cells form longitudinal stripes in the wing and thus oriented cell division is indicated. Calculations based on average clone size in relation to the overall size of an organ estimate cell numbers at various ages. Using this method after irradiating early embryos, Bryant (1970) estimates that the early wing bud has 11 cells since the average clone produced constitutes 1/11th of the adult mesothoracic surface. A similar estimate of 12 initial blastoderm nuclei comes from using landmarks on gynandromorphs (Garcia-Bellido and Merriam, 1969). They suggest that two cell divisions take place so that by the first larval instar the disc contains nearly 50 cells. Bryant (1970) finds 70 at 48 hours, the time of the first moult. Ripoll (1972) finds that the minimal spots of male, marked tissue occupy 1/47 of the adult surface and suggests 47 as the number of presumptive cells in the disc. He divides this into 30 wing cells and 17 for the notum. Bryant (1970) indicates that by 72 hours, when wing forming tissue is first physically separating, by a cross-ridge from the notum, there are 300+ cells which form identifiable clones. By 120 hours when the pouch is growing rapidly, there are about 9600 .

The number of cells in the adult mesothorax and wing have been measured directly (Garcia-Bellido and Merriam, 1971). Overall there are 52,000 of which 30,000 are in the wing.

Such estimates can then be used to calculate such growth parameters as cell-cycle time and the number of mitotic divisions. It is usually assumed that growth is not continuous in the usual exponential sense. Instead mitosis appears to take place in waves producing synchronous division so that the growth model which applies is 2^n times the initial cell number where n is the number of mitotic divisions. Ripoll(1972) finds that with 16-30 presumptive wing cells 10.5 divisions are needed to produce the adult wing; he calculates 9 for the notum. Cell cycle time estimates vary from 8.5 (Garcia-Bellido) to 9.6 hours (Bryant).

A detailed fate map of the wing disc has been published by Bryant (1975) . He used the implantation method described above. He dissected the discs into various segments, implanted the pieces into larvae directly and assayed the cuticular structures produced after metamorphosis. (See attached copy.)

In the same paper he presented detailed results on regeneration and duplication of the wing disc. This time the disc fragments were grown in adult hosts previous to being metamorphosed by implantation into mature larvae. The results were that disc fragments when permitted healing and growth either regenerate the missing part or duplicate themselves in mirror image fashion. Which event occurs depends on the position of the cut in relation to a central "high point" of the disc. If the cut edge faces away from the center, it regenerates; otherwise duplicates occur. Either case requires intercalary growth to replace missing tissue and to re-form the continuity of positional information. The most recent model (French, Bryant, and Bryant, 1976) predicts duplication or regeneration on a "shortest interval-

ation rule" - that is, if the required positions on a gradient are considered as the numbers on a clock face, removal of a segment from 3 to 6 would yield regeneration of the remainder while the segment itself would heal together along the 3-6 border and then replace the 4 and 5 positions leading to duplication of the segment.

Background C: The Cell Death Hypothesis for Mutant Formation

The mechanism for formation of mutants has been observed since the very early studies of the morphogenesis of the wing structure. Auerbach (1936) describes discs of Beadex, vestigial, and Dumpy mutants. Waddington (1940) continues the study of Dumpy, several vein mutants, and the scalloping mutants vestigial, cut, Beadex, and Lyra mutants during the pupal stage. Waddington's theory of margin mutant formation is that the initial fold was controlled and different in the mutant. Goldschmidt (1935) ~~1937)~~ had previously suggested that development was normal in mutants initially but that subsequent degeneration at certain places along the borders sculptures the wing and causes the characteristic notches.

This disagreement formed the basis for the recent work of Fristrom (1968, 1969). She examined several mutants with electron microscopy and discovered unique bodies which appeared to represent degenerating cells. At more advanced stages these bodies look like an autophagic vacuole; i.e., an intracellular body bounded by a single membrane. Thus degeneration in imaginal discs is unique in that dying cells are continuously phagocytized by neighboring cells. These bodies show up in mutant forms concentrated in areas corresponding to the phenotypic modification of the forms. She found no evidence, however, of cell death in normal wings. Spreij (1970, 1971) continued the study of the exact location of degenerating cells using fluorescence microscopy. He found evidence of cell death in normal as well as mutant forms in studies of Calliphora as well as Drosophila.

