No nominations submitted for 1982
5/14/82
Dr. Clark.
Please
handle.
mmw/g.

AMERICAN ASSOCIATION OF UNIVERSITY WOMEN
EDUCATIONAL FOUNDATION
2401 VIRGINIA AVENUE, N.W. WASHINGTON, D. C. 20037

March 1982

Dear Colleague:

On behalf of the American Association of University Women Educational Foundation, I would like to thank you for helping us to identify a young scholar of great merit. I am sorry, however, that we were unable to choose your nominee for the Recognition Award; many outstanding women were nominated and the competition was very keen.

This year's recipient of the Recognition Award for Young Scholars is Phyllis Jo Baunach, a correctional research specialist with the National Institute of Justice. Julia Montgomery Walsh, a noted stockbroker who is chairman of the board of her own investment company, Julia M. Walsh & Sons, Inc., will receive the companion Achievement Award. Women of such high caliber as Baunach and Walsh continue to be identified because of your assistance. We thank you for your efforts.

Once again I would like to invite you to nominate a young woman for the 1983-84 Recognition Award for Young Scholars. The woman selected will be honored at the AAUW biennial convention to be held in San Francisco in June 1983.

The Recognition Award for Young Scholars carries an honorarium of $1000. A nomination form is enclosed and should be accompanied by three letters of recommendation. One should be from the president or an equally qualified official of an accredited academic institution, if the nominee is affiliated with such an institution. A second recommendation should come from the nominator; and if the nominee is affiliated with a nonacademic institution, a third should be submitted from a person in a top managerial position or an authority in her field. Nominees for the award must meet the following conditions:

1. Less than 35 years of age at the time of nomination.

2. Earned a doctorate or its equivalent.

3. At least one outstanding contribution in the academic and/or professional field of her choice.

4. Potential for achievement (indicated by past accomplishments and letters of recommendation) in an academic discipline or professional field.

AAUW invites you to submit the name and supporting data for a woman you think qualifies for this award. Please send your nomination to me at the above address no later than May 15, 1982.

Sincerely,

Beverly A. Harden

Beverly A. Harden, Ph.D.
Director
Educational Foundation Programs

Enclosures

RECIPIENTS OF AMERICAN ASSOCIATION OF UNIVERSITY WOMEN

RECOGNITION AWARD FOR YOUNG SCHOLARS

1973-83

1972-73 Mary Eva Swigar, M.D.
Assistant Professor
Department of Psychiatry in Obstetrics and Gynecology
Yale University School of Medicine
New Haven, CT

1973-74 Dr. Susan M. Hartmann
Associate Professor of History
University of Missouri
St. Louis, MO

1974-75 Dr. Donella H. Meadows
Assistant Professor of Environmental Studies
Dartmouth College
Hanover, NH

1975-76 Dr. Edna Bonacich
Assistant Professor of Sociology
University of California
Riverside, CA

1976-77 Dr. Donna E. Shalala
Associate Professor of Politics and Education
Teachers College, Columbia University
New York, NY

1977-78 Dr. Sarah Woodin
Assistant Professor, Marine Biology
Department of Earth and Planetary Sciences
The Johns Hopkins University
Baltimore, MD

1978-79 Dr. Martine Watson Brownley
Assistant Professor of English
Emory University
Atlanta, GA

1979-80 Dr. Bennetta Jules-Rosette
Associate Professor of Sociology
University of California, San Diego
La Jolla, CA

1980-81 Dr. Sandra Bem
Associate Professor
Department of Psychology
Cornell University
Ithaca, NY

1981-82 Dr. Benita Katzenellenbogen
Associate Professor
Department of Physiology and Biophysics
University of Illinois
Urbana, IL

1982-83 Dr. Phyllis Jo Baunach
Correctional Research Specialist
National Institute of Justice
Washington, D.C.

AMERICAN ASSOCIATION OF UNIVERSITY WOMEN EDUCATIONAL FOUNDATION

NOMINATION FOR AAUW RECOGNITION AWARD

To a young American woman scholar under 35 who has
demonstrated great potential for achievement

Name

Address

City State Zip

Date and place of birth

Education (institution, field of study, degree, date received)

Professional experience (position, institution or organization, dates of employment)

Honors, publications, and other professional recognition

Basis for nomination: Outstanding contributions to area of specialization
(Please attach newsclippings and other supporting materials)

Future career plans and potential

Nominated by (name and institution)

Date

May 13, 1981

MEMORANDUM

TO: J. E. Legates
Dean, School of Ag. & Life Sciences

FROM: Lawrence M. Clark
Assistant Provost

RE: Nominees for the AAUW Recognition
Award for Young Scholars

This year three excellent nominees were received from the campus, they were as follows:

1) Dr. Cathy Lee Crossland, Associate Professor of Curriculum and Instruction, School of Education

2) Dr. Cathy C. Laurie-Ahlberg, Assistant Professor of Genetics, School of Agriculture and Life Sciences

3) Dr. Sarah A. Rajala, Assistant Professor of Electrical Engineering, School of Engineering

Dr. Cathy Crossland was selected and her nomination sent forward as North Carolina State University's candidate. This decision was made because in subsequent years she would not be eligible because of her age.

Since Dr. Laurie-Ahlberg has such outstanding credentials, we would like to nominate her if we have the opportunity in the 1982-1983 academic year. At that time we would hope to update her application.

We appreciate such an outstanding nominee. We need to find ways to enhance such persons visibly, especially used for role models for other females.

CJ

May 13, 1981

MEMORANDUM

TO: Larry K. Monteith
Dean, School of Engineering

FROM: Lawrence M. Clark
Assistant Provost

RE: Nominees for the AAUW Recognition
Award for Young Scholars

We appreciate receiving the nomination of Dr. Sarah A. Rajala as a candidate from your school for the AAUW Recognition Award for Young Scholars. We had two other outstanding nominees also, they were as follows:'

1) Dr. Cathy Lee Crossland, Associate Professor of Curriculum and Instruction, School of Education

2) Dr. Cathy C. Laurie-Ahlberg, Assistant Professor of Genetics, School of Agriculture and Life Sciences

Dr. Cathy Crossland was chosen because in subsequent years she would not be eligible because of her age. We would like to update Dr. Rajala's vitae and application for a subsequent year provided we have the opportunity to make such nominations in the future.

This nominee seems to be an asset to your school and this sounds a positive beat for the School of Engineering in the age of equal opportunity.

CJ

North Carolina State University
School of Agriculture and Life Sciences

Department of Genetics
Box 5487, Raleigh, NC 27650
(919) 737-2292

April 22, 1981

Dr. Beverly A. Harden, Director
Educational Foundation Programs
American Association of University Women
2401 Virginia Avenue, N.W.
Washington, D. C. 20037

Dear Dr. Harden:

It is a great pleasure and honor for me to support the nomination of Dr. Cathy Laurie-Ahlberg for the "Recognition Award for Young Scholars" of the American Association of University Women Education Foundation for 1981-1982. Dr. Laurie-Ahlberg is a young, enthusiastic and productive scientist, and we have every indication that she will continue to be productive and in the forefront of modern genetics.

Dr. Laurie-Ahlberg received her B.S. degree (summa cum laude) in zoology in 1971 and her Ph.D. degree in genetics in 1976; both at the University of Minnesota. She was an NIH Postdoctoral Research Fellow in Genetics at the University of Wisconsin (1976-1977), and in 1977 she joined the Department of Genetics here as Assistant Professor.

Cathy's major research interests and training are in the area of experimental population genetics, particularly the extent and adaptive significance of enzyme variability, both structural and regulatory, in natural populations of Drosophila. Her research effort to date, since joining our department, has led to three published papers and two others currently in press; all in the most prestigious refereed journals in her field.

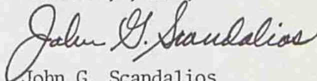
Dr. Laurie-Ahlberg has recently received a three year Research Grant from the National Institutes of Health to support her research program. In addition, she has participated in an ongoing NIH-sponsored Program Grant in quantitative genetics, which is jointly operated by the departments of Genetics and Statistics. It is not an overstatement in saying that Cathy is the most prolific experimentalist in the Program Grant and was a significant factor in its recent renewal. She has interacted very well with other members of our department. This interaction has led to her success in interfacing certain aspects of biochemical and population genetics.

Cathy, in collaboration with her associates, has recently demonstrated the existence of high levels of quantitative genetic variation in the catalytic activities of a number of enzymes in Drosophila populations. Most of the enzymes investigated show activity variations attributable to factors that are not linked to the respective structural genes. These unlinked activity modifiers identify possible "regulatory" elements. These findings will certainly open up new vistas for investigations in population and quantitative genetics.

In addition to her active research program, Dr. Laurie-Ahlberg teaches a course on "Evolution" and a minicourse on "Population Genetics." She is an extremely competent, conscientious, and popular teacher.

In general, we are very fortunate to have this young person on our faculty. She contributes positively to the intellectual atmosphere of the department, and is a pleasant and valued colleague. There is no doubt that Cathy is rapidly attaining significant stature in the area of experimental population genetics. I therefore recommend her for this honor with a great deal of enthusiasm.

Sincerely yours,



John G. Scandalios
Professor and Head
Department of Genetics

JGS:jgb

North Carolina State University

School of Agriculture and Life Sciences
Academic Affairs, Extension & Research

Office of the Dean
Box 5847, Raleigh 27650
Tel: 919-737-2668

April 24, 1981

Dr. Beverly A. Harden, Director
Educational Foundation Programs
American Association of University Women
2401 Virginia Avenue, N.W.
Washington, D. C. 20037

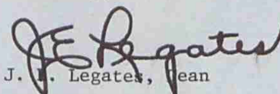
Dear Dr. Harden:

It is a distinct privilege to endorse the nomination of Dr. Cathy Laurie-Ahlberg for the "Recognition Award for Young Scholars" of the American Association of University Women Education Foundation for 1981-82. Dr. Laurie-Ahlberg has developed an imaginative and productive research program since joining our faculty in Genetics in 1977. Her research has brought into focus a new investigative arena involving biochemical and population genetics.

Dr. Laurie-Ahlberg is a dedicated scientist and teacher who contributes freely and significantly to our academic community. She is deeply committed to a scientific career, and I predict she will become an outstanding experimental population geneticist. I might add that my own field of inquiry was involved in population genetics.

We are pleased to have this opportunity to submit her nomination.

Sincerely,



J. E. Legates, Dean

JEL:bdf

AMERICAN ASSOCIATION OF UNIVERSITY WOMEN EDUCATIONAL FOUNDATION

NOMINATION FOR AAUW RECOGNITION AWARD

To a young American woman scholar under 35 who has demonstrated great potential for achievement

Name Cathy C. Laurie-Ahlberg

Address Department of Genetics, North Carolina State University

City Raleigh State NC Zip 27650

Date and place of birth August 27, 1949
Minneapolis, Minnesota

Education (institution, field of study, degree, date received)

University of Minnesota, Zoology, B.S. 1971

University of Minnesota, Genetics, Ph.D. 1976

Professional experience (position, institution or organization, dates of employment)

Jan. 1977 - Present Assistant Professor, Dept. of Genetics, NCSU
Aug. 1976 - Jan. 1977 NIH Postdoctoral Research Fellow, Dept. of Medical Genetics, University of Wisconsin-Madison
Sept. 1971 - Aug. 1976 Graduate Student, Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul

Honors, publications, and other professional recognition

B.S. awarded summa cum laude
NIH individual Postdoctoral Fellowship 1976 (declined)
(see attached list of Publications)

Basis for nomination: Outstanding contributions in area of specialization
(Please attach news clippings and other supporting materials)

She has, in a short period, developed and maintained an excellent research program. She has managed to obtain support, train graduate students and teach effectively.

Future career plans and potential

To continue her career in research and teaching. Her potential in becoming a future leader in the area of experimental population genetics is excellent.

Nominated by (name and institution)

School of Agriculture and Life Sciences
North Carolina State University

Date April 22, 1981

Cathy C. Laurie-Ahlberg
Department of Genetics
North Carolina State University

PUBLICATIONS

Laurie-Ahlberg, C. C., J. H. Williamson, B. J. Cochrane, A. N. Wilton and F. E. Chasalow. 1981. Autosomal factors with correlated effects on the activities of the glucose-6-phosphate and 6-phosphogluconate dehydrogenases in *Drosophila melanogaster*. Submitted to Genetics.

Curtsinger, J. W. and C. C. Laurie-Ahlberg. 1981. Genetic variability in flight metabolism in *Drosophila melanogaster*. I. Characterization of power output during tethered flight. Submitted to Genetics.

Laurie-Ahlberg, C. C., G. Maroni, G. C. Bewley, J. C. Lucchesi and B. S. Weir. 1980. Quantitative genetic variation of enzyme activities in natural populations of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA *77*:1073-1077.

Laurie-Ahlberg, C. C. and B. S. Weir. 1979. Allozymic variation and linkage disequilibrium in some laboratory populations of *Drosophila melanogaster*. Genetics *92*:1295-1314.

Laurie-Ahlberg, C. C. and D. J. Merrell. 1979. Aldehyde oxidase allozymes, inversions and DDT resistance in some laboratory populations of *Drosophila melanogaster*. Evolution 33:342-349.

CURRICULUM VITAE

Cathy C. Laurie-Ahlberg

Personal Data:
- Born: August 27, 1949
  Minneapolis, Minnesota
- Married, no children
- U. S. Citizen

Education:
- B. S. in Zoology, 1971, University of Minnesota, Twin Cities
- Ph.D. in Genetics, 1976, University of Minnesota, Twin Cities

Honors:
- B. S. awarded summa cum laude
- NIH Individual Postdoctoral Fellowship 1976 (declined)

Major Research Interest:

Experimental population genetics, particularly the extent and adaptive significance of enzyme variability, both structural and regulatory, in natural populations of Drosophila.

Professional Experience:

Jan. 1977 - present	Assistant Professor of Genetics; Department of Genetics, North Carolina State University, Raleigh, NC
Aug. 1976 - Jan. 1977	NIH Postdoctoral Research Fellow; Department of Medical Genetics; University of Wisconsin at Madison; Madison, Wisconsin; worked with J. F. Crow
Sept. 1971 - Aug. 1976	Graduate student; Department of Genetics and Cell Biology; University of Minnesota; St. Paul, Minnesota; Advisor D. J. Merrell

Publications:

Laurie-Ahlberg, C. C., J. H. Williamson, B. J. Cochrane, A. N. Wilton and F. E. Chasalow. 1981. Autosomal factors with correlated effects on the activities of the glucose-6-phosphate and 6-phosphogluconate dehydrogenases in Drosophila melanogaster. Submitted to Genetics.

Curtsinger, J. W. and C. C. Laurie-Ahlberg. 1981. Genetic variability in flight metabolism in Drosophila melanogaster. I. Characterization of power output during tethered flight. Submitted to Genetics.

Laurie-Ahlberg, C. C., G. Maroni, G. C. Bewley, J. C. Lucchesi and B. S. Weir. 1980. Quantitative genetic variation of enzyme activities in natural populations of Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 77:1073-1077.

Laurie-Ahlberg, C. C. and B. S. Weir. 1979. Allozymic variation and linkage disequilibrium in some laboratory populations of Drosophila melanogaster. Genetics 92:1295-1314.

Laurie-Ahlberg, C. C. and D. J. Merrell. 1979. Aldehyde oxidase allozymes, inversions and DDT resistance in some laboratory populations of Drosophila melanogaster. Evolution 33:342-349.

Current Grants:

PHS GM 11546; Quantitative Genetics Research Program
(parent grant, C. Clark Cockerham Director);
Genetics of Populations of Drosophila (subproject);
Annual direct costs for subproject $91,407.00;
Period of support: Dec.1, 1980 - Nov. 30, 1983;
50% effort.

PHS GM 28455; Genetic Variation of Flight Metabolism in Drosophila;
C. Laurie-Ahlberg, Principal Investigator;
Annual direct costs, $53,439.00;
Period of support: Feb. 1, 1981 - Jan. 31, 1984.
35% effort

North Carolina State University
School of Engineering

Department of Electrical Engineering

P. O. Box 5275
Raleigh, NC 27650

April 27, 1981

Dr. Beverly A. Harden
American Association of University
Women Educational Foundation
2401 Virginia Ave., NW
Washington, DC 20037

Dear Dr. Harden:

It is with great pleasure that I nominate Dr. Sarah A. Rajala for the 1981-82 Recognition Award for Young Scholars.

Dr. Rajala received her PhD in electrical engineering from Rice University in 1979. While at Rice she was a Rice University Fellow as well as an elected member of Sigma Xi, Tau Beta Pi, and Eta Kappa Nu. Dr. Rajala joined NCSU as an assistant professor of electrical engineering in August 1979 and has been actively involved in our teaching and research programs.

Dr. Rajala has been singled out by her students as an extremely effective instructor. She has taught several key courses in the department and has participated in the development of new courses. In particular, she has played a major role in the development of a new course on VLSI design in which students have designed state-of-the-art integrated circuits which are presently being fabricated by GE.

Her research work has resulted in four paper presentations at various conferences and symposia since 1979. She has participated actively on various research programs and has been, or is currently, co-principal investigator on two projects.

"A Study of Real-Time Image Graphics Display Technology for Aeronautical Applications," NASA-Langley Research Center, $25,014

"Computer Analysis of Time-Varying Imagery," Army Research Office, $158,448

She has played a leadership role in the ongoing development of the computing facilities for VLSI design for the Microelectronics Center of North Carolina. She is presently serving as co-chairman

of the committee developing VLSI design capabilities in North Carolina.

