National Library of Canada

Acquisitions and Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada

Direction des acquisitions et des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Canada

UNIVERSITY OF ALBERTA

PTERIDINE ACCUMULATION AND ITS RELATION TO METABOLIC RATE IN HOUSE FLIES (MUSCA DOMESTICA L.)

BY

GRANT S. McINTYRE

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**.

DEPARTMENT OF **ENTOMOLOGY**

Edmonton, Alberta
FALL 1993

ISBN 0-315-88154-2

UNIVERSITY OF ALBERTA

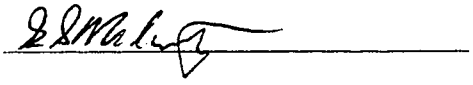
RELEASE FORM

NAME OF AUTHOR: **Grant S. McIntyre**

TITLE OF THESIS: **Pteridine accumulation and its relation to metabolic rate in house flies (*Musca domestica* L.)**

DEGREE: **Master of Science**

YEAR THIS DEGREE GRANTED: **Fall 1993**



11615-134 St.
Edmonton, AB
Canada
T5M 1J3

DATE: August 30, 1993

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faulty of Graduate Studies and Researh for acceptance, a thesis entitled **PTERIDINE ACCUMULATION AND ITS RELATION TO METABOLIC RATE IN HOUSE FLIES (*MUSCA DOMESTICA* L.)** submitted by **GRANT S. MCINTYRE** in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**.

R. H. Gooding
Dr. R. H. Gooding

B. K. Mitchell
Dr. B. K. Mitchell

Dr. L. Wang

DATE August 3rd, 1993

ABSTRACT

Pteridine accumulation in the head capsule of adult house flies, *Musca domestica* L., was examined using fluorescence spectroscopy. Head capsule fluorescence (HCF) was affected by age, temperature, size, sex, and duration of development. The rate of HCF accumulation in house flies decreased rapidly with age, at 17, 22, and 27C, but decreased less rapidly with age in male wild type and *salmon* mutant tsetse flies, *Glossina morsitans morsitans*, at 24C. Young adult *salmon* mutants had higher HCF than did wild type flies, but accumulated HCF at a lower rate. In house flies, the time from egg deposition to eclosion varied by 30 to 46% depending upon temperature. Flies that eclosed later had higher HCF at eclosion than did those that eclosed earlier and this difference persisted throughout the first 15 days of life at 22 and 27C. In two-day-old male house flies, rates of oxygen consumption were greater at 27C than at 17C by 115%. Confinement of flies in 1 ml mesh cages reduced oxygen consumption by 35%, and holding flies in total darkness reduced oxygen consumption by 33%. At 27C flies that had their activity limited through maintenance in the dark or through surgical removal of part of their wings exhibited greater longevity, but did not differ in HCF, from non-mutilated controls maintained under a 16L:8D photoperiod. Differences in HCF were not detected among 20-day-old flies that experienced differing degrees of wing damage and loss of flight ability. The log of total oxygen consumption of males was related to log (HCF) at 27C but not at 17C, in experiments in which oxygen consumption was measured for 30 min every 4 hours, between 24 and 96 hr after eclosion. In an experiment measuring total oxygen consumption at 17C between ages 24 and 96 hours, a significant relationship existed between the log of "above basal" oxygen consumption (total oxygen consumption less an estimate of basal oxygen consumption) and the log of HCF but not between the log of "basal" oxygen consumption and the log of HCF. The relationship of pteridine accumulation, activity, and metabolic rate is discussed.

ACKNOWLEDGEMENTS

I wish to express my thanks to those who helped and prodded me to complete this project. Dr. Ron H. Gooding offered consistent, kind and patient supervision during the course of this study. Brian Rolseth gave many practical suggestions for completing the laboratory work involved, and Drs. Larry Wang and Bev K. Mitchell provided valuable insights into the nature of pteridine accumulation in house flies.