In Fristrom's studies (1968, 1969) degenerating cells were found in several scalloping mutants during the late third instar (196 to 120 hours. They becam even more numerous during the prepupal stage . Spreij found degenerating cells visible throughout the third instar period (72 to 120 hours). It hould be noted that the bodies Fristrom observes result from phagocytosis of already dead cells by their neighbors. Thus there is probably some delay in their appearance past the actual cell death. Cell death, then, appears to occur at least from mid-third instar through prepups.

Thus, the general mechanism of cell death in these border mutants seems well established. (Bryant, 1976). Santamaria (1976) shows that adult wings in $\underline{Bx^J}$ and $\underline{ct^6}$ actually have fewer cells than normal wings. The interesting question now is how cell death is confined to particular areas. In this connection the crosses I have made between $\underline{Ly}$ and $\underline{ct^6}$ are relevant. The missing border areas characteristic of each mutant simply add up in the double mutant and the wing has both cut off longitudinal edges and a point distally.

Background D : Review of the Literature Regarding the possible Existence of Compartments

The recent proposal by Lawrence and Crick (1975), based on the work of Garcia-Bellido, Ripoll, and Morata (1973 and 1976), that a series of boundaries established in early development progressively restrict clonal growth patterns and that the resulting compartments are basic units in insect development is an hypothesis of interest to us - particularly since a portion of the proposed dorso-ventral compartment boundary is the wing margin.

An operational definition in terms of Garcia-Bellido's experiments is perhaps the best way of describing what compartments are supposed to be. He worked with a system of non-Minute clones in flies of Minute genotype produced as explained above, in the second experiment. These rapidly growing clones when initiated after certain critial times in development regularly failed to cross certain boundary lines. Instead "Such clones may border a demarcation line for as many as a thousand cells" (Lawrence and Crick, 1975)

The actual observation and mapping of the lines was done by selecting a number of landmarks well distributed over the wing surface. Members within all the possible pairs of landmarks were then compared for the frequency with which both occurred in a single clone. When that frequency declined to 0, a segment of a clonal boundary is defined somewhere between the two landmarks dated by the earliest irradiation time which causes them never to occur in the same clone. These demarcation lines tend to occur at well defined times, in a fixed succession and in

constant locations with respect to other wing structures. Further they appear to be straighter than the boundaries of ordinary clones. Similar boundaries have been reported recently in Drosophila eye and head development (Baker, 1976).

One other line of evidence which may support the compartment theory is the existence of several of the homeotic mutants in which substitution of whole compartments appears to occur. Engrailed, for example, causes the usual posterior portion of the wing to be replaced by a repetition of the anterior.

Ordinary clones (that is, marked clones growing at the same rate as the cells surrounding them) behave in a way which is not inconsistent with Garcia-Bellido's clones. It has long been known, for example, (Bryant, 1970) that clones do not cross the wing margin from dorsal to ventral surface unless the clones have been formed very early.) Naturally, the small size of ordinary clones does not permit them to display clone boundaries so dramatically. In their case, however, the progressive restriction of clones to particular regions seems quite what should be expected from two-dimensional cell growth uniformly distributed over a surface; even the appearance of straight line demarcation lines can probably be shown to result from simple geometric properties of the growth pattern in connected clones given only the possibilities of oriented cell divisions and regionally differential cell growth rates (Malitz and Ehrenfeucht, 1976).

One other important feature of Garcia-Bellido's system is of direct importance to our interest in mechanisms for control of wing shape. He reports that despite the large size of his

clones "the overall size and shape of the wing was not altered" (Lawrence and Crick, 1975). Informal reports at Irvine last summer raised some doubts as to the strict accuracy of this statement which is the reason for my attempting to repeat the experiments as controls to the Lyra wing non-Minutes. Illustrations in the draft of a paper I received very recently from Baker show no distortion due to overgrowing clones in eye shape.