Dr. Rajala has played a leadership role in recruiting qualified female students into engineering both formally through the Society of Women Engineers and informally through personal contacts. She is an exemplary role model for future female engineers and has demonstrated a true commitment to promoting engineering as an exciting and rewarding career for women.

In summary, Dr. Rajala shows tremendous potential for achievement in her professional field. I am proud to have such an outstanding and dedicated individual on our faculty, and I take great pleasure in submitting her name for consideration for the 1981-82 Recognition Award for Young Scholars.

Sincerely,

N. A. Masnari

N.A. Masnari
Department Head

NAM:hwj

Old Heritage
GRADE
Bond
25% COTTON FIBRE

North Carolina State University
School of Engineering

Office of the Dean

P. O. Box 5518
Raleigh, N. C. 27650

April 29, 1981

Dr. Beverly A. Harden, Director
Educational Foundation Programs
American Association of University Women
Educational Foundation
2401 Virginia Avenue, NW
Washington, DC 20037

Dear Dr. Harden:

I am pleased to support the nomination of Dr. Sarah A. Rajala for the 1981-82 Recognition Award for Young Scholars.

Dr. Rajala joined NCSU as an assistant professor of electrical engineering in August 1979 and has already made major contributions in both teaching and research at the University. Her classes have consistently rated her an outstanding instructor. She has also worked effectively directing individual students on special projects. She has demonstrated an ability to communicate with students and has earned their respect. Her research activities have resulted in several papers being presented at various meetings during the past two years. She has been a co-principal investigator on various research projects amounting to over $180,000 since joining NCSU.

Dr. Rajala has been one of the moving forces in the development of the VLSI design activities within the Microelectronics Center of North Carolina. Not only is she serving as co-chairman of an important committee developing VLSI design capabilities in North Carolina, but in collaboration with other faculty members she has introduced a VLSI course into our curriculum. She has also played a major role in attracting female students into engineering.

Dr. Rajala has all of the attributes necessary for making major contributions in her professional field. I am pleased to support her nomination for the Recognition Award.

Sincerely,

Larry K. Monteith

Larry K. Monteith
Dean of Engineering

Neenah Bond
25% COTTON FIBER

D R A F T

Dr. Beverly A. Harden
American Association of University
Women Educational Foundation
2401 Virginia Ave., NW
Washington, DC 20037

Dear Dr. Harden:

I am pleased to recommend Dr. Sarah A. Rajala for the 1981-82 Recognition Award for Young Scholars.

Since joining NCSU in August 1979 Dr. Rajala has been a leader in the teaching and research programs of the university. She has also played a major role in the development of the microelectronics programs in the state of North Carolina through her involvement in the Microelectronics Center of North Carolina. She has worked closely with various organizations to promote engineering as a rewarding professional career for women. Dr. Rajala has clearly established an ability to relate to students and to guide them through their academic careers both in the classroom and in directed research projects. She shows promise for outstanding achievement in the field of electrical engineering, and NCSU is proud to submit her name for consideration for the 1981-82 Recognition Award.

AMERICAN ASSOCIATION OF UNIVERSITY WOMEN EDUCATIONAL FOUNDATION

NOMINATION FOR AAUW RECOGNITION AWARD

To a young American woman scholar under 35 who has demonstrated great potential for achievement

Name Sarah A. Rajala

Address Elec. Engr. Dept. - PO Box 5275
North Carolina State University
City Raleigh State NC Zip 27650

Date and place of birth 5/10/53 Skandia, Michigan

Education (institution, field of study, degree, date received)

Michigan Technological University, Electrical Engineering, BS, 1974
Rice University, Electrical Engineering, MS, 1977
Rice University, Electrical Engineering, PhD, 1979

Professional experience (position, institution or organization, dates of employment)

Rice University Fellow, Rice University, 1974-79
Engineering Assistant, General Electric, 6/73-8/73

Honors, publications, and other professional recognition

SEE ATTACHED

Basis for nomination: Outstanding contributions in area of specialization
(Please attach news clippings and other supporting materials)

SEE ATTACHED

Future career plans and potential

SEE ATTACHED

Nominated by (name and institution)

Date

Honors, publications, and other professional recognition

ACADEMIC HONORS

Rice University Fellow, Sigma Xi, Tau Beta Pi, Eta Kappa Nu

PUBLICATIONS

Discrete Nonlinear Inverse Filtering, Masters' Thesis, April 1977, Rice University, R.F.P. de Figueiredo, advisor.

Adaptive Nonlinear Image Restoration by a Modified Kalman Filtering Approach, Ph.D. Thesis, June 1979, Rice University R.J.P. de Figueiredo, advisor.

R.J.P. de Figueiredo and S.A. Rajala, "An Approach to Cancellation of Nonlinear Distortion in Communication Networks," IEEE International Symposium on Circuits and Systems, New York, NY, May 17-19, 1978.

R.J.P. de Figueiredo, R.W. Wendt, and S.A. Rajala, "Nonlinear Equalizers for Solid State Devices and Circuits," IEEE International Symposium on Circuits and Systems, Tokyo, Japan July 17-19, 1979.

S.A. Rajala and R.J.P. de Figueiredo, Adaptive Nonlinear Image Restoration by a Modified Kalman Filtering Approach, Technical Report EE - 7904, Rice University, June 1979.

S.A. Rajala and R.J.P. de Figueiredo, "An Approach to Adaptive Nonlinear Image Restoration," 1980 ICASSP, Denver, Colorado.

S.A. Rajala and W.E. Snyder, "Image Modeling the Continuity Assumption and Tracking", The International Conference on Pattern Recognition, Miami, December 1980.

Basis for nomination

- Outstanding contributions in teaching

  EE 201, Electric Circuits I, FA 79, FA 80

  EE 302, Numerical Applications in Elec. Engr., SS 80

  EE 435, Elements of Control, FA 80

  EE 492, Special Topics in Elec. Engr., SP 80, SP 81

  EE 592, Special Topics in Elec. Engr., SP 80

  EE 559, Pattern Recognition, SP 81

- Leadership role in the development of facilities for VLSI design in the Microelectronics Center of North Carolina

- Co-principal investigator on two research programs

  "A Study of Real-Time Image Graphics Display Technology for Aeronautical Applications," NASA-Langley Research Center, $25,014

  "Computer Analysis of Time-Varying Imagery," Army Research Office, $158,448

Future career plans and potential

Dr. Rajala is interested in applications and fundamental research in the areas of image processing and digital signal processing and communications. Her recent research efforts have included analysis of time-varying imagery with applications in segmentation and tracking and development of software and hardware for a real-time graphic display system. Her evolving research interests include the development of more exact techniques for the restoration of two-dimensional images. This activity will focus on research in feature extraction, pattern recognition and human visual system responses. She is presently developing research and teaching plans in the area of VLSI design as part of the Microelectronics Center of North Carolina.

Dr. Rajala has committed herself to an academic career in electrical engineering.

May 5, 1981

MEMORANDUM

TO: Mrs. Linda Nipper
Assistant to the Chancellor

FROM: Larry Clark
Assistant Provost

SUBJECT: Campus Nominations for the 1981-82 Recognition Award for Young Scholars of American Association of University Women Educational Foundation

We have received from the schools three nominations for this award. The nominees are as follows:

(1) Dr. Cathy Lee Crossland, Associate Professor of Curriculum and Instruction, School of Education

(2) Dr. Cathy C. Laurie-Ahlberg, Assistant Professor of Genetics, School of Agriculture and Life Sciences

(3) Dr. Sarah A. Rajala, Assistant Professor of Electrical Engineering, School of Engineering

The Provost and his staff have reviewed the supporting data on each, and recommend that Chancellor Thomas place in nomination Dr. Cathy Crossland as NCSU's candidate.

Attached is the necessary supporting information for Dr. Crossland which includes,

a) A draft letter of nomination for the Chancellor
b) Two supporting letters
c) Application form completed, and
d) Dr. Crossland's resume

Attached also is background information on the other two campus nominees, Chancellor Thomas may care not to review information on these two. If you have any questions please contact me.

MN
Attachments

John G. Scandalios
North Carolina State University
Department of Genetics
Box 5487, Raleigh, North Carolina 27650

Dr. Beverly A. Harden, Director
Educational Foundation Programs
American Association of University Women
2401 Virginia Avenue, N.W.
Washington, D. C. 20037

North Carolina State University
School of Agriculture and Life Sciences
Office of the Dean
Box 5847, Raleigh, North Carolina 27650

Dr. Beverly A. Harden, Director
Educational Foundation Programs
American Association of University Women
2401 Virginia Avenue, N.W.
Washington, D. C. 20037

DRAFT

Dr. Beverly A. Harden, Director
Educational Foundation Programs
American Association of University Women
2401 Virginia Avenue, N.W.
Washington, D. C. 20037

Dear Dr. Harden:

I wish to nominate Dr. Cathy Laurie-Ahlberg, Assistant Professor of Genetics, North Carolina State University, for the "Recognition Award for Young Scholars" of the American Association of University Women Education Foundation for 1981-82. This nomination is supported by Dr. J. E. Legates, Dean, School of Agriculture and Life Sciences, and by Dr. J. G. Scandalios, Head, Department of Genetics.

ok JEL

Dr. Laurie-Ahlberg is a productive and enthusiastic young scientist and is the top candidate for this honor from our University. She reflects her profession and the University in a most positive manner by her scholarly contributions and her personal integrity.

For these reasons, and for those stated in the attached supporting letters and credentials, we recommend Dr. Laurie-Ahlberg for this honor very enthusiastically, and ask that she be given serious consideration.

Sincerely,

Joab L. Thomas
Chancellor

North Carolina State University

Department of
Curriculum and Instruction
402 Poe Hall, Box No. 5096
Raleigh, N. C. 27650
Phone: (919) 737-3221

School of Education

April 29, 1981

MEMORANDUM

To: Dr. Clark

From: Barbara Parramore

Re: AAUW Nomination for Young Scholar's Award

In response to your memo of April 10 the Administrative Council of the School of Education decided to forward Dr. Cathy L. Crossland' name to you for the campus nomination. The appropriate nomination form and supporting letters are attached.

Thank you for this opportunity to present Dr. Crossland for consideration.

cc: Dean Dolce

North Carolina State University
P. O. Box 5067, Raleigh, N. C. 27650

Office of the Provost
and Vice-Chancellor

April 10, 1981

MEMORANDUM

TO: School Deans

FROM: Lawrence M. Clark
Assistant Provost

RE: 1981-82 Recognition Award for
Young Scholars

The American Association of University Women Educational Foundation is inviting women nominees for the 1981-82 Recognition Award for Young Scholars. The woman selected will be honored at the AAUW State President's Conference to be held in Washington, D.C. in June 1982. This award carries an honorarium of $1,000.

Along with the enclosed form, three letters of recommendation are required. These letters should be from the Department Head, the Dean and the Chancellor. The nominee for the award should meet the following conditions:

1. Less than 35 years of age at the time of nomination.

2. Earned doctorate or its equivalent.

3. At least one outstanding contribution in the academic and/or professional field of her choice.

4. Potential for achievement (indicated by past accomplishments and letters of recommendation) in an academic discipline or professional field.

If you have such a candidate, would you please prepare the appropriate supporting letters, including a draft for the Chancellor and forward to me by April 25, 1981. After all nominees on campus are submitted to the Chancellor, he will select one to be forwarded as NCSU's nominee.

CJ
cc: Dr. Nash N. Winstead

DRAFT

Dr. Beverly A. Harden, Director
Educational Foundation Programs
American Association of University Women
2401 Virginia Avenue, N.W.
Washington, D. C. 20037

Dear Dr. Harden:

I am pleased to nominate Dr. Cathy Lee Crossland, Associate Professor of Education and Coordinator of the Graduate Program in Special Education at North Carolina State University, for the 1981-82 Recognition Award for Young Scholars to be given by the American Association of University Women Educational Foundation. This nomination is supported by Dr. Carl J. Dolce, Dean, School of Education, and by Dr. Barbara M. Parramore, Head, Department Curriculum and Instruction.

Dr. Crossland is a productive and enthusiastic young educator and scholar and is the top candidate for this honor from our University. She reflects her profession and the University in a most positive manner by her service and scholarly contributions and her personal integrity. For these reasons, and for those stated in the attached supporting letters and credentials, we recommend Dr. Crossland for this honor very enthusiastically, and ask that she be given serious consideration.

Sincerely,

Jeab L. Thomas
Chancellor

CC: Provost Nash Winstead
Dean Carl J. Dolce
Dr. Barbara M. Parramore
Dr. Cathy L. Crossland

Draft of Chancellor's Letter

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DRAFT (for Chancellor's letter)

Dr. Beverly A. Harden, Director
Educational Foundation Programs
American Association of University Women
2401 Virginia Avenue, N. W.
Washington, D. C. 20037

Dear Dr. Harden:

It is a pleasure for me, as Chancellor, to place in nomination the name of Dr. Cathy Lee Crossland for the 1981-82 Recognition Award for Young Scholars to be given by the American Association of University Women Educational Foundation. Dr. Crossland, Associate Professor of Education and Coordinator of the Graduate Program in Special Education at North Carolina State University, has made a significant contribution to her field, to the State of North Carolina, to the University and has given evidence of the potential for making a rather special contribution in years to come in selected areas in which she has chosen to work.

Professor Crossland is a respected member of our faculty not only in the School of Education but across campus. During 1980 she served ably and well on a special University Committee to study the administrative structure for research and graduate education. At graduation on May 16 she will be given University recognition as an outstanding teacher, having been nominated by students and selected for this honor by students, faculty and alumni.

As major universities have tried to be responsive to the most pressing social and economic problems of the larger society these institutions have discovered that abstract knowledge must be made useful through a creative process of interpretation that will ensure effective application by

those who have the responsibility for managing these problems in the world of practice. Furthermore, the acquisition of concrete knowledge about these problems, as well as interventions intended to ameliorate them, require a close cooperation between university faculty and those in the practical world who have these responsibilities. Sometimes young faculty members find the work of knowledge application neither interesting nor rewarding. The risks to young faculty are great. This sort of work requires a certain level of maturity and the self-confidence that one's skills are sufficient to meet the demands of the university's constituency. Moreover, one has to have the belief in the ultimate importance of these extra-university affiliations.

Dr. Crossland is an unusual faculty member in that she has not only taken on the direction of an educational program that is receiving considerable recognition in her school, the larger University of North Carolina System, and the Nation, but she has launched a set of programmatic applications of her discipline to a cluster of societal problems that have only begun to be recognized and dealt with throughout the United States.

My major comment about Professor Crossland is about her leadership in policy development for special education programs in the State of North Carolina. She has served for two years, at the request of the President of the sixteen-campus University of North Carolina system, as the chairperson of the Cooperative Planning Consortium of Special Education Training Programs which represents the fourteen public institutions and eleven private institutions with training programs in special education in North Carolina. This group has conducted studies, prepared position papers,

drafted guidelines for teacher certification and teacher training programs and assured the availability of high quality teacher training programs across the State. The provision of appropriate and adequate educational services for special needs students in the public schools of North Carolina has been a primary goal and Dr. Crossland has made a substantial contribution to this goal. The entire State's educational community is in her debt.

Among other important statewide appointments, Dr. Crossland has been a member of the Governor's Task Force on Mental Retardation. She is a frequent consultant to our State General Assembly and the North Carolina Department of Public Instruction. In taking her expertise and ideas outside the University Dr. Crossland has shown an impressive degree of skill in working with persons from other human services fields, including the field of medicine.

When a young faculty member demonstrates the courage to extend her efforts beyond her own discipline, and when this same faculty member demonstrates both a willingness and a high degree of skill in working beyond the walls of the university in partnership with persons in other disciplines who seek practical solutions to concrete problems, such a faculty member is indeed rare. Dr. Crossland is a person who has demonstrated a keen sense of what a University is all about -- what its role in the larger society ought to be. She has employed her considerable administrative talents in the development of a strong academic program here on campus and she has taken her program into the surrounding community, State and region and developed a model of knowledge application that is targeted on a set of problems of

critical importance to those who deal with exceptional children.

I am pleased to offer the name of Dr. Cathy Crossland for your consideration for this national award to be presented in recognition of past contributions in an academic or professional field and a demonstrated potential for future accomplishment. No one better exemplifies these qualities than this nominee. The nomination form shows the breadth of Professor Crossland's achievements which reflect a dedication to scholarship and service to her profession unequalled by others of her rank and experience. She represents a young American scholar who has demonstrated great potential for achievement. We commend her to you and the selection committee.

Yours truly,

Joab L. Thomas
Chancellor

RECIPIENTS OF AAUW ACHIEVEMENT AWARD and of

RECOGNITION AWARD FOR YOUNG SCHOLARS

1973-80

	Achievement Award	Recognition Award for Young Scholars
1972-73	Dr. Helen H. Nowlis Director, Drug Abuse Education US Office of Education* *On leave from University of Rochester, Rochester, NY	Mary Eva Swigar, M.D. Assistant Professor Department of Psychiatry in Obstetrics and Gynecology Yale University School of Medicine New Haven, CT
1973-74	Dr. Mamie Phipps Clark Executive Director, Northside Center for Child Development New York, NY	Dr. Susan M. Hartmann Associate Professor of History University of Missouri St. Louis, MO
1974-75	The Honorable Edith Green Representative United States Congress Washington, DC	Dr. Donella H. Meadows Assistant Professor of Environmental Studies Dartmouth College Hanover, NH
1975-76	Dr. Dixy Lee Ray Assistant Secretary of State for Oceans and International Environmental and Scientific Affairs US Department of State Washington, DC	Dr. Edna Bonacich Assistant Professor of Sociology University of California, Riverside Riverside, CA
1976-77	Dr. Jessie Bernard Visiting Scholar US Civil Rights Commission Washington, DC	Dr. Donna E. Shalala Associate Professor of Politics and Education Teachers College, Columbia University New York, NY
1977-78	Dr. Gisela Konopka Director of Center for Youth Development and Research Professor of Social Work University of Minnesota St. Paul, MN	Dr. Sarah Woodin Assistant Professor, Marine Biology Department of Earth and Planetary Sciences The Johns Hopkins University Baltimore, MD
1978-79	Dr. Margaret Mead Anthropologist American Museum of Natural History New York, NY	Dr. Martine Watson Brownley Assistant Professor of English Emory University Atlanta, GA
1979-80	Dr. Hanna H. Gray Historian and University President University of Chicago Chicago, IL	Dr. Sandra L. Bem Psychologist, Associate Professor of Psychology and Women's Studies Cornell University Ithaca, NY

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GENETIC VARIABILITY OF FLIGHT METABOLISM IN DROSOPHILA MELANOGASTER

I. CHARACTERIZATION OF POWER OUTPUT DURING TETHERED FLIGHT[1].

James W. Curtsinger[2] and Cathy C. Laurie-Ahlberg

Department of Genetics

North Carolina State University

Raleigh, N.C. 27650

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2. Present address: Department of Zoology, University of Texas, Austin, Texas 78712.

RUNNING HEAD: FLIGHT METABOLISM IN DROSOPHILA

Address editorial correspondence to Curtsinger at Texas

ABSTRACT

The mechanical power imparted to the wings during tethered flight of *Drosophila melanogaster* is estimated from wingbeat frequency, wing stroke amplitude and various aspects of wing morphology by applying the steady-state aerodynamics model of insect flight developed by Weis-Fogh (1972,1973). Wingbeat frequency, the major determinant of power output, is highly correlated with the rate of oxygen consumption. Therefore, estimates of power generated during flight are expected to closely reflect rates of ATP production in the flight muscles, since flies do not acquire an oxygen debt or accumulate ATP during flight. In an experiment using 21 second chromosome substitution lines, lines were a significant source of variation for all flight parameters measured. Broad-sense heritabilities ranged from 0.16 for wing stroke amplitude to 0.44 for inertial power. The variation among lines is not explained by variation in total body size (i.e. live weight). Line differences in flight parameters are robust with respect to age, ambient temperature and duration of flight. These results indicate that characterization of the power output during tethered flight will provide a sensitive experimental system for detecting the physiological effects of variation in the structure or quantity of the enzymes involved in flight metabolism.

INTRODUCTION

It is a fundamental tenet of modern evolutionary theory that natural selection directs the process of gene frequency change only indirectly, by discriminating among phenotypes, rather than genotypes. The relationship between genotypic, phenotypic, and fitness variation is obscure; in fact, it is seldom possible to describe the effect of a single gene substitution on a fitness-related quantitative character. Here we present the first of a series of studies, the ultimate goal of which is to describe the way that variation in the structure and quantity of some specific gene products, the enzymes involved in flight metabolism, causes variation in a fitness-related metrical phenotype, the power output of the flight muscles.

The flight metabolism of *Drosophila melanogaster* is a desirable experimental system for the detection of physiological effects of variation in enzyme structure and regulation for several reasons:

(1) The metabolic pathways are well characterized, and the specific functions of many enzymes are known. Of the more than 30 enzymes with well-defined roles in generating ATP for flight in the Diptera (Sacktor, 1964, 1970, 1974, 1975; Crabtree and Newsholme, 1975), twelve have been assayed and genetically mapped in *D. melanogaster* (O'Brien and MacIntyre, 1978; Voelker *et al*, 1978; Oliver, Huber and Williamson, 1978).

(2) The many biophysical investigations of insect flight provide a basis for estimating the mechanical power imparted to the wings during flight. Weis-Fogh (1972, 1973) has presented formulae for the power output of the flight muscles that derive from a model of the hovering or slow forward flight practiced by many small insects, including *Drosophila*.

The relevant parameters are listed in Table 1.

(3) The flight muscles of the Diptera, including Drosophila, exhibit metabolic rates that are among the highest known in any tissue (Sacktor, 1965). As a result, the rate of ATP production by the flight muscles is expected to be very sensitive to functional differences among enzyme variants.

(4) The power output of the flight muscles is at least potentially a fitness-related phenotype. Since flight behavior is an integral part of feeding, mating, dispersal, and oviposition, it is likely, though not demonstrated, that variation in power output is ultimately related to variation in reproductive success.

The bulk of this paper is devoted to describing our methods of characterizing the power output of the flight muscles. We also present evidence that the flight variables measured reflect the rate of ATP production in the flight muscles, and are subject to genetic modification. These are preliminary, but critical, steps towards relating genetic variation in enzyme activity and structure to the flight phenotype.

A brief description of some of this work has appeared elsewhere (Curtsinger and Laurie-Ahlberg, 1980).

MATERIALS AND METHODS

Experimental stocks: Flies used were from isogenic second chromosome substitution lines, the origin and construction of which has been described by Laurie-Ahlberg et. al. (1980). The isogenic lines are homozygous for the same X and third chromosomes, but are homozygous for different second chromosomes derived from natural populations. Stocks were routinely maintained at 25° C. on cornmeal-molasses medium.

Flight observations: Unless otherwise stated, subjects for flight observation were six-day old males reared at 25° C. on a 12-12 light-dark cycle, and observed within four hours of incubator "dawn". Flies were lightly etherized and tethered to a fine syringe-cleaning wire, approximately .10 mm in diameter, with Permount histological mounting medium (Wigglesworth, 1949). The wire was attached to the mesonotum perpendicular to the long axes of the body and the extended wing, and did not appear to interfere with wing movement in that position. Approximately 85% of the several thousand tethered individuals observed began beating the wings spontaneously after recovering from the ether.

Tethered "flight" was observed in a temperature-controlled stock room, 25° C., relative humidity 61-68%. The long axis of the body was positioned horizontally in the viewing field of a 16x dissecting microscope. Wingbeat frequency was measured by adjusting the flashing rate of a variable speed stroboscope (General Radio Model GR 1531-AB "Strobotac") until a single stationary image of the wings was observed. Any integer sub-multiple flashing rate of the stroboscope relative to the true wingbeat frequency results in a single wing image. The true wingbeat frequency is

estimated as the fastest flashing rate of the stroboscope that gives a single wing image. The stroboscope was calibrated with a crystal-controlled frequency counter and found to be accurate within .1% in the range $115\ s^{-1}$ to $250\ s^{-1}$. Wingbeat frequencies were recorded to the nearest $1.67\ s^{-1}$.

Under constant illumination the movement of the wings creates an envelope of blurred motion, the boundaries of which are clearly visible under slight magnification from a frontal view. Wing tips invariably meet at the top of the wing stroke, generally directly above the thorax, but the lower boundary varies among individuals. The wing stroke amplitude, which is the angle corresponding to the arc described by the wing tip during a complete stroke, was measured on both sides with a camera lucida and protractor, to the nearest 5°. Flight observations were always completed within one hour from the time the individual was tethered.

Wing measurements: After the flight observations, flies were removed from the tethering wire and one wing was clipped off at the thorax with micro-scissors. Each wing was mounted between two microscope slides sealed with transparent tape, magnified 50X with a profile projector (Ehrenreich Photo-Optical Industries Model LP-2), and traced. The coordinates of the points of intersection of the wing tracing and several transects taken perpendicular to the long axis of the wing at 1/2" intervals were entered into a mini-computer with a Tektronics 4956 Graphics Tablet. The transects divided the wing into two triangles and 6-8 trapezoids (as shown in Figure 1). The height of the distal triangle was between 1/10 and 2/10 inch. The height of the distal trapezoid, which was usually less than 1/2 inch, was

determined by the length of the wing. The origin or point of rotation of the wing was assumed to lie inside the thorax, 1/10" from the basal termination of the wing membrane on the tracing. The total wing length, L, is the distance from the origin to the tip. Wing width, W_5 in Tables 1 and 2, is the width of the fifth transect.

An approximate expression for the wing chord at a perpendicular distance r from the origin, c(r), can be obtained by linear interpolation as the width of the trapezoid or triangle bounded by the wing chords $c(R_i)$ and $c(R_{i-1})$ (where $R_{i-1}<r<R_i$, see Figure 1). Then the area (A) and the second and third moments of the area (S and T) can be calculated for each segment by solving the following integral in closed form, where y equals 1, r^2 or r^3 for A, S or T, respectively:

$$\int_{R_{i-1}}^{R_i} c(r)\, y \, dr \qquad (1)$$

The sum over segments then gives the area and the moments for the whole wing.

Respirometry: Oxygen consumption rates were measured on single tethered flies with a horizontal capillary - differential syringe manometer (Roger Gilmont Instruments, Inc., Model W-4200), modified as described by Peterson et. al. (1967). The tethering wire was mounted in a cork that fitted snugly in the well of a reaction vessel. Manometric fluid was 0.2% Liquinox in distilled water. CO_2 absorption medium was 10% KOH on Whatman #40 filter paper. Measurements were taken in a water bath at 25° C. With slight magnification, it is possible to observe the movement of the wings in the submerged reaction vessel, thus allowing simultaneous measurement of

oxygen consumption rate and wingbeat frequency.

Power calculations: Aerodynamic, inertial, and total power were calculated as described by Weis-Fogh (1972, 1973), with some simplifications, as follows:

Referring to the symbols defined in Table 1, the aerodynamic power, which is the power required to overcome drag on the wing, is

$$P_a = \left(\frac{2}{3}\right) \rho_a \, C_d \, T \, \Pi^2 \, WBF^3 \, \theta^3 \qquad (2)$$

See Weis-Fogh, 1973, equation (19). Here we assume that ρ_a and C_d are constants, where $\rho_a = 1.17 \ kg\,m^{-3}$ is the density of moist air at 25° C. and $C_d = 0.6$. The estimate of C_d, which depends on shape, comes from the work of Vogel (1967b) on D. virilis. All other variables in equation (2) are estimated from observations on tethered flies and wing measurements.

The general expression for inertial power, which is the power required to decelerate and accelerate the wing mass at the top and bottom of each stroke, follows directly from Weis-Fogh's (1973) equation (20), and is explicitly derived by Alexander (1977):

$$P_i = 2 \, I \, \Pi^2 \, WBF^3 \, \theta^2 \qquad (3)$$

Here I is the mass moment of inertia, defined by equation (4) where ρ_w is the mass density of the wings, c(r) is the wing chord at a distance r from the axis of rotation, D(r) is the thickness of the wing at that distance r, and L is the total length of the long axis of the wing.

$$I = \rho_w \int_0^L c(r)\, D(r)\, r^2\, dr \qquad (4)$$

We assume that $\rho_w D$ is a constant, estimated to be 4.0×10^{-4} kg m^{-2} from the weight and area of twenty wings. It then follows from the definition of the second moment of the wing area that $I = \rho_w DS$. Thus the equation directly used to estimate inertial power is

$$P_i = 2\, \rho_w\, D\, S\, \Pi^2\, WBF^3\, \Theta^2 \qquad (5)$$

Total power, which includes both aerodynamic and inertial components, is estimated using Weis-Fogh's (1973) equations (23) and (33). The approximation of total power gives a measure in arbitrary units and depends on N, the ratio of maximum aerodynamic and inertial bending moments:

$$N = \frac{4\, \rho_w\, D\, S}{\rho_a\, C_d\, T\, \Theta} \qquad (6)$$

The total power imparted by the thorax to the wings is then

$$P_t = WBF \left[\frac{6N^2 + 13N + 14}{12(N+1)} \right] \qquad (7)$$

RESULTS

Characteristics of tethered flight

Long flights: Continuous flights of six-day old males can last up to four hours, as shown in Figure 2. Flies assume a characteristic posture in tethered flight, with the prothoracic and mesothoracic legs tucked against the body, and the metathoracic legs extended behind and parallel to the abdomen.

WBF is relatively constant during the first hour of long flights. Rates of change of WBF during that period have been observed to lie in the range $-2\times10^{-3}s^{-2}$ to $7\times10^{-4}s^{-2}$, based on observation of 25 males from six isogenic lines at five minute intervals. In no cases were the regression coefficients of WBF on time significantly different from zero for the first hour of tethered flight. A similar constancy of WBF has been reported for *D. funebris* (Williams, Barnes, and Sawyer, 1943), but other species of *Drosophila* show a rapid decline of WBF during tethered flight (Chadwick and Gilmour, 1940; Chadwick, 1947).

Following the initial period of relative constancy, WBF gradually declines to about 120 s^{-1}, at which point wing beating stops. Wigglesworth (1949) showed that flies in this state of "exhaustion" can be induced to beat the wings briefly by feeding specific carbohydrates, suggesting that depletion of metabolic reserves is a limiting factor in the later stages of long flights.

The total wing stroke amplitude does not vary measurably during long flights, even during the period of WBF decline, suggesting that WBF and wing stroke amplitude are independently controlled (Chadwick, 1953). However, the amplitude on the left or right side can change during a long flight, possibly as a result of attempts to turn on the tethering wire.

Ontogeny of WBF: One-hundred and seventy-two males from six isogenic lines were collected at emergence, aged under standard conditions for one to eight days, and then tethered for three replicated WBF observations. The line means and overall means for each age group are shown in Figure 3. WBF increases during the first and second days of the imago, and is otherwise independent of age over the range tested. Analysis of variance omitting days one and two gives a highly signifigant line effect ($F = 38.1$, $P < .001$) but no statistically significant effect of age or line x age interaction.

Temperature dependence: WBF of thirty-eight individuals from three isogenic lines all reared at 25°C were observed at each of three temperatures, with three replications per individual per temperature. Flies were allowed at least one minute to acclimate to temperature shifts, but the WBF change was almost immediate. Analysis of variance shows a highly significant line effect and temperature effect, but no significant line x temperature interaction. Results are shown in Figure 4. In a similar experiment with outbred flies, 258 individuals from 40 iso-female lines were each observed at 15°, 20°, 25°, and 30°C, with three replications per individual per temperature. The empirically determined lower limit of flight for *D. melanogaster* is near 15°C. Analysis of variance again shows highly significant line and temperature effects, but no significant line x temperature interaction.

Oxygen consumption rate: WBF and oxygen consumption rates were measured on twenty-eight individuals from four isogenic lines. The results are shown in Figure 5, where each point represents the average of at least two WBF observations and two corresponding O_2 consumption rates measured on single individuals for five minute or greater intervals. The relatively small sample size is due to frequent difficulty in stimulating flight once the fly is mounted in an air-tight reaction vessel submerged in a water bath. Chadwick and

Gimour (1940) and Reed *et al* (1942) have constructed simple models of flight energetics that predict a positive correlation between WBF^2 and O_2 consumption (or work) per wing stroke. If the work or oxygen consumption per stroke is proportional to the second power of the WBF, then the work or consumption per unit time is expected to vary with WBF^3. That prediction has been verified in observations on *D. virilis*, *D. americana*, and *D. repleta* (Chadwick and Gilmour, 1940;Chadwick, 1947). Our observations on *D. melanogaster* show proportionality between rates of O_2 consumption and WBF, WBF^2, and WBF^3 as equally acceptable hypotheses: all three product moment correlation coefficients lie in the range 0.82 - 0.84 ($p < .001$).

We were unable to obtain reliable estimates of wing stroke amplitude on flies tethered in the respirometer. Consequently, it is not possible at this time to test hypotheses concerning the relationship bewteen power output and O_2 consumption.

A multiple line study

Design: A set of observations consists of tethering one six-day old male from each of 21 isogenic second-chromosome substitution lines, recording WBF and wing stroke amplitude from those that start wing-beating spontaneously, and recording the wing size and shape parameters from one wing from each of the fliers. WBF and wing stroke amplitude were recorded with three replications at one minute intervals for each flier, which were averaged to obtain one value per variable per fly for the analyses reported here. Sixty-four sets were observed, four per day, four days a week, for four weeks. The flies for each week of observations were reared in a different set of four half-pint bottles. The flies used on each of the four days within each week came from the same set of four bottles but differ in time of emergence from those bottles. Because of variations in the number of spontaneous fliers, the resulting data structure is unbalanced.

Analyses of Variance: A total of 1166 fliers were observed, with 46 to 63 observations per line. Line means are shown in Table 2, where symbols and units are as defined in Table 1. The wing stroke amplitude reported in Table 2 is the average of the left and right sides, in radians. The live weights reported in Table 2 are based on 10 males reared simultaneously with the flies tethered for flight observations, with 16 replications (one for each day of flight observations). The distribution of line means for each of the variables is approximately continuous except for WBF and the three powers that depend on it, because of a single outlier (Figure 6). The WBF outlier, KA16, has been excluded from all the analyses involving WBF, P_a, P_i, and P_t.

The model equation for analysis of the multiple line study is:

$$Y_{ijklm} = \mu_i + \alpha_{ij} + \beta_{ik} + (\alpha\beta)_{ijk} + \gamma_{il} + (\alpha\gamma)_{ijl} + (\beta\gamma)_{ikl} + (\alpha\beta\gamma)_{ijkl} + \epsilon_{ijklm}$$

where Y corresponds to the value of the ith variable in the jth week, k^{th} day, l^{th} line and m^{th} fly; i = 1, ... 10, corresponding to the ten variables listed in Table 2 other than Wt, j and k = 1, ... 4, l = 1, ... 21, and m = 1, 2, 3, or 4 (unbalanced). For the analysis of variance of the raw data, the method of unweighted means was used (Neter and Wasserman, 1974, p. 615).

In addition to the analysis of raw data, weight-adjusted data were also tested, in order to remove any general body size effects. Linear regressions of $\bar{Y}_{ijkl\cdot}$ on weight (Wt_{jkl}) were performed over lines for each of the 16 week x day combinations and the sums of products were pooled to obtain a single estimate of the regression coefficient for each dependent variable (b_i). Adjusted variables were then obtained by the expression $\hat{Y}_{ijkl} = \bar{Y}_{ijkl\cdot} - b_i(Wt_{jkl})$. θ was not weight-adjusted because no overall correlation between those two variables was observed. All other flight variables were positively and significantly correlated with weight (see below). Analysis of the weight-adjusted variables follows the model equation above, but the $(\alpha\beta\gamma)_{ijkl}$ and ϵ_{ijklm} effects are, of course, confounded.

Significance tests and variance components are shown in Table 3, for both raw and adjusted data. Because all five wing morphology variables are very highly correlated (see below), only the analysis for wing area is given. The last columns of Table 3 show two kinds of variance component ratio: K, defined below, and H^2, the usual broad sense heritability.

$$H^2 = \hat{\sigma}^2_l / (\hat{\sigma}^2_l + \hat{\sigma}^2_{wl} + \hat{\sigma}^2_{dl} + \hat{\sigma}^2_{wdl} + \hat{\sigma}^2_e)$$

where $\hat{\sigma}^2_l$ is the estimated line variance component, $\hat{\sigma}^2_{wl}$, $\hat{\sigma}^2_{dl}$ and $\hat{\sigma}^2_{wdl}$ are the components for the interactions involving lines and $\hat{\sigma}^2_e$ is the variance among individuals within a week x day x line combination.

$$K = \hat{\sigma}^2_l / (\hat{\sigma}^2_l + \hat{\sigma}^2_{wl} + \hat{\sigma}^2_{dl} + \hat{\sigma}^2_{wdl} + \hat{\sigma}^2_{\bar{e}})$$

where $\hat{\sigma}^2_{\bar{e}}$ is the error variance of the week x day x line means. K is the proportion of variance among the week x day x line means corrected for week and day effects that is attributable to lines; it was computed in order to provide a measure of the effect of weight-adjustment on the line component of variance.

Lines are a highly significant component of variance for all the flight variables as well as for weight. Most of the week x line interaction terms are significant, which indicates a line-specific sensitivity to variations in rearing conditions. The main effects for the environmental factors, days and weeks, are only significant in a few cases but the week x day interaction is significant for most variables. It is important to note that lines remain highly significant when the observations are weight-adjusted. Comparison of the variance component ratio K for raw and weight-adjusted observations shows that adjustment decreases the line component in all cases, but not to a great extent. Therefore, while most of the flight variables are positively correlated with weight, variation in weight can only account for a small part of the genetic variation observed.

Correlations: The five wing variables A, WS, L, S, and T, are highly intercorrelated, the minimum product-moment correlation being that for L X WS line means ($r = 0.886$, $P < .001$). Product-moment correlations over line means between WBF, θ, wing area, and the power calculations are shown above the main diagonal in Table 4. The three power measures are highly intercorrelated, and also highly correlated with WBF. Because all the variables other than θ are significantly correlated with Wt, the partial correlation coefficients of line means with weight as a constant were computed, as shown below the main diagonal in Table 4. It is clear that the A x P_a

and A x P_i line mean correlations are high because A, P_a, and P_i are all highly correlated with Wt; the corresponding partial correlations are not statistically significant. Wing stroke amplitude is negatively correlated with weight, and shows a closer relationship to the power variables when weight effects are held constant. WBF is the variable most closely related to the calculated power variables, especially P_t, for which the partial correlation is very high.

Correlations between line effects, γ_{il}, are shown above the main diagonal in Table 5. These are not true product-moment correlations, but are computed from the covariance and variance component estimates, as follows: if $\hat{\sigma}_{ii'}$ is the line covariance component estimate and $\hat{\sigma}_i$ and $\sigma_{i'}$ are the square roots of the line variance component estimates for variables i and i', then $r_{ii} = \sigma_{ii'} / \sigma_i \sigma_{i'}$. No significance test is known, but the standard errors can be computed (Mode and Robinson, 1959). Comparison of Tables 4 and 5 show that the correlations of line effects are very similar to the correlations of line means.

The correlations of error effects are shown below the main diagonal in Table 5. These correlations were obtained by computing the sums of squares and products for each week x day x line combination separately and pooling the sums to obtain a product-moment correlation for each line. They were then tested for homogeneity and averaged over lines by Fisher's z-transform method. Note that the line effects correlation between θ and WBF is quite different from the corresponding error effects correlation. One possible explanation is that the total power is relatively constant for a given line, such that any change in θ is accompanied by an opposite and compensating change in WBF. Between lines there is no constancy of flight power output, and θ and WBF vary more or less independently (and probably from a variety

of causes). The WBF x A line effects correlation also differs considerably from the corresponding error effects correlation, possibly as a result of weight variations over lines. Within lines, the WBF x A correlation loses statistical significance. The most surprising feature of Table 5 is the significant negative correlation of P_t and θ error effects. It appears that the negative correlation of WBF and θ error effects causes increased θ to result in reduced WBF and consequently reduced P_t; perhaps the formula for P_t is inherently more sensitive to variation in WBF than in wing stroke amplitude.

DISCUSSION

The studies presented here have established three points that are fundamental to our goal of relating variation in enzyme structure and quantity to variation in the power output of the flight muscles.

First, wingbeat frequency and wing stroke amplitude measurements are robust. Within rather broad limits, age, ambient temperature, and duration of flight have little effect on line differences in flight parameters. As a result, line effects can be interpreted as differences in flight physiology that persist over a variety of environmental conditions. Tethered "flight" is admittedly artificial, but it is easily standardized, facilitates measurement of the critical flight parameters, and probably gives an accurate approximation of the flight variables in free flight (see Vogel, 1966).

Second, the estimate of power output of the flight muscles reflects the metabolic rate. Drosophila do not acquire an oxygen debt during flight (Chadwick, 1947), nor do the Diptera accumulate ATP during flight (Sacktor, 1974), so oxygen consumption rates must be directly related to rates of ATP production. Wingbeat frequency is highly correlated with oxygen consumption rates, both within individuals during long flights (Reed, Williams, and Chadwick, 1942; Chadwick and Gilmour, 1940; Chadwick, 1947) and among individuals from different lines, as shown in Figure 5. Further, as seen in Table 4, wingbeat frequency is the major determinant of power output. Motivational variables are not expected to greatly influence wingbeat frequency, since the Diptera have the myogenic type of flight muscle stimulation by which contractions are not individually triggered by nerve impulses, but are continued in a self-oscillatory manner (Pringle,

1947). Consistent with this expectation are the observations of Vogel (1967a, b) and Götz (1968) that wingbeat frequency variation is not used for controlling the relative magnitudes of lift and thrust vectors in *Drosophila*.

Third, there is genetic variation for the flight variables that is attributable to chromosomes derived from natural populations. Lines are a highly significant source of variation for all flight variables in the multiple line study, for both raw and weight-adjusted data. The broad sense heritabilities range from 0.16, for wing stroke amplitude, to 0.44 for inertial power, as shown in Table 3. The relatively low genetic component for wing stroke amplitude is possibly due to the effect of behavioral modifications of that variable; it appears that flies alter the stroke amplitude, increasing on one side and decreasing on the other, as part of a turning maneuver (Götz, 1968). Attempts to turn on the tethering wire could inflate the error variance and reduce the genetic component.

There are several limitations to our methods for estimating the power generated by the flight muscles. Weis-Fogh's power formulae (1972, 1973) are based on the assumptions of steady-state aerodynamics, simple harmonic motion of the wings, and a free moving wing-hinge (i.e., elastic forces are ignored). Furthermore, we have assumed constant wing thickness, wing density, and coefficient of drag. Using estimates of flight parameters obtained from several groups of flying insects, including *Drosophila*, Weis-Fogh (1972, 1973) concluded that the steady-state models provide an adequate energetic description of flapping flight -- the calculated lift is sufficient to sustain hovering and the power requirements are consistent with known metabolic rates. To some extent our work is limited by the fact

that the mechanics of insect flight are incompletely understood; in particular, the consequences of the "clap-fling" mechanism of lift generation used by <u>Drosophila</u> and other insects is unknown (Weis-Fogh 1972, 1973).

In spite of possible inaccuracies and the complexity of total power estimation, we and others have provided strong evidence for a close relationship between metabolic rate, as measured by O_2 consumption, and wingbeat frequency. We have devised measurement techniques that are highly repeatable, feasible for large scale replication, and sufficient for the estimation of power output of the flight muscles. Experiments involving characterization of the activity of some enzymes involved in flight metabolism and their effects on power output are in progress.

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TABLE 1.

PARAMETERS FOR THE COMPUTATION OF THE POWER OUTPUT OF THE FLIGHT MUSCLES.

MEASUREMENTS OBTAINED	PARAMETER	SYMBOL	UNITS (SI)
FROM TETHERED INDIVIDUALS:	WINGBEAT FREQUENCY	WBF	s^{-1}
	WING STROKE AMPLITUDE	Θ	RADIANS
FROM WING TRACINGS:	WING AREA	A	m^2
	WING LENGTH	L	m
	WING WIDTH	W5	m
	SECOND MOMENT OF AREA	S	m^4
	THIRD MOMENT OF AREA	T	m^5
COMPUTED FROM ABOVE:	AERODYNAMIC POWER	P_a	W (WATT)
	INERTIAL POWER	P_i	W (WATT)
	TOTAL POWER	P_t	ARBITRARY
FROM GROUPS OF 10 MALES:	LIVE WEIGHT	Wt	kg
CONSTANTS:	COEFFICIENT OF DRAG	C_d	DIMENSIONLESS
	AIR DENSITY	ρ_a	kg m^{-3}
	WING DENSITY	ρ_w	kg m^{-3}
	WING THICKNESS	D	m

TABLE 2.

LINE MEANS OF FLIGHT PARAMETERS FROM THE MULTIPLE LINE STUDY.

LINE	WBF (s^{-1})	θ (rads)	Wt (10^{-6}Kg)	L (10^{-3}m)	A ($10^{-6}m^2$)	W5 (10^{-3}m)	S ($10^{-12}m^4$)	T ($10^{-15}m^5$)	P_a (10^{-7}W)	P_i (10^{-7}W)	P_t
KA06	217	2.26	6.68	1.90	1.12	0.80	1.42	1.92	10.6	5.8	280
KA16	155	2.28	6.71	1.90	1.14	0.81	1.43	1.94	4.2	2.3	201
KA21	203	2.33	7.26	1.89	1.14	0.81	1.42	1.91	9.7	5.2	262
KA25	213	2.22	6.85	1.97	1.28	0.87	1.73	2.43	12.0	6.5	274
KA27	220	2.17	7.29	1.89	1.13	0.80	1.41	1.89	9.9	5.7	289
KA33	238	2.32	7.71	1.96	1.22	0.84	1.64	2.29	18.4	9.6	305
MN08	223	2.14	7.46	1.95	1.22	0.85	1.63	2.27	11.4	6.6	291
MN12	228	2.12	7.95	1.96	1.27	0.86	1.69	2.35	12.4	7.1	297
NC12	233	2.45	7.41	1.93	1.22	0.85	1.59	2.19	19.0	9.5	296
NC14	210	2.13	7.29	1.98	1.27	0.86	1.73	2.43	10.1	5.7	271
NC16	230	2.19	7.48	1.94	1.21	0.85	1.60	2.21	13.3	7.4	297
NC35	220	2.23	7.17	1.92	1.20	0.84	1.55	2.11	11.7	6.5	286
RI03	242	2.20	7.15	1.94	1.23	0.85	1.63	2.24	16.1	8.9	313
RI05	232	2.29	7.91	2.00	1.29	0.87	1.77	2.50	17.7	9.2	295
RI08	207	2.36	7.32	1.91	1.15	0.82	1.47	2.00	11.1	5.8	266
RI16	217	2.22	7.79	1.95	1.23	0.85	1.64	2.27	12.2	6.7	281
RI23	210	2.29	6.30	1.90	1.15	0.81	1.44	1.94	10.2	5.6	272
RI28	223	2.14	7.03	1.92	1.17	0.81	1.49	2.03	10.7	6.2	292
RI31	223	2.20	7.61	1.96	1.24	0.85	1.66	2.31	12.6	7.0	287
RI36	238	2.36	7.45	1.97	1.24	0.85	1.68	2.35	19.2	9.9	304
RI46	212	2.28	7.12	1.94	1.17	0.81	1.51	2.07	11.0	6.0	272
MEAN	222	2.25	7.24	1.94	1.20	0.84	1.58	2.17	12.5	6.8	282

TABLE 3.

SIGNIFICANCE TESTS AND VARIANCE COMPONENT ESTIMATES FOR THE MULTIPLE LINE STUDY.

VARIABLE	SOURCES OF VARIATION							VARIANCE COMPONENT RATIOS	
	WEEK	DAY	WxD	LINE	WxL	DxL	WxDxL	K	H^2
WBF	.039	.049	-.038	4.17***	.516***	.038	-.178	0.63	0.39
	-.022	.133**	-.034	2.89***	.633***	.132	-	0.51	
θ	1.99*	.112	1.34***	6.47***	2.27**	.251	1.83	0.32	0.16
A	.078	.233	.302***	2.55***	.319***	.246***	-.004	0.55	0.33
	-.086	-.084	.283***	1.46***	.021	.094	-	0.46	
P_a	.706	-.069	.790***	9.50***	2.36***	.078	-.555	0.61	0.39
	.397	-.066	.661***	7.36***	2.28***	-.003	-	0.55	
P_i	.114	-.002	.111***	2.16***	.488***	-.006	-.107	0.65	0.44
	.051	.008	.085***	1.57***	.475***	-.016	-	0.58	
P_t	-.021	.018	.012	1.84***	.206**	.070	-.036	0.57	0.33
	-.029	.048*	.024	1.27***	.278***	.107	-	0.46	
Wt	.031	.561***	.025*	2.06***	.257***	.107***	-	0.73	

Analyses of θ, A, and Wt include all 21 lines; for the other variables, KA16 was excluded. For each variable, the first row represents analysis of raw data, and the second row represents analysis of weight-adjusted data (see text).

Variance component ratios are defined in the text.

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

TABLE 4

CORRELATIONS OF LINE MEANS (ABOVE DIAGONAL) AND PARTIAL CORRELATIONS OF LINE MEANS WITH WEIGHT AS THE CONSTANT VARIABLE (BELOW DIAGONAL).

	WBF	θ	A	P_a	P_i	P_t	Wt
WBF	---	.049	.443	.828***	.892***	.987***	.539*
θ	.197	---	-.223	.508*	.392	-.088	-.210
A	.160	-.119	---	.508*	.548*	.403	.627**
P_a	.774***	.697***	.316	---	.990***	.728***	.461*
P_i	.852***	.594**	.340	.989***	---	.810***	.511*
P_t	.981***	.030	.102	.641**	.739***	---	.537*

Correlations were computed excluding line KA16

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

TABLE 5

CORRELATIONS OF LINE (GENETIC) EFFECTS (ABOVE DIAGONAL) AND AVERAGE CORRELATIONS OF ERROR EFFECTS (BELOW DIAGONAL).

	WBF	θ	A	P_a	P_i	P_t	Wt
WBF	---	.034	.485	.843	.904	.991	.574
		±.244	±.185	±.071	±.045	±.005	±.160
θ	-.302	---	-.249	.486	.368	-.090	-.239
	***		±.234	±.187	±.211	±.244	±.231
A	-.026	.052	---	.524	.569	.455	.623
				±.175	±.163	±.194	±.143
P_a	.348	.652	.468	---	.990	.754	.478
	***	***	***		±.005	±.107	±.182
P_i	.543	.486	.480	.974	---	.832	.530
	***	***	***	***		±.077	±.170
P_t	.974	-.502	-.099	.132	.346	---	..579
	***	***	**	***	***		±.161

For the correlation of error effects, the significance level of the z-test is given; for the correlation of line effects, the standard error is given. The correlations of error effects were averaged over all lines except for A x P_i, for which inclusion of line KA16 (r = .016) caused significant heterogeneity. The WBF x P_t error effects correlations are significantly heterogeneous over lines, ranging from .917 to .989; each correlation is significant at the 0.001 level. Correlations of genetic effects were computed excluding line KA16.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

FIGURE LEGENDS

1. A typical wing tracing on the left and, on the right, the transects that define the points used for estimating wing length, width, area, and the second and third moments of area.

2. Wingbeat frequency recorded at five-minute intervals during a continuous four-hour flight of an individual male. Long flights are characterized by relatively constant WBF during the first hour, then gradual decline to cessation. Wing stroke amplitude remains relatively constant during long flights.

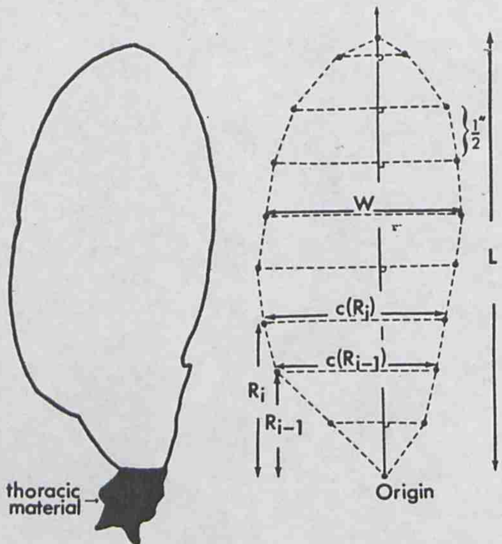
3. Ontogenic development of wingbeat frequency. WBF increases during the first two days of the imago but is otherwise independent of age over the range tested.

4. Wingbeat frequency as a function of ambient temperature. Individuals were observed three times at each temperature. Vertical bars indicate the standard error of line means.

5. Correlation between oxygen consumption rate and wingbeat frequency. Each point represents the average of at least two simultaneous WBF and consumption measurements on an individual tethered inside a respirometer. The product moment correlation coefficient is highly significant ($r = 0.82$, $p < .001$).

6. Wingbeat frequency distribution of line means from the multiple line study. Line means are based on three observations on each of 46 - 63 individuals per line. Horizontal bars indicate two standard deviations. With the exception of the obvious outlier, WBF line means are approximately continuously distributed.

①



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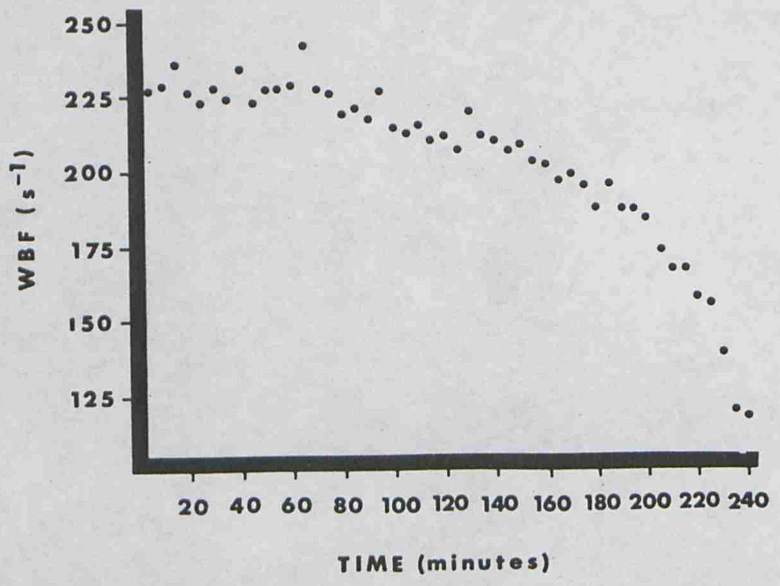

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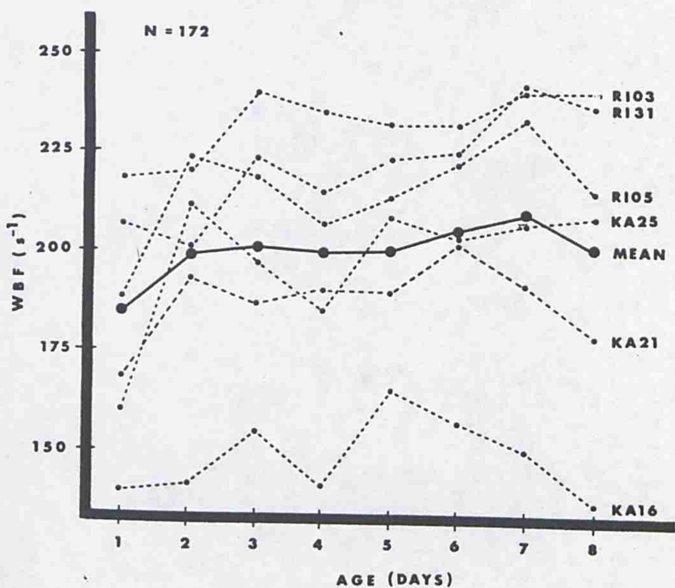

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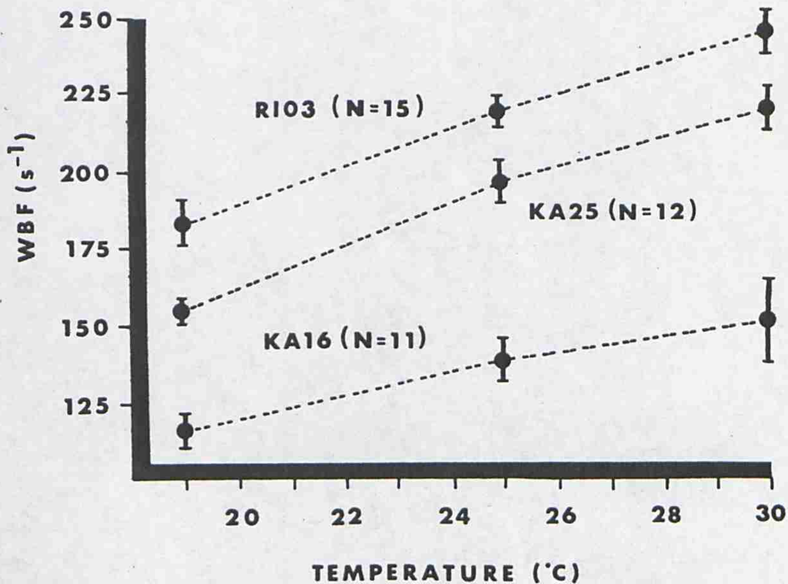

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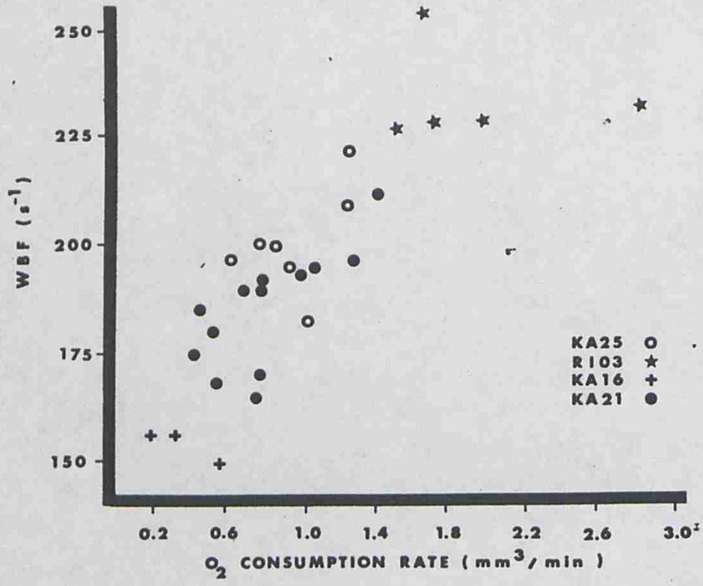

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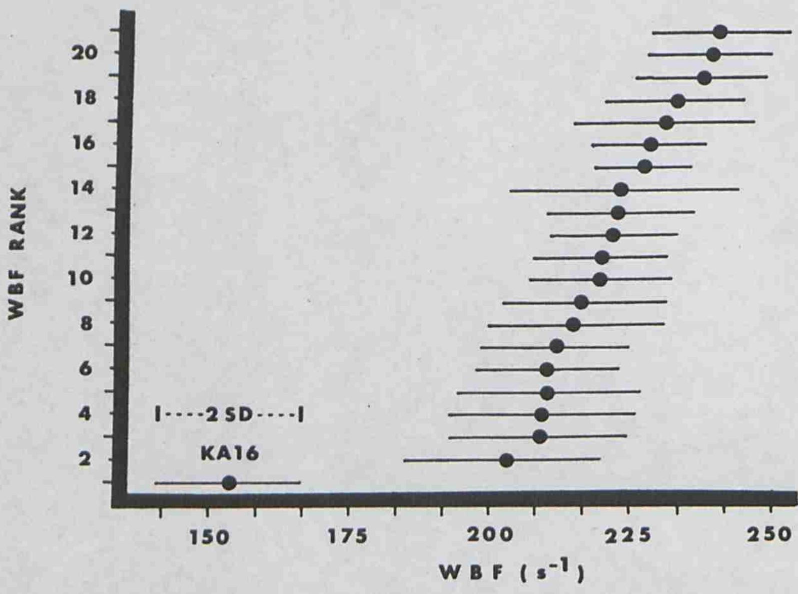

Autosomal Factors with Correlated Effects on the Activities of the Glucose-6-phosphate and 6-Phosphogluconate Dehydrogenases in *Drosophila melanogaster*[1]

C. C. Laurie-Ahlberg[2], J. H. Williamson[3], B. J. Cochrane[4], A. N. Wilton[2], and F. I. Chasalow[5]

[1]Paper No 6843 of The Journal Series of the North Carolina Agricultural Research Service, Raleigh, North Carolina.

[2]Department of Genetics, North Carolina State University, Raleigh, North Carolina 27650.

[3]Department of Biology, University of Calgary, Calgary, Alberta, Canada T2N 1N4

[4]Curriculum in Genetics and Department of Zoology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514.

[5]Chemistry and Life Sciences Division, Research Triangle Institute, P. O. Box 12194, Research Triangle Park, North Carolina 27709.

Running Head: Drosophila G6PD and 6PGD

ABSTRACT

Isogenic lines, in which chromosomes sampled from natural populations of *D. melanogaster* are substituted into a common genetic background, were used to detect and partially characterize autosomal factors that affect the activities of the two pentose phosphate pathway enzymes, glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD). The third chromosome effects on G6PD and 6PGD are clearly correlated; the second chromosome effects, which are not as great, also appear to be correlated but the evidence in this case is not as strong. Examination of activity variation of ten other enzymes revealed that G6PD, 6PGD is not the only pair of enzymes showing a high positive correlation, but it is among the highest in both sets of lines. In addition, there was some evidence that the factor(s) affecting G6PD and 6PGD may also affect two other metabolically related enzymes, transaldolase and phosphoglucose isomerase — Rocket immunoelectrophoresis was used to estimate specific CRM levels for three of the enzymes studies - G6PD, 6PGD and ME. This experiment shows that a large part of the activity variation is accounted for by variation in CRM level (especially for third chromosome lines), but there remains a significant fraction of the genetic component of activity variation that is not explained by CRM level. — These results suggest that the autosomal factors are modifiers that are involved in regulation of the expression of the X-linked structural genes for G6PD and 6PGD, but a role in determining part of the enzymes' primary structure cannot be excluded with the present evidence.

INTRODUCTION

Some basic problems in evolutionary biology are to determine the amount and the nature of genetic variation in natural populations and to understand the roles that different types of variation have played in long-term evolutionary changes. In recent years, approaches to this problem have focused primarily on genetic variation in the structure of enzymes, which has proven very extensive by electrophoretic and other criteria (cf. Lewontin, 1974). In contrast, very little information is available about the amount or nature of variation in regulatory sequences, which may constitute a much larger fraction of the eukaryotic genome than sequences coding for the primary structure of proteins. This gap in knowledge appears particularly significant in view of the possibility that polymorphism of regulatory elements may be a much more important source of variation for adaptive evolutionary change than structural variability (Britten and Davidson, 1969; Wilson, 1976).

Some of the reasons why population studies of regulatory elements have not proceeded are, of course, that the mechanisms of regulation in eukaryotes are not well understood and the phrase "regulatory element" does not have a precise definition in molecular terms in the way that "structural element" does. Here we will use the term modifier gene, rather than regulatory element, to mean a locus that affects enzyme activity levels without affecting the primary structure of the polypeptide(s) at the time of translation (with no implication about molecular mechanisms). We have initiated a study of genetic variation of enzyme activities in natural populations of *Drosophila melanogaster* in order to detect and characterize polymorphism of modifiers. To date we have discovered extensive genetic variation in the activities of ten different enzymes; 9 of those 10 show evidence of variation of modifiers

that are not linked to the structural locus of the enzyme (Laurie-Ahlberg et al., 1980 and unpublished). This paper is a progress report on our efforts to characterize the autosomal factors that affect the activity levels of glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44), which catalyze the first and third steps, respectively, of the pentose phosphate pathway.

The *Drosophila melanogaster* G6PD and 6PGD enzymes have been the subject of a large number of genetic, biochemical and physiological studies, which have recently been reviewed (Lucchesi et al., 1979 and Geer et al., 1980). Two naturally occurring electrophoretic variants at each locus were used to assign the structural locus for 6PGD (*Pgd*) to the tip of the X chromosome (1-0.6, Young et al., 1964 and 2D3-5, Gerasimova and Ananiev, 1972) and the structural locus for G6PD (*Zw*) to the proximal end of the X (1-63, Young, 1966 and 17B-18F, Stewart and Merriam, 1974). Subsequently, several null and low activity variants of both enzymes have been induced on the X chromosome (see Lucchesi et al., 1979), some of which also alter electrophoretic mobility (Gvozdev et al., 1976 and 1977). Only one of the two enzymes is affected by any one of these variants.

Both enzymes have been purified and characterized to some extent biochemically. The 6PGD has been purified by Williamson et al. (1980), who report a native molecular weight of 105,000 with subunits of 55,000 and 53,000. Hori and Tanda (1980) also report a native molecular weight of 105,000 but find only one subunit of 58,000. The 6PGD thus appears to be a dimer, which is further supported by the observation of an intermediate hybrid band in females heterozygous for the two naturally occurring allozymes (Young et al., 1964). The two naturally occurring allozymes of G6PD do

not exhibit a hybrid band in heterozygotes. Steele et al. (1968) originally showed that the B (slow) form has a native molecular weight of 317,000 and the A (fast) form, 147,000. They concluded that the polymorphism is due to the instability of subunit association, which has recently been confirmed by Hori and Tanda (1980). Lee et al. (1978) and Hori and Tanda (1980) report a single subunit after SDS electrophoresis of purified enzyme, whereas Williamson (in Geer et al., 1980) finds two similar subunits of 61,000 and 66,000. The B variant is therefore apparently a tetramer and the A variant is a dimer.

The pentose phosphate pathway consists of an oxidative branch in which glucose-6-phosphate is converted to ribulose-5-phosphate with the reduction of $NADP^+$ in the reactions catalyzed by G6PD and 6PGD and a non-oxidative branch that allows for interconversion of glycolytic intermediates with pentose phosphates. The metabolic role of the pathway is generally considered to be the production of NADPH for lipid biosynthesis and pentose phosphate for nucleotide synthesis. The importance of G6PD and 6PGD in producing NADPH for lipid biosynthesis in *D. melanogaster* is well documented (see review by Geer et al., 1980), but their role is not essential since stocks that are null for both enzymes are viable and fertile. Stocks null for 6PGD only are, however, lethal or semi-lethal, apparently due to the toxic effects of 6-phosphogluconate accumulation (Hughes and Lucchesi, 1977 and Gvozdev et al., 1976 and 1977).

The mechanisms that regulate or cause variation in the activity levels of these enzymes are being investigated at several levels. With respect to the enzyme activities at a given stage in the life cycle, both environmental and genetic causes of variation have been identified as well as causes that involve an interaction between environment and genotype. Two types of environmental

variation can be distinguished - short-term fluctuations in the concentrations of metabolites that result in allosteric modulation of activity levels and long-term influences of the diet that may cause variation in the rates of synthesis or degradation of the enzymes. For example, both G6PD and 6PGD are competitively inhibited by NADPH, from which Geer et al. (1980) have concluded that the NADPH/$NADP^+$ ratio probably regulates flux through the pentose shunt. Dietary modulation provides a much coarser type of control. Geer et al. (1976) and subsequently others have reported large changes in the activities of G6PD and 6PGD in response to changes in the concentrations of dietary factors. Increases in sucrose, for example, increase the activities of both enzymes, and also cause an increase in the rate of lipid synthesis. The increase in G6PD activity is accompanied by a change in the level of cross-reacting material, indicating a change in the rate of synthesis or degradation of the enzyme (Geer et al., 1980).

The genetic causes of activity variation can be divided into sex-specific and sex-nonspecific effects. Because the structural genes for G6PD and 6PGD appear to be X-linked, they have been the subject of many studies of dosage compensation (see reviews by Lucchesi, 1977 and by Stewart and Merriam, 1980). Even though normal males have only one X chromosome and females have two, the activities of G6PD and 6PGD as well as other X-linked enzymes are equal in the two sexes in similar tissues. Examples of sex-nonspecific effects are the lack of dosage compensation within each sex (Lucchesi, 1977) and the differences in activities between the A and B allozymes of 6PGD (Bijlsma, 1979 and Cavener and Clegg, 1981). An example of an environment x genotype interaction effect is the genetic variation with respect to the inducing effect of dietary sucrose on G6PD and 6PGD activities (Cochrane and Lucchesi, 1980). The work reported

here deals primarily with the detection and characterization of genetic effects on G6PD and 6PGD activities in a standard laboratory culture environment. Particular attention is given to the possibility of coordinate genetic control because of the closely related functions of these two enzymes.

In an earlier report (Laurie-Ahlberg et al., 1980), we described an experiment in which 50 second and 50 third chromosome isogenic substitution lines were screened for variation in the activities of 7 enzymes, including G6PD and 6PGD. In that experiment, a significant genetic component to the variation in G6PD activity was found in both sets of lines and in both sexes but no significant genetic component to the 6PGD variation was found. However, the two activities were highly correlated: $r = .81$ for females and $r = .71$ for males for second chromosome lines and $r = .64$ for females and $r = .80$ for males for third chromosome lines ($p<0.001$ for all four). These observations suggested the possible existence of polymorphic autosomal factors that have correlated effects on the activities of G6PD and 6PGD. In order to investigate this possibility, the five lines with the highest G6PD activity and the five with the lowest activity (after weight-adjustment) were selected from each set of chromosome substitution lines and used for the experiments reported here (along with *Ho-R*, the genetic background line). Because the environmental component of enzyme activity variation is greater for adult females than males, only males were used in this study.

MATERIALS AND METHODS

1. *General Procedures*

Stocks: Two sets of isogenic chromosome substitution lines were used in this study. The constitution of a line of each type is: $i_1/i_1;+_2/+_2;i_3/i_3$ (referrred to as a second chromosome line) and $i_1/i_1;i_2/i_2;+_3/+_3$ (a third chromosome line), where i refers to a chromosome from a highly inbred line (*Ho-R*) and + refers to a chromosome sampled from a natural population. The + but not the i chromosomes vary within a set of lines. Both sets of lines contain the same X chromosome (from *Ho-R*), which carries the "A" (fast) electromorph for both G6PD and 6PGD. The construction and electrophoretic analysis of these lines is described in Laurie-Ahlberg et al. (1980).

Rearing Conditions and Sampling: All of the flies used for enzyme assays were raised at North Carolina State University on standard cornmeal-molasses medium. The standard procedure for obtaining samples from the isogenic lines is to place 50 pairs of parents in a half-pint bottle for 48 hours, rear the offspring at 25°C, collect them within 18 hours of emergence, age the imagos for X days (usually X = 6) in vials (15 per vial), weigh the live flies and freeze the samples at -70°C.

Statistical Analyses: The genetic correlations and their standard errors were computed with our own FORTRAN program. All other analyses were performed by using various procedures of the "SAS" statistical analysis system (Helwig and Council, 1979).

Chemical Abbreviations: DTT: DL-dithiothreitol, EDTA: ethylenediamine tetracetic acid (disodium salt), SDS: sodium dodecyl sulfate, PMSF: phenylmethylsulfonyl

fluoride, BSA: bovine serum albumin, DCIP: 2,6-dichlorophenol-indophenol, TAPS: tris (hydroxymethyl) methylaminopropane sulfonic acid, PIPES: piperazine-N,N'-bis(2-ethane sulfonic acid).

2. *Experiment I*

Sample Collection: Performed in April, 1979. Two separate experiments with the same design were used to investigate G6PD and 6PGD activity variation among 10 second chromosome lines plus *Ho-R* and among 10 third chromosome lines plus *Ho-R*. On each of two days ("blocks"), three bottles of parents for each of the 11 lines of chromosome type were set up. The flies from each set of 3 bottles were pooled to obtain three samples of 6-day-old males, which were weighed and frozen whole in sets of 10.

Extraction and Assay: The frozen flies were homogenized and assayed at the University of North Carolina - Chapel Hill by the methods described by Lucchesi and Rawls (1973). Units of activity are μmoles $NADP^+$ reduced per minute at 30°C.

3. *Experiment II*

Sample Collection and Preparation: Performed in January, 1980. On each of three days within each of two weeks, four bottles for each of 21 lines (10 second chromosome, 10 third chromosome and *Ho-R*) were set up. Nineteen days after a group of bottles was set up, 25 each of 5-, 6-, 7- and 8-day-old males were pooled, weighed as a set of 100, homogenized in 1.25 ml of 0.01 M Potassium Phosphate buffer, pH 7.4 and then centrifuged for 30 min. at 12,000 xg. The supernatant was split into 4 aliquots, which were diluted 1:1 with the homogenization buffer (buffer A), buffer A with 2.0 mM EDTA (buffer B), buffer A with 0.2 mM

DTT (buffer C) or buffer A with 2.0 mM EDTA and 0.2 mM DTT (buffer D). These four types of samples were split into a total of twenty 95 μl aliquots and frozen for enzyme assays, rocket immunoelectrophoresis and general protein determination.

Enzyme Assays: The enzyme assays were performed at the Research Triangle Institute with a GeMSAEC centrifugal fast analyzer. This instrument, developed at Oak Ridge National Laboratory, is an automated spectrophotometric system, allowing simultaneous measurement of 16 reaction rates (Anderson, 1969). The samples for a given enzyme (total of 132) were all assayed on the same day at 30^{0}C. The 12 enzymes in Table 1 were assayed by the methods described below. The AOX reaction was monitored at 600 nm, all others were monitored at 340 nm. In all cases substrate concentrations are saturating for crude extracts of *Ho-R*. For all assays, 10 μl of sample were used in a total reaction volume of 128 μl. Concentrations are for the total reaction mixture.

ADH: Sample buffer B. Reaction mixture: 90.0 mM ethanol, 1.4 mM NAD^{+}, 0.9 mM EDTA in 0.04 M Glycine-NaOH, pH 9.5.

AK: Sample buffer C. Reaction mixture: 4.7 mM arginine, 0.44 mM NADH, 0.4 mM ATP, 8.0 mM $MgSO_4$, 80 mM KCl, 1.1 mM phosphoenolypyruvate, 1.7 units/ml pyruvate kinase and 0.4 units/ml lactate dehydrogenase (Sigma Co. P-1381) in 0.02M TAPS-PIPES, pH 7.2.

ALD: Sample buffer D. Reaction mixture: 0.5 mM fructose-1,6-diphosphate, 0.29 mM NADH, 2.6 units/ml α-glycerophosphate dehydrogenase and 24.7 units/ml triosephosphate isomerase (Sigma Co. G-1881) in 0.02 M TAPS-PIPES, pH 7.6.

AOX: Sample buffer A. Reaction mixture: 25.0 mM acetaldehyde, 0.13 mM phenazine methosulfate, 36.0 μM $DCIP_{ox}$, 0.04 mM EDTA in 0.1 M Tris-HCl, pH 7.4 with 1.0 mg/ml BSA.

FUM: Sample buffer C. Reaction mixture: 6.0 mM fumarate, 0.6 mM $NADP^+$, 0.8 mM $MgCl_2$, 0.5 units/ml malic enzyme (Sigma Co. M-5257) in 0.02 M TAPS-PIPES, pH 7.8.

GDPH: Sample buffer C. Reaction mixture: 13.4 mM α-glycerophosphate, 2.26 mM NAD^+ in 0.04 M Glycine-NaOH, pH 9.5.

G6PD: Sample buffer D. Reaction mixture: 3.44 mM glucose-6-phosphate, 0.2 mM $NADP^+$, 18.8 mM $MgCl_2$, 0.78 mM DTT, 0.02 M TAPS-PIPES, pH 7.8.

IDH: Sample buffer C. Reaction mixture: 2.62 mM isocitrate, 0.36 mM $NADP^+$, 0.78 mM $MgSO_4$, 1.17 mM DTT in 0.02M TAPS-PIPES, pH 9.0.

ME: Sample buffer D. Reaction mixture: 47.0 mM malate, 0.68 mM $NADP^+$, 2.0 mM $MgCl_2$ in 0.02 M TAPS-PIPES, pH 8.0.

PGI: Sample buffer B. Reaction mixture: 2.34 mM fructose-6-phosphate, 0.38 mM $NADP^+$, 1.37 units/ml glucose-6-phosphate dehydrogenase (Sigma Co. G-5760) in 0.02 M TAPS-PIPES, pH 8.3.

TA: Sample buffer D. Reaction mixture: 21.6 mM fructose-6-phosphate, 1.0 mM erythrose-4-phosphate, 0.4 mM NADH, 0.2 units/ml α-glycerophosphate dehydrogenase and 0.78 units/ml triosephosphate isomerase (Sigma Co. G-1881) in 0.02 M TAPS-PIPES, pH 7.0.

6PGD: Sample buffer A. Reaction mixture: 0.3 mM 6-phosphogluconate, 0.3 mM $NADP^+$,

3.12 mM $MgSO_4$ in 0.02 M TAPS-PIPES, pH 8.2.

Rocket immunoelectrophoresis: Levels of specific immunologically cross-reacting material (CRM) were determined at the University of Calgary by one-dimensional immunoelectrophoresis (Laurell, 1966) with subsequent staining for specific activity of the antigen-antibody complexes for G6PD, 6PGD or ME. Gels were prepared by heating 30 ml of 1.0% agarose suspension on 0.088 M Tris, 2.8 mM EDTA, 0.025 M Acetate buffer, pH 8.6, to 90^oC. After cooling to 55^oC in a waterbath, antiserum was added (40 μl for malic enzyme gels, 100 μl for 6PGD gels and 120 μl for G6PD gels). Five-microliter samples of crude homogenate (buffer A for 6PGD, buffer D for G6PD and ME) were placed in wells and the gels were electrophoresed for 21 hours at 100 V in Bio-Rad Model 4200 chambers. Specific enzyme activities were visualized by staining the gels with specific substrate (1.5 mM L-malate, 7.0 mM 6-phosphogluconate or 6.0 mM glucose-6-phosphate), 35.0 mM $MnCl_2$, 0.35 mM $NADP^+$, 0.6 mM phenazine ethosulfate, 2.4 mM nitroblue tetrazolium in 0.06 M Tris-HCl, pH 7.9.

For each of the 6 sampling days of the experiment, there is a corresponding measurement day on which one sample per line was split in two and each run on a different immunoelectrophoresis gel. Each gel contained 6 control samples and 14 experimental samples; thus, three gels per day were run. The controls were three replicates each of two concentrations of an *Oregon-R* mass homogenate. Analyses of variance of the control rocket heights showed a highly significant component of variation among gels within a day for G6PD and 6PGD but no significant variation for ME. Therefore, the experimental rocket heights for G6PD and 6PGD were adjusted by the control rocket heights for each gel separately: $\hat{R} = (R-a)/b$ where $\hat{R}$ is the adjusted rocket height, and a and b are the intercept and slope, respectively, of the regression of control rocket height on the concentration of the control homogenate (50% or 100%). The $\hat{R}$ values for G6PD and 6PGD, which are on an arbitrary scale, and the original R values for ME (in mm)

were used in all subsequent analyses and are hereafter referred to as CRM level. For the statistical analyses reported here, the mean of the two CRM levels determined for a given line on one day were used.

Antisera: The production of antiserum specific to ME has been described by Geer et al. (1979). Antisera to G6PD and 6PGD were produced by subcutaneous injection of 100 μg of pure enzyme carried in Freund's complete adjuvant into young female San Juan rabbits. A second injection of 50 μg of pure enzyme in Freund's incomplete adjuvant was administered to each rabbit 14 days after the first injection. Ten days after the second injection, samples of antiserum produced strong, single precipitin bands on Ouchterlony double diffusion plates. Blood was collected by heart puncture, allowed to clot at room temperature for three hours and at 4°C for 24 hours. The serum was collected by centrifugation at 4000 xg for 10 minutes and frozen in 1.0 ml aliquots. Both G6PD and 6PGD were purified 400-600-fold by sequential purification on 2',5'-ADP Sepharose 4B and Blue Sepharose CL 6B (Williamson et al., 1980 and unpublished).

Protein determination: Protein concentrations were determined at the University of Calgary, by the dye-binding method of Bradford (1976), using BSA as the standard. Triplicate assays were performed on each sample for each day that immunoelectrophoresis gels were run.

Dissections: On each of the 6 sampling days, 20 males from each of 4 lines were dissected into 5 body parts: head, thorax, alimentary tract, the reproductive organs and the abdominal wall. The 4 lines were *RI09II* (a second chromosome line), *RI22III* and *KA27III* (third chromosome lines) and *Ho-R*. The 20 body parts were

homogenized in 500 μl of buffer D, centrifuged for 10 min. at 12,000 xg and the supernatant was frozen. The protein determinations were done by the method of Lowry et al. (1951) using BSA as the standard. The enzyme assays were performed at N. C. State University by monitoring the reduction of $NADP^+$ at 340 nm and 30^oC. For both assays, 100 μl of sample in a total reaction volume of 1.0 ml was used. The total reaction mixture for G6PD contained 1.62 mM glucose-6-phosphate, 0.16 mM $NADP^+$, 16.7 mM $MgCl_2$ in 0.05 M Tris-HCl, pH 7.6. The total reaction mixture for 6PGD contained 0.30 mM 6-phosphogluconate, 0.14 mM $NADP^+$, 1.51 mM $MgSO_4$ in 0.05 M Tris-HCl, pH 7.6.

4. *Effects of Extraction Buffers on G6PD and 6PGD Assays*

Sample collection and preparation: On each of two consecutive days, 4 bottles for each of 5 lines were set up. Nineteen days after a group of bottles were set up, 40 each of 5-, 6-, 7- and 8-day-old males were pooled and then divided into 16 sets of 10 flies each; the same was done for females. Two replicate homogenates using 8 different buffers for each sex x line combination were made on each of the two days of the experiment. The 8 buffers represent all combinations of 0 or 0.4 mM PMSF, 0 or 1.0 mM DTT and 0 or 1.0 mM $NADP^+$ in 0.01 M Potassium Phosphate, pH 7.4 with 1.0 mM EDTA. Each set of 10 flies was homogenized in 0.25 ml of buffer, centrifuged for 20 minutes at 12,000 xg and then split into two aliquots (one for G6PD, one for 6PGD) and frozen. The lines were *RI09II* (a second chromosome line), *RI22III*, *KA27III*, *RI06III* (third chromosome lines) and *Ho-R*.

Enzyme Assays: The assays for each enzyme (G6PD, 6PGD) were performed on two different days (corresponding to the two sampling days) at N. C. State University. For both assays, 80 μl of sample were used in a total reaction volume of 1.08 ml. The concentrations of reactants are the same as reported above for the centrifugal fast analyzer assays.

5. *Acrylamide Gel Electrophoresis*

Vertical slab acrylamide gel electrophoresis was performed at the University of North Carolina - Chapel Hill with the buffer system of Davis (1964). The gel consisted of 10% acrylamide and 0.8% bis with a 5% stacking layer. The electrode buffer contained 26 μM $NADP^+$. The homogenization buffer and staining solution are described by Faizullin and Gvozdev (1973). Ten males were homogenized in 100 μl of buffer (with 10% sucrose) and 5 μl were loaded in each slot. When PMSF was used in the homogenization buffer, it was added in the form of a 4.0 mM solution in isopropanol to a concentration of 0.8 mM.

6. *Specificity of the G6PD Assay*

In this study, G6PD activity was estimated by monitoring the reduction of $NADP^+$ that accompanies the conversion of glucose-6-phosphate to 6-phosphoglucono-lactone. In crude extracts, the 6-phosphogluconolactone is probably converted to 6-phosphogluconate by lactonase (Hughes and Lucchesi, 1978), which can allow reduction of $NADP^+$ by 6PGD. The possible contribution of 6PGD to the assay of G6PD in crude extracts was investigated by three methods: (1) Four assays were performed in the G6PD reaction buffer containing $NADP^+$: (a) the no substrate blank, (b) saturating concentrations of both glucose-6-phosphate and 6-phosphogluconate, (c) a saturating concentration of 6-phosphogluconate only and (d) a saturating concentration of

glucose-6-phosphate only (the standard G6PD assay). It should be noted that neither 6-phosphogluconate or ribulose-5-phosphate inhibit *Drosophila* G6PD (Geer et al., 1980). If 6PGD contributes to the apparent G6PD rate, then the sum of (c) and (d) will be greater than the sum of (a) and (b). The two sums were very similar both for high and low activity lines. (2) The amount of purified yeast 6PGD (Sigma Co. P-0632) that is saturating with respect to the reduction of $NADP^+$ at 0.3 mM 6-phosphogluconate and under the G6PD assay conditions was first determined (1.0 units/ml). The addition of this amount of pure 6PGD to the standard G6PD assay had no effect on the reaction rate for either high or low activity lines. (3) Crude extract from a double mutant strain (Pgd^n Zw^n), which contains no G6PD or 6PGD activity but does contain 6-phosphogluconolactonase activity (Hughes and Lucchesi, 1978), was supplemented with either purified *Drosophila* G6PD only or with purified *Drosophila* G6PD and 6PGD. There was no difference between the two rates. We therefore conclude that any contribution of 6PGD to the apparent G6PD rate in crude extracts is negligible.

RESULTS

Experiment I

The following model was used for analyses of variance and estimation of covariance components:

$$Y_{ijkl} = \mu_i + \beta_{ij} + \tau_{ik} + (\beta\tau)_{ijk} + \varepsilon_{ijkl}$$

where μ_i is the mean of the ith variable (i = 1, 2 for G6PD, 6PGD), β_{ij} is the effect of the jth block for the ith variable (j = 1, 2), τ_{ik} is the effect of the kth line (k = 1, . . ., 11), $(\beta\tau)_{ijk}$ is the interaction effect and ε_{ijkl} is the error effect (l = 1, 2, 3). For adjustment of activities (Y) by live weight (WT), regression of $\bar{Y}_{ijk\cdot}$ on $\overline{WT}_{jk\cdot}$ was performed for each of the two blocks (j = 1, 2); the sums of squares and products were then pooled over blocks to obtain a single regression coefficient, b_i. Adjusted variables ($\hat{Y}$) were then obtained as follows:

$$\hat{Y}_{ijkl} = Y_{ijkl} - b_i(WT_{jkl} - \overline{WT}_{...})$$

The ranges of line means (in units x 10^3/fly) are 1.80-4.37 and 1.48-2.75 for G6PD and 6PGD, respectively for the second chromosome lines. Excluding one line with an extremely high weight (line R in the figures), the third chromosome ranges are 1.78-3.68 and 1.51-2.63 for G6PD and 6PGD, respectively. Weight-adjustment has very little effect on the magnitude of these ranges.

The results of the analyses of variance are summarized in Table 2. Lines are a highly significant component of variance for both raw and weight-adjusted G6PD and 6PGD activities in both sets of lines. The correlations over line means between live weight and activity are r = 0.29 and r = 0.37 ($p>0.05$ for both) for G6PD and 6PGD, respectively, for second chromosome lines and r = 0.77 ($p<.01$) and r = 0.71 ($p<0.05$) for G6PD and 6PGD, respectively for third chromosome lines. The higher correlations for the third chromosome lines are largely due to the high weight outlier, line R. The variance component ratio K, where

$K=\hat{\sigma}^2_l/(\hat{\sigma}^2_l+\hat{\sigma}^2_{bxl}+\hat{\sigma}^2_e)$, is the proportion of variance among the observations (based on 10-fly homogenates) within a block that is attributable to lines ($\hat{\sigma}^2_l$ is the estimated line component of variance, $\hat{\sigma}^2_{bxl}$ is the block x line component and $\hat{\sigma}^2_e$ is the error mean square). The relative magnitudes of K for raw and weight-adjusted activities indicate that weight-adjustment was only effective in substantially reducing the line component for G6PD, third chromosome lines. However, even in this case, most of the effect was due to line R and the K value for the weight-adjusted G6PD is still quite large (0.48).

Figure 1 shows the relationship between the line means for G6PD and 6PGD raw activities. The corresponding correlations are $r = 0.78$ ($p<0.01$) for the second chromosome lines and $r = 0.96$ ($p<0.001$) for the third chromosome lines. The correlations over weight-adjusted line means are very similar: $r = 0.76$ ($p<0.01$) for second and $r = 0.90$ ($p<0.001$) for third chromosome lines. The product-moment correlations over line means are not necessarily good estimates of the correlation between the line (genetic) effects on activity. Therefore, the genetic correlations (r^*) were computed as follows: Let $\hat{\sigma}_{xl,yl}$ be the estimated covariance of line effects on enzymes x and y and $\hat{\sigma}^2_{xl}$, $\hat{\sigma}^2_{yl}$ be the estimated variance components for lines. Then $r^* = \hat{\sigma}_{xl,yl}/(\hat{\sigma}_{xl}\hat{\sigma}_{yl})$. Because the quantity r^* is not necessarily bounded by -1 and +1 and may not even be defined for negative variance component estimates, no test of the hypothesis that the true value of r^* equals zero is available although the standard errors were computed by the method of Mode and Robinson (1959). The genetic correlations for the weight-adjusted activities are $r^* = 0.83 \pm 0.13$ for second and $r^* = 0.98 \pm 0.05$ for third chromosome lines. These results provide clear evidence for autosomal factors with correlated effects on G6PD and 6PGD. Experiment II, which includes measurements of 12 different enzyme activities, was undertaken to investigate the specificity of this relationship.

Experiment II

The following model was used for analyses of variance and estimation of covariance components.

$$Y_{ijkl} = \mu_i + \beta_{ij} + \alpha_{ijk} + \tau_{il} + (\beta\tau)_{ijl} + \varepsilon_{ijkl}$$

where μ_i is the mean of the ith variable (i = 1, ..., 12), β_{ij} is the effect of the jth week of sampling (j = 1, 2), α_{ijk} is the effect of the kth day within the jth week (k = 1, 2, 3), τ_{il} is the effect of the lth line of a chromosome type (l = 1, ..., 11), $(\beta\tau)_{ijl}$ is the week x line interaction and ε_{ijkl} is the residual.

Two kinds of adjustments of the raw variables were made. Activities and CRM levels were adjusted for general protein or live weight and activities were also adjusted for CRM level. Let Y be the dependent variable to be adjusted and X be the independent variable. The regression of Y on X over lines was performed for each of the 6 days of the experiment; the sums of squares and products were then pooled over days to obtain a single regression coefficient, b_i. Adjusted variables ($\hat{Y}$) were then obtained as follows:

$$\hat{Y}_{ijkl} = Y_{ijkl} - b_i(X_{ijkl} - \bar{X}....)$$

The ranges of line means for G6PD and 6PGD are similar to those in Experiment I but are somewhat smaller: 2.18-3.85 for G6PD and 2.21-3.48 for 6PGD in second chromosome lines and 2.14-4.23 for G6PD and 1.89-2.89 for 6PGD in third chromosome lines (in units x 10^3 per fly).

The results of linear regression using the line means (N=11) of the 12 raw activities and the CRM levels on live weight or protein are summarized in Table 3. Many of the regression coefficients are not significantly different from zero, but more of the significant regressions are of activity or CRM on protein rather than on weight. These results suggest that adjustment by protein may be more effective than adjustment by weight.

The results of the analyses of variance of raw and protein-adjusted activities are summarized in Table 4. Lines are a significant component of variance for all of the raw variables. The variance component ratio $K = \hat{\sigma}^2_l/(\hat{\sigma}^2_l+\hat{\sigma}^2_{wxl}+\hat{\sigma}^2_e)$ is the proportion of variance among the observations within a day that is due to differences among lines, (where $\hat{\sigma}^2_l$ is the estimated line component of variance, $\hat{\sigma}^2_{wxl}$ is the week x line interaction component and $\hat{\sigma}^2_e$ is the error mean square). The values of K for raw variables range from 0.15 to 0.93. In most cases the effect of weight or protein adjustment on the significance level of the line component or on the value of K is small. In some cases, however, lines become non-significant after adjustment. In particular, the second chromosome line component for G6PD and 6PGD loses significance after protein-adjustment (but not after weight adjustment). For the third chromosome lines, however, both G6PD and 6PGD have highly significant line components after either protein or weight adjustment and the K values are quite large: 0.69 and 0.60 for protein-adjusted G6PD and 6PGD, respectively. These results suggest that the second chromosome line effects on G6PD and 6PGD may be due to non-specific body size and tissue quantity variation. However, the week x line interactions for both G6PD and 6PGD in the second chromosomes are significant even after protein adjustment. This result indicates not only that there are some non-additive genetic effects that cannot be removed by protein-adjustment but it also means that the F-test for the main effect of lines is much less powerful than if the week x line interaction were non-significant.

The plots of the line means of the raw G6PD vs. 6PGD activities from Experiment II are shown in the lower half of Figure 1. The 2 or 3 lines at either extreme maintain their positions in both Experiments I and II. The correlations are also similar, although somewhat lower: $r = 0.77$ ($p<0.01$) for second chromosome lines (compared with $r = 0.78$ in Experiment 1) and $r = 0.71$ ($p<0.05$) for

third chromosome lines (compared with $r = 0.90$ in Experiment II).

Table 5 shows the partial correlations over line means between pairs of enzymes with protein as the fixed variable ($r_{xy.p}$). For the second chromosome lines, only one of the partial correlations is significantly different from zero (ADH, AOX) and the numbers of positive and negative estimates are about equal (34 and 32, respectively). Although the G6PD, 6PGD partial correlation over second chromosomes is not significantly different from zero, it is the fourth highest (out of 66) and the G6PD, TA correlation is third highest. For the third chromosome lines, there are 47 positive and 19 negative estimates and most of the negative estimates involve AOX. Three negative estimates are significantly different from zero, each involving AOX. The four positive estimates that are significant are between ADH and TA, G6PD and TA, G6PD and 6PGD, and TA and PGI. These results show that G6PD, and 6PGD is not the only pair of enzymes showing a high positive correlation but it is among the highest in both sets of lines and most of the other high positive correlations involve enzymes that are also closely related in function. It should be noted that the correlations between G6PD and 6PGD and FUM (the only other enzyme in the set known to be X-linked) are small.

As mentioned above, the correlations of line means are not necessarily good estimates of the correlations of line (genetic) effects. However, in this experiment the two types of correlations are very similar. For example, the genetic correlations between protein-adjusted G6PD and 6PGD are $r^* = 0.55 \pm 0.41$ and $r^* = 0.71 \pm 0.17$ for second and third chromosomes, respectively, compared with $r_{xy.p} = 0.58$ and $r_{xy.p} = 0.66$ for partial correlations over line means.

The analyses of variance of the CRM levels for G6PD, 6PGD and ME are summarized in Table 6. Lines are a significant component of variance for all the raw and weight-adjusted CRM levels in both sets of lines and for all the protein-adjusted CRM levels except for ME in the second chromosome lines. Note that the line components for second chromosome G6PD, 6PGD and ME protein-adjusted CRM levels are all significant, although this was not the case for the corresponding activities. Similarly, the second chromosome week x line interactions are significant for protein-adjusted G6PD and 6PGD activities but not for the corresponding CRM levels.

The plots of the line means of activity versus CRM level are shown in Figure 2 and the corresponding linear regression analyses are summarized in Table 7. There is a significant regression of activity on CRM level for both G6PD and ME in both sets of lines and for 6PGD in the third chromosome lines but not the seconds. The multiple regression of activity on CRM level and protein shows that, in each case, CRM level is accounting for variation in activity that is not accounted for by variation in protein (i.e. the partial regression coefficients are significant). The partial correlations between activity and CRM level with protein fixed are all quite high, except for 6PGD in the second chromosome lines.

The results of the analyses of variance of the CRM-adjusted activities are shown in Table 4. CRM-adjustment, like protein-adjustment, causes lines to lose significance for G6PD and ME in the second chromosome set. Since there was no significant regression of 6PGD activity on CRM for the second chromosome lines, CRM-adjustment did not change the line component appreciably. Even though the CRM level and activity were strongly associated for all three enzymes in the third chromosome lines (especially ME), lines remained highly significant for all three CRM-adjusted activities. These results for the third chromosome lines indicate that even though variation in CRM level can account for a large part

of the variation in activity level, there is a genetic component to the activity variation that is not explained by CRM level, presumably some type of structural variation. The results for the second chromosome lines are not as clear-cut, but they also provide indications of activity variation not accounted for by CRM level: the significant week x line interactions for G6PD and 6PGD activity, which are not significant for the corresponding CRM levels, and the lack of significant regression of 6PGD activity on CRM level.

The correlations over line means between G6PD and 6PGD CRM levels are very similar to the correlations between the activities for the third chromosome lines: $r = .71$ for raw activities, $r = 0.73$ for raw CRM level, $r_{xy.p} = .68$ for the partial correlation between activities with protein fixed and $r_{xy.p} = .60$ for CRM levels with protein fixed. For the second chromosome lines, the correlations between CRM levels are less than between activities: $r = 0.64$ and $r_{xy.p} = 0.58$ for activities and $r = 0.31$ and $r_{xy.p} = 0.26$ for CRM levels.

One of the possible non-specific causes of variation in enzyme activities is variation in the amount of a tissue type in which the enzyme is expressed. This situation could also explain parallel variation between activity and CRM levels as well as correlations between functionally related enzymes. We therefore dissected adult males from two high (*RI09II* and *KA27III*) and two low (*Ho-R* and *RI22III*) activity lines (based on Experiment I) into several body parts, which were then assayed for G6PD, 6PGD and general protein. The body parts are the abdominal wall (which contains most of the adult adipose tissue as well as several other tissue types), the alimentary tract (midgut and hindgut), the head, the reproductive organs, and the thorax (which consists mainly of flight muscle). The data for each high vs. low activity pair of lines were analyzed separately. Table 8 gives the difference between lines for each body part (with its standard error and a test of significance). The results of the F-tests of lines and the

line x body part interaction from the ANOVA of all body parts together are also given. These results show that the activity differences between lines are not simply due to variation in size or amount of a certain body part. For each pair of lines and for each enzyme, more than one body part shows a significant difference, whereas none of the protein differences are significant.

Effect of PMSF on Activity and Electrophoretic Mobility

Hori and Tanda (1980) have recently reported a small change in the electrophoretic mobility of both A and B allozymes of G6PD that occurs during storage of the crude extract at $4^{o}C$. Each allozyme normally has one or two sub-bands that migrate slightly faster than the major band. After storage of crude extract at $4^{o}C$ for 2 days, Hori and Tanda find virtually all the activity shifted to the position of the sub-band. This change in mobility is apparently due to proteolytic activity in the crude extract since it is prevented by the addition of 0.4 mM PMSF and it can be duplicated in a short time by the addition of trypsin.

Hori and Tanda's results suggested that proteolytic activity in our crude extracts might explain some of the activity variation. We duplicated their electrophoretic conditions and observed the same pattern of faintly staining sub-bands (Figure 3). Although the amount of activity present in the sub-bands varies somewhat (independent of the presence of 0.8 mM PMSF in the crude extract), there was no indication of consistent differences among lines (either second or third chromosomes) with respect to the distribution of activity among the major and sub-bands or with respect to the mobilities of the bands. We also investigated the effect of having PMSF in the extraction buffer on the activities of 5 lines — two typically low activity lines and three typically high activity lines. The experiment was set up with a factorial design, with two levels of each of three chemicals added to the extraction buffer - 0 or 0.4 mM

PMSF, 0 or 1.0 mM DTT and 0 or 1.0 mM $NADP^+$. In the analyses of variance of both G6PD and 6PGD, none of the three line x chemical interactions were significant and neither of the main effects for PMSF or DTT were significant but $NADP^+$ showed a small, significant enhancement of activity (about 2% for G6PD and 4% for 6PGD). We therefore have no evidence that proteolytic activity in the crude extract contributes to variation among lines, although a PMSF-insensitive protease could, of course, be involved.

DISCUSSION

The results presented here clearly demonstrate genetic variation in natural populations of *D. melanogaster* for autosomal factors that affect the activities of G6PD and 6PGD. The existence of such factors for G6PD has been suggested by others (Steel et al., 1979; Rawls and Lucchesi, 1974; Bijlsma, 1980), but no systematic efforts were made to isolate and characterize particular variants, with one exception. Belote and Lucchesi (1980) have recently induced and characterized male-specific lethal mutants on the second chromosome that affect the rate of X chromosome transcription as well as the activities of three X-linked enzymes (G6PD, 6PGD, FUM) in homozygous male larvae, but not in females. These mutants are therefore implicated in the process of dosage compensation. Although we did not measure activity levels in females, it is unlikely that the autosomal factors investigated in this study are involved in dosage compensation because the correlations between either G6PD or 6PGD and FUM are very small.

Because it is generally accepted that the structural genes that code for the subunits of G6PD and 6PGD are located on the X chromosome, the autosomal factors could be considered modifiers that are somehow involved in the regulation of gene expression. However, we must consider the evidence for each enzyme that there is just one structural gene located on the X chromosome that codes for its primary structure. The evidence, most of which has already been referenced in the introduction, is very similar for both enzymes: (1) The common, naturally occurring allozymes map to the X chromosome. (2) Several null and low activity alleles have been induced on the X chromosome, some of which also alter electrophoretic mobility. (3) There is dosage compensation

between the sexes but within each sex there is dependence on the dosage of the chromosomal region to which the allozyme variants have been localized. (4) The biochemical evidence is somewhat ambiguous: some workers report a single band after SDS electrophoresis of purified enzyme, indicating identical subunits, whereas Williamson and coworkers report two bands for both G6PD and 6PGD (which could be due to proteolytic breakdown during purification or to an *in vivo* post-translational process). (5) Giesel (1976) has speculated that *Zw* is not the structural locus for G6PD but rather regulates the expression of two autosomal loci, one coding for the A and one for the B form. The basis for this suggestion is the apparent segregation of variants by both A and B forms, but the evidence provided is far from convincing and no independent support has come from the many laboratories that have worked extensively with this enzyme. Hori and Tanda (1980) have suggested that Giesel's hypothesis is due to a misinterpretation of the zymogram since the number and relative mobility of Giesel's "alleles" coincide with the proteolytic breakdown products they observe. In conclusion, there is certainly no compelling evidence on which to base a rejection of the hypothesis that *Zw* and *Pgd* are the sole structural genes for G6PD and 6PGD, respectively, but there definitely are plausible alternatives. Therefore, we cannot rule out the possibility that the autosomal factor(s) code for some part of the primary structure of the enzyme.

The third chromosome factors clearly have correlated effects on G6PD and 6PGD and the second chromosome factors seem to as well, but the evidence in the latter case is not as strong. Experiment II was designed primarily to investigate the specificity of this relationship. The selection of enzymes was made to include one of the non-oxidative pentose shunt enzymes (TA); some related glycolytic enzymes (ALD, PGI); GPDH, which probably plays a role in lipid as

well as carbohydrate metabolism; the NADP-dependent enzymes ME and IDH, which, like G6PD and 6PGD, are believed to be important in providing NADPH for lipid synthesis and which are co-induced or co-repressed with G6PD and 6PGD by dietary factors (see Geer et al., 1976); as well as a few other enzymes with no obvious or intimate metabolic connection with the pentose shunt (ADH, AK, AOX, FUM). The results show that G6PD, 6PGD is not the only pair of enzymes showing a high positive correlation, but it is among the highest in both sets of lines. Moreover, many of the other high correlations are also between enzymes with closely related functions. For example, the TA, G6PD correlation is high in both sets of lines (0.60 in seconds and 0.78 in thirds), as is the TA, PGI correlation in the third chromosome lines (0.75). It is possible that the third chromosome factor(s) with correlated effect(s) on G6PD and 6PGD are also affecting TA and PGI. It is certainly conceivable that the enzymes in intimately interconnected pathways such as glycolysis and the pentose shunt are regulated in such a way as to maintain some constancy in relative amounts, and the genetic factors we have identified may be involved in such a process. This suggestion is only a speculation at present, but we are currently investigating it further by attempting to map the activity factors for all four enzymes (PGI, TA, G6PD, 6PGD). Whether or not they all map to the same location will be very informative with respect to this hypothesis.

A large part of the activity variation among third chromosome lines for G6PD, 6PGD and ME is accounted for by variation in CRM level, but there is a significant fraction of the genetic component of activity variation that is not explained by CRM level. This result suggests the existence of structural variability that influences catalytic efficiency. Because the structural gene for ME is located on the third chromosome, some variation in enzyme structure

is not unexpected. In the case of G6PD and 6PGD, some type of post-translational modification such as that reported for XDH (Finnerty and Johnson, 1979) could be involved. An intensive search for direct evidence of structural variation of G6PD and 6PGD among these lines is in progress.

Two small experiments reported here argue against non-specific causes of the correlated genetic effects on G6PD and 6PGD activities. The dissection of high and low activity lines indicate that the activity differences are not simply due to a gross change in the amount of one particular tissue although more work of this type is clearly needed to characterize the extent of systematic versus tissue-specific effects. The lack of an effect of the protease inhibitor, PMSF, on activity or electrophetic mobility indicates that proteolytic breakdown in crude extracts is not responsible for activity variation among the lines.

A basic requirement of any model of regulation of eukaryotic gene expression is some mechanism to account for the coordinate control of functionally related enzymes. Therefore, the detection of variants with correlated effects on enzymes of the same or related pathways is potentially very useful for investigating the mechanisms of regulation as well as the importance of regulatory variation in evolutionary change. We conclude that continued characterization of the autosomal factors reported here is likely to provide some unusual opportunities to investigate these problems.

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TABLE 1

Enzymes Assayed in This Study

Name	Abbreviation	E. C. #	Map Position[†]
Alcohol dehydrogenase	ADH	1.1.1.1	2-50.1
Arginine kinase	AK	2.7.3.3	-
Aldolase	ALD	4.1.2.13	3R
Aldehyde oxidase	AOX	1.2.3.1	3-56.6
Fumarase	FUM	4.2.1.2	1-19.9
α-Glycerophosphate dehydrogenase	GPDH	1.1.1.8	2-20.5
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	1-63
NADP-dependent Isocitrate dehydrogenase	IDH	1.1.1.42	3-27.1
Malic enzyme	ME	1.1.1.40	3-53.1
Phosphoglucose isomerase	PGI	5.3.1.9	2-58.7
Transaldolase	TA	2.2.1.2	-
6-phosphogluconate dehydrogenase	6PGD	1.1.1.44	1-0.64

[†]O'Brien and MacIntyre (1978) and Voelker et al. (1978)

TABLE 2

Summary of Analyses of Variance for Experiment I†

Variable		Chromosome 2 Lines				Chromosome 3 Lines			
		Block	Line	Block x Line	K	Block	Line	Block x Line	K
G6PD	RAW	ns	***	ns	0.38	ns	***	ns	0.76
	WT-ADJ	*	***	ns	0.44	***	***	ns	0.48
6PGD	RAW	ns	***	ns	0.49	ns	***	ns	0.57
	WT-ADJ	ns	***	ns	0.47	**	***	ns	0.52
WT	RAW	ns	ns	***	0.39	*	*	***	0.43

†The significance levels of the F-tests and a variance component ratio $K = \hat{\sigma}_l^2/(\hat{\sigma}_l^2+\hat{\sigma}_{bxl}^2+\hat{\sigma}_e^2)$ are given for raw and weight-adjusted variables.

* $p<0.05$

** $p<0.01$

*** $p<0.001$

ns $p>0.05$

TABLE 3

Linear Regression of Activities and CRM Level on Weight or Protein†

	Chromosome 2				Chromosome 3			
	Weight		Protein		Weight		Protein	
Variable	$H_o{:}\beta=0$	R^2	$H_o{:}\beta=0$	R^2	$H_o{:}\beta=0$	R^2	$H_o{:}\beta=0$	R^2
ADH	*	.51	ns	.24	ns	.16	ns	.26
AK	ns	.09	*	.40	ns	.34	*	.42
ALD	ns	.28	**	.62	ns	.32	ns	.18
AOX	ns	.19	ns	.01	ns	.29	*	.54
FUM	ns	.09	ns	.32	ns	.11	ns	.30
GPDH	ns	.02	ns	.06	**	.69	ns	.23
G6PD	ns	.02	*	.39	ns	.17	ns	.13
IDH	ns	.28	ns	.01	ns	.02	ns	.01
ME‡	**	.54	**	.57	ns	.20	ns	.00
PGI	*	.38	***	.78	ns	.34	**	.59
TA	ns	.08	ns	.01	ns	.22	*	.50
6PGD	ns	.05	**	.66	ns	.24	ns	.21
CRM-G6PD	ns	.02	*	.49	ns	.02	*	.39
CRM-6PGD	ns	.02	ns	.03	ns	.09	ns	.29
CRM-ME	*	.40	**	.71	ns	.14	ns	.00

†Regression over line means (10 or 11) from Experiment II. Significance levels of the regression coefficients (β) and the coefficients of determination (R^2) are given.

‡The third chromosome ME null line is excluded.

TABLE 4

Summary of Analyses of Variance for Experiment II[†]

Variable[§]	Chromosome 2 Week	Day in Week	Line	Week x Line	K	Chromosome 3 Week	Day in Week	Line	Week x Line	K
ADH(2) RAW	**	ns	***	***	0.93	ns	*	***	ns	0.64
PROT-ADJ	ns	**	***	*	0.85	ns	***	***	ns	0.61
AK RAW	ns	***	***	ns	0.49	*	***	***	ns	0.62
PROT-ADJ	*	***	**	ns	0.19	*	***	***	ns	0.54
ALD(3) RAW	ns	***	**	ns	0.48	ns	*	**	ns	0.28
PROT-ADJ	ns	***	ns	ns	0.13	ns	**	**	ns	0.23
AOX(3) RAW	ns	***	*	ns	0.26	ns	***	***	ns	0.68
PROT-ADJ	ns	***	*	ns	0.26	ns	***	***	ns	0.53
FUM(X) RAW	***	ns	***	ns	0.32	ns	**	***	ns	0.49
PROT-ADJ	ns	*	*	ns	0.22	ns	***	***	ns	0.41
GPDH(2)RAW	***	ns	***	*	0.82	ns	ns	*	*	0.52
PROT-ADJ	ns	*	***	**	0.82	ns	***	***	ns	0.51
G6PD(X)RAW	***	ns	*	*	0.40	**	ns	***	ns	0.66
PROT-ADJ	ns	ns	ns	*	0.25	ns	ns	***	ns	0.69
CRM-ADJ	**	ns	ns	*	0.10	ns	ns	***	ns	0.49
IDH(3) RAW	*	***	***	ns	0.88	ns	ns	***	ns	0.81
PROT-ADJ	*	**	***	ns	0.88	ns	ns	***	ns	0.84
ME[‡](3) RAW	***	ns	*	ns	0.15	ns	ns	***	ns	0.90
PROT-ADJ	ns	**	ns	ns	<0	ns	ns	***	ns	0.91
CRM-ADJ	ns	***	ns	ns	<0	ns	ns	***	ns	0.39
PGI(2) RAW	*	**	***	*	0.47	ns	***	***	ns	0.73
PROT-ADJ	ns	***	*	ns	0.09	ns	***	***	ns	0.56
TA RAW	ns	*	***	ns	0.51	ns	***	***	ns	0.70
PROT-ADJ	ns	ns	***	ns	0.54	ns	***	***	ns	0.63
6PGD(X)RAW	**	ns	**	**	0.53	ns	ns	***	ns	0.63
PROT-ADJ	ns	*	ns	*	0.20	ns	***	***	ns	0.60
CRM-ADJ	***	ns	**	*	0.50	ns	***	**	ns	0.40
WT RAW	***	ns	***	***	0.80	ns	**	***	ns	0.82
PROT RAW	*	***	***	ns	0.48	**	ns	ns	*	0.07

[†]The significance levels of the F-tests and a variance component ratio $K = \hat{\sigma}_l^2/(\hat{\sigma}_l^2+\hat{\sigma}_{wxl}^2+\hat{\sigma}_e^2)$ are given for raw and adjusted variables

[‡]The third chromosome ME null line is excluded.

ns $p>0.05$
* $p<0.05$
** $p<0.01$
*** $p<0.001$

§ The chromosomal location of the structural gene, if known, is given in parentheses

TABLE 5

Partial Correlations over Line Means with Protein as the Fixed Variable[†]

	ADH	AK	ALD	AOX	FUM	GPDH	G6PD	IDH	ME	PGI	TA	6PGD
ADH	___	-.31	-.15	.66*	-.09	-.33	.32	-.10	.34	.03	.03	-.05
AK	-.14	___	.32	-.18	.48	.58	.05	.25	-.42	.31	-.00	.14
ALD	-.38	.63	___	-.03	-.48	-.21	-.05	-.05	.34	.42	.40	-.37
AOX	-.34	-.24	-.32	___	.29	-.41	-.17	.18	.47	-.22	-.26	-.05
FUM	-.01	.46	.25	-.47	___	.46	-.26	.40	-.46	-.40	-.61	.22
GPDH	.01	.58	.52	-.03	.35	___	.05	-.15	-.49	.29	-.28	.17
G6PD	.46	.07	-.31	-.34	-.12	.16	___	.38	-.24	.25	.60	.58
IDH	.13	.26	-.22	-.40	.07	-.31	.57	___	-.28	-.49	.05	.50
ME	.35	.23	.43	-.69*	.63	.46	.06	.02	___	.12	-.19	-.35
PGI	.16	.42	.20	-.70*	.61	.42	.60	.51	.46	___	.48	.19
TA	.65*	.03	-.37	-.51	.31	.15	.78*	.51	.61	.75*	___	.26
6PGD	.54	.34	.13	-.68*	.07	.13	.66*	.55	.30	.53	.49	___

[†]Second chromosome lines above diagonal; third chromosome lines below.

*p<0.05

TABLE 6

Summary of Analyses of Variance of CRM Level[†]

		CHROMOSOME 2					CHROMOSOME 3				
Variable		Week	Day (Week)	Line	Week x Line	K	Week	Day (Week)	Line	Week x Line	K
CRM-G6PD	RAW	*	**	***	ns	0.35	ns	*	***	ns	0.48
	PROT-ADJ	ns	***	*	ns	0.18	ns	***	***	ns	0.41
CRM-6PGD	RAW	ns	***	*	ns	0.21	ns	***	***	ns	0.61
	PROT-ADJ	ns	***	*	ns	0.21	ns	***	***	ns	0.54
CRM-ME	RAW	ns	***	***	ns	0.36	ns	***	***	ns	0.77
	PROT-ADJ	ns	***	ns	ns	0.10	ns	***	***	ns	0.78

[†]The significance levels of the F-tests and a variance component ratio $K = \hat{\sigma}^2_l/(\hat{\sigma}^2_l + \hat{\sigma}^2_{wxl} + \hat{\sigma}^2_e)$ are given for raw and adjusted variables.

ns p>0.05

* p<0.05

** p<0.01

*** p<0.001

TABLE 7

Linear Regressions Over Line Means of Activity on CRM Level and Protein

	Activity on CRM Level Only[†]		Multiple Regression of Activity on CRM Level and Protein[‡]			
Chromosome 2	$H_0:\beta=0$	R^2	CRM Level $H_0:\beta_1=0$	Protein $H_0:\beta_2=0$	$r_{AC\cdot P}$	R^2
G-6PD	**	0.69	*	ns	0.71*	0.69
6-PGD	ns	0.19	ns	**	0.50	0.74
ME	***	0.78	*	ns	0.69*	0.78
Chromosome 3						
G-6PD	*	0.49	*	ns	0.65*	0.50
6-PGD	**	0.56	*	ns	0.67*	0.56
ME[§]	*	0.83	***	ns	0.91***	0.83

[†]Significance levels of the regression coefficient (β) and the coefficient of determination (R^2) are given.

[‡]Significance levels of the partial regression coefficients (β_i), the partial correlation between activity and CRM level with protein fixed ($r_{AC\cdot P}$) and the coefficient of multiple determination (R^2) are given.

[§]The third chromosome ME null line is excluded.

ns $p>0.05$

* $p<0.05$

** $p<0.01$

*** $p<0.001$

TABLE 8

Localization of Activity Differences to Body Parts of Adult Males[†]

Variable	Abdominal Wall	Alimentary Tract	Head	Reproductive Organs	Thorax	Whole Body	All Body Parts[‡] Line	Line x Body Part
Lines RI09II-Ho-R								
G6PD	0.87±0.17***	0.18±0.07*	0.07±0.03*	0.10±0.03***	0.00±0.04	1.55±0.42**	*	***
6PGD	0.24±0.09*	0.10±0.02**	0.04±0.02	0.02±0.01	-0.01±0.02	0.74±0.17**	**	***
PROT	-1.6±1.1	-0.1±0.5	-0.4±1.0	-1.8±0.8	-3.4±1.8	2.5±3.9	ns	ns
Lines KA27III-RI22III								
G6PD	0.86±0.20***	0.10±0.08	-0.10±0.04*	0.05±0.02	0.03±0.02	4.90±3.17	**	***
6PGD	0.76±0.12***	0.02±0.03	-0.03±0.03	0.01±0.12	-0.04±0.01**	4.68±1.46*	ns	***
PROT	3.0±2.0	-1.8±0.8	-1.0±1.1	-1.1±2.1	-2.6±1.2	1.0±3.6	ns	**
Lines KA27III - Ho-R								
G6PD	0.63±0.18**	0.25±0.07***	0.12±0.03***	0.08±0.02**	0.04±0.03	4.53±2.96	***	***
6PGD	0.44±0.14**	0.10±0.03**	0.07±0.02**	0.01±0.01	-0.01±0.02	2.55±1.46	***	***
PROT	2.0±1.8	-0.6±0.5	0.6±1.2	-1.8±1.9	-3.2±1.8	1.1±4.3	ns	ns
Means Over All 4 Lines[§]								
G6PD	1.23	0.29	0.35	0.23	0.20	2.67		
6PGD	0.79	0.19	0.21	0.13	0.10	2.41		
PROT	10.1	4.5	8.1	6.6	10.6	32.7		

[†]The mean difference ± s.e. is given with significance level indicated when $p<0.05$. For G6PD, N=5 for body parts and N=6 for whole body; for 6PGD, N=6 for body parts and whole body; for protein, N=6 for body parts and N=18 for whole body. Activities are units x 10^3/body part and protein is μg/body part.

[‡]F-tests from ANOVAs of all 5 body parts for 2 lines.

ns $p>0.05$
* $p<0.05$
** $p<0.01$
*** $p<0.001$

[§]The body parts and whole body assays were performed at different times and with somewhat different methods so the sum of body parts is not necessarily less than the whole body.

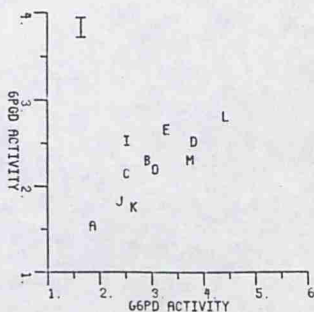
FIGURE LEGENDS

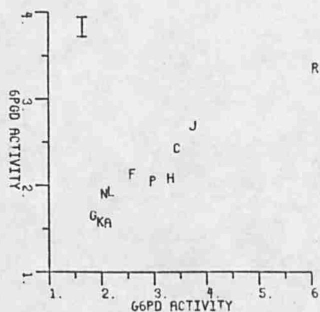
Figure 1. Plots of the line means of 6PGD versus G6PD activity for Experiments I (upper half) and II (lower half) and for both sets of chromosome substitution lines (chromosome 2 on left, chromosome 3 on right). Each letter represents a line. Second and third chromosome lines are represented by the same letter if they were derived from the same isofemale line. The only line that is identical between the second and third chromosome sets is the isogenic background stock, *Ho-R*, represented by the letter A. Both activities are in units x 10^3 per fly.

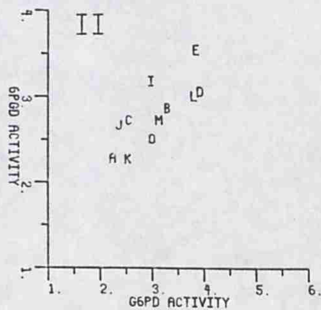
Figure 2. Plots of enzyme activity versus CRM level for G6PD, 6PGD and ME with the corresponding regression lines. Each letter represents a line, as in Figure 1.

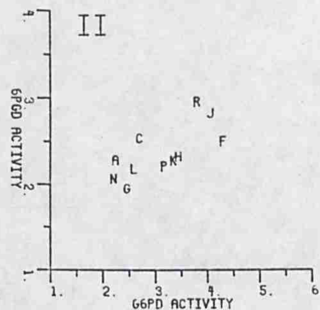
Figure 3. Polyacrylamide gel stained for G6PD activity. Lane 1 (left) is a homozygous Zw^B (slow) line for comparison with the 11 third chromosome isogenic lines (Zw^A, fast) in lanes 2-12.

SECOND CHROMOSOME LINES THIRD CHROMOSOME LINES









SECOND CHROMOSOME LINES

THIRD CHROMOSOME LINES