TABLE OF CONTENTS

INTRODUCTION AND LITERATURE REVIEW 1
AGE GRADING METHODS IN INSECTS 1
THE PTERIDINE METHOD OF AGE DETERMINATION 2
THE BIOLOGY OF PTERIDINES IN INSECTS 5
LIPOFUSCIN AND ITS RELATION TO PTERIDINES 6
AGEING IN HOUSE FLIES 7
STATEMENT OF PURPOSE 9

METHODS COMMON TO THE DESCRIBED EXPERIMENTS 10
COLONY HISTORY AND MAINTENANCE 10
HEAD CAPSULE FLUORESCENCE ASSAY 10
RESPIROMETRY 11

THE PATTERN OF PTERIDINE ACCUMULATION IN HOUSE FLIES 12
METHODS 12
RESULTS 12
DISCUSSION 13

PTERIDINE ACCUMULATION IN *GLOSSINA MORSITANS MORSITANS* 18
METHODS 18
RESULTS 19
DISCUSSION 19

EFFECTS OF DURATION OF DEVELOPMENT AND ACTIVITY ON PTERIDINE ACCUMULATION 24
METHODS 25
Effect of Development Temperature 25
Effect of Development Duration 25
Effect of Presence and Absence of Light 26
Effect of Wing Removal 26
Effect of Loss of Flight Ability 26
RESULTS 26
Effect of Development Temperature 26
Effect of Development Duration 27
Effect of Presence or Absence of Light 28
Effect of Wing Removal 28
Effect of Loss of Flight Ability 28
DISCUSSION 28

THE RELATIONSHIP BETWEEN HEAD CAPSULE FLUORESCENCE AND OXYGEN CONSUMPTION 39
METHODS 39
Effects of confinement, temperature and light on oxygen consumption 39
Periodic measurement of oxygen consumption 39
Hourly measurement of oxygen consumption 40
RESULTS 40
Effects of confinement, temperature and light on oxygen consumption 40
Periodic Measurement of Oxygen consumption 41
Continuous Measurement of Oxygen consumption 42
DISCUSSION 42

GENERAL DISCUSSION 56

REFERENCES 61

APPENDIX A 68

APPENDIX B 70

APPENDIX C 71

APPENDIX D 73

LIST OF TABLES

Table 1 Mean (sem) relative fluorescence for male and female house flies maintained at two temperatures and two diets. 15

Table 2 Anova table of HCF of adult house flies examining effects of sex, temperature, age, and interactions. 15

Table 3. Comparison of mean (sem) age at death and mean size for *salmon* and wild type male *G. m. morsitans*. 21

Table 4. Comparisons of regression coefficients of head capsule fluorescence on age, for salmon and wild type male *G. m. morsitans* divided into young (up to 10-days-old) and old (more than 10-days-old) age classes. 21

Table 5. Emergence data for house flies mass reared at 17, 22, and 27C. 31

Table 6. Mean head width and HCF at eclosion for earliest emergent male and female house flies mass reared at 17 and 27C. 31

Table 7. Anova table for effects of emergence cohort, age, sex, and temperature on HCF of house flies. 32

Table 8. Mortality and mean (sem) HCF for male house flies maintained in the presence or absence of light. 33

Table 9. Mortality and HCF means (sem) for wing-clipped and non-mutilated flies. 33

Table 10. Head capsule fluorescence values for 20-day-old male house flies grouped by wing damage rating. 34

Table 11. Mean change in oxygen volume of control vessels (µl/hr). 45

Table 12. Anova of oxygen consumption of male house flies with confinement method, light conditions, and temperature as variables. 45

Table 13. Mean and range of oxygen consumption (µl/mg/hr), measured over three days, for male house flies at two temperatures, based on 1/2 hour measurement periods every four hours. 45

Table 14. Head capsule fluorescence of *M. domestica* males maintained under different light and temperature treatments. 46

Table 15. Comparisons made between head capsule fluorescence of *M. domestica* males for light and temperature treatments. 46

Table 16. Pearson correlation coefficients between weight, head width, and RF for one-day-old male house flies 47

LIST OF FIGURES

Figure 1 The effect of the presence of dietary protein on the accumulation of pteridines in (A) female and (B) male house flies at 27C. 16

Figure 2 The effect of age on head capsule fluorescence in (A) female and (B) male house flies at 17 and 27C, including regressions of log(HCF) on log(age). 17

Figure 3 Relationship of A) relative fluorescence and B) head capsule fluorescence with age, for male wild type and *salmon* mutants of *G. m. morsitans*. 22

Figure 4. Regressions of HCF on age for young (10 days old or less) and old (greater than 10 days old) male *G. m. morsitans*. 23

Figure 5. Emergence patterns for male and female house flies mass-reared at 17, 22, and 27C. 35

Figure 6. Emergence pattern of house flies mass-reared at 27C. 36

Figure 7. The relationship of HCF, at one day of age, to duration of preadult development for house flies at 27C. 37

Figure 8. Relationship of log(HCF) and log(age) for house flies emerging on consecutive days from the same egg collection, maintained at two temperatures. 38

Figure 