Auerbach, C. (1936) The development of the legs, wings, and halteres in wild type and some mutant strains of Drosophila melanogaster, Roy. Soc. of Edinb. Transac. 58: 787-

Baker W. K. (1976) A polyclonal basis for early developmental restriction in the head of Drosophila, in preparation.

Becker, H. (1957) Über Röntgenmosaikflecken und Defektmutationen am auge von Drosophila und die entwicklungsphysiologie des auges, Z. Verebungslehre 88: 333-379

Becker, H. J. (1969) The influence of heterochromatin, inversion heterozygosity and somatic pairing on X-ray induced mitotic recombination in Drosophila melanogaster Molec. Gen Genetics 105: 203-218.

Becker, H. (1974) Mitotic recombination maps in Drosophila melanogaster, Naturwissenschaften 61: 441-448

Bryant P. (1970) Cell lineage relationships in the imaginal wing disc of Drosophila melanogaster, Dev. Biol. 22: 389-411

Bryant, P. (1975) Pattern Formation in the imaginal wing disc of Drosophila melanogaster: Fate map, regeneration and duplication, J. Expt. Zool. 193(1): 49-78.

Bryant, P. (1976) personal communication

Dobzhansky, T. (1929) The influence of the quantity and quality of the chromosomal material on the size of cells of Drosophila melanogaster Wilh. Roux Arch. 115: 363-379.

Ephrussi, B. and Beadle, G. (1936) A Technique of transplantation for Drosophila, Amer. Naturalist 70: 218-225.

French, V., Bryant, P., Bryant S. (1976) Pattern regulation in epimorphic fields Science 193: 969-981

Fristrom, D. (1965) Cellular degeneration in wing development of the mutant vestigial of Dros. melanogaster, J. Cell Biol. 39: 488-491

Fristrom, D. (1969) Cellular degeneration in the production of some mutant phenotypes in Drosophila melanogaster Molec. Gen. Genetics 103: 363-379

Fristrom, D. (1972) Chemical modification of cell death in Drosophila Mol. Gen. Genetics 115: 10-18

Garcia-Bellido, A. & Merriam, J. (1969) Cell lineage in the imaginal discs of Drosophila gynandromorphs, J. Exp. Zool: 170: 61-76.

Garcia-Bellido, A. and Merriam, J. (1971) Parameters of the wing imaginal disc development of Drosophila melanogaster, Dev. Biol. 24: 61-87.

Garcia-Bellido, A., Ripoll, P., Morata G. (1973) Developmental compartmentalization of the wing disc of Drosophila, Nat. New Biol. 245: 251-253.

Garcia-Bellido. A, Ripoll, P. and Morata, G. (1976) Developmental compartmentalization in the dorsal mesothoracic disc of Drosophila, Dev. Biol. 48: 132-147

Gehring, W. (1967) Clonal analysis of determination dynamics in culture of imaginal discs in Dros. mel. Devel. Biol. 16: 438-456

Goldschmidt, R. (1935) Gen und Au Benchara Kter (II (Untersuchuften an Drosophila, Biol. Zebl. 55: 535-554.

Herman, G. and Schiff, G. (1975) Simulation of organisms using L-systems, J. Theor. Biol. 54: 35-46.

Kauffman, S. (1973) Control Circuits for determination and transdeterminations, Sci. 181: 310-318

Kauffman, S. (1976) Personal communication

Trabert, Shainka & Kauffman, S. (1976) Characteristic wave patterns and compartmentalization in Drosophila development in preparation

Lindenmayer, A. (1968) Mathematical models for cellular interactions in development I. Filaments with one-sided inputs, J. Theor. Bid. 18: 280-099.

Lindenmayer, A. (1975) Developmental Algorithms for Multicellular Organisms: A Survey of L-systems, J. Theor. Biol. 54: 3-22

Lawrence, P. and Crick, F. (1975) Compartments and Polyclones in insect development, Sci. 189: 340-347.

Malitz, J. (1976). personal communication & Ehrenfeucht, A.

Ransom, R. (1975) Computer Analysis of division patterns in the Drosophila head disc, J. Theor. Biol. 53: 455-462.

Ripoll, P. (1972) The embryonic organization of the imaginal wing disc of Drosophila melanogaster, Wilh. Roux Arch. 169: 200-215

Saunders, J.W. Jr. (1966) Death in embryonic systems, Sci. 154: 604-612

Santamaria, P. and Garcia-Bellido, A. (1975) Developmental analysis of two wing scalloping mutants ct^6 and Bx^J of Drosophila melanogaster, Wilh. Roux Arch. 178: 233-245.

Spreij (or Sprey), T.E. (1970) Morphological and histochemical changes during the development of some of the imaginal discs of Calliphora erythrocephala, Neth. J. Zool., 20: 283-275.

Spreij, T.E. (1971) Cell death during the development of the imaginal discs of Calliphora erythrocephala, Neth. J. Zool. 21(3): 221-264 (also includes Drosophila)

Stern, C. (1936) Somatic Crossing-over and segregation in Drosophila melanogaster, Genetics 21: 625-730.

Stern, C. (1970) The prospective significance of imaginal discs in Drosophila, J. Morphol. 67:107-122.

Thompson, D'Arcy W. (1971) in On Growth and Form, J.T. Bonner, ed. Cambridge Univ. Press

Ulam, S. (1970) in Essays on Cellular Automata, A. Burks, ed.

Ursprung, H. (1967) In-vivo culture of Drosophila imaginal discs (in Methods in Developmental Biology

Waddington, C.H. (1940) The Genetic control of wing development in Drosophila, J. Gen. 41: 75-139

Wehman, H.J. (1969) Fine structure of Drosophila wing imaginal discs during early stages of metamorphosis, Wilhelm Roux Arch. 163: 375-390

Wilby, O.K., and Ede, D. (1975) A model generating the pattern of cartilage skeletal elements in embryonic chick limb J. Theor. Biol. 52: 199-217.

Wolpert, L. (1969) Positional Information and the Spatial Pattern of Cellular Differentiation J. Theor. Biol. 25: 1

Wolpert, L. (1972) In: Current Topics in Developmental Biology, vol. 6, A.A. Moscona and A. Monroy, ed., Academic Press, N.Y. pp. 183-224.

Summary – Chronology of Development

Age hours (days)	Development Stage	Wing Disc Development	# cells in wing disc	Frequency of clones	Compartment boundaries
2 (0)	Syncytial blastoderm (1)	not separated from blastoderm (3)	10–11 (2)	0.1% (2)	anterior–posterior (5)
24 (1)	hatching → 1st instar (1)	wing bud = oval group of cells clearly demarcated, situated between muscle & fat body; no lumen. (3)	10–11 (2)	0.7% (2)	30 hours: Dorso-ventral and wing-notum (5)
48 (2)	1st moult → 2nd instar (1)	lumen formed with cells regularly arranged about it (3)	70 (2)	6.1% (2)	
72 (3)	2nd moult → 3rd instar (1)	wing bud differentiated into disc and peripodial membrane. First cross-ridges appear separating wing from thorax. (3)	370 (2)	29.7% (2)	in notum: Sectum – post sectum and Scutellum – ant scutell (5)
96 (4)	mature 3rd instar (1)	further folding → concentric folds and wing depression (2) and (3)	770 (2)	63.3%	Proximal-distal in win (5)
120 (5) = P	Puparium formation	beginning of evagination of wing blade pouch. (3)	9300 (2)	708%	
P+6 hrs	Prepupa (4)	Pouch enlarges; cells each divide once; pouch "ballooning" as stretching occurs. Prepupal veins corresponding to L1 & L3 only are present. (4)	18600 (?)		
P+18 hrs	Pupa	final cell divisions; end "ballooning" and collapse of pouch to L → adult-veins.	30,000 (2)	No further clones after P+18 hours (2)	
9 days	Adult		since 9		

Legend: for Chronology of Development

Age in hours: = hours after egg laying at 25°C.

# of cells is computed as the reciprocal of the fraction of the adult wing occupied by the average patch.

frequency = # clones / wing.

Numbers in parentheses refer to the following references:

(1.) Bodenstein, in Demerec, Biology of Drosophila

(2.) Bryant, P. (1970)

(3.) Auerbach, C. (1936)

(4.) Waddington, C.H. (1940)

(5.) Kauffman, S. (1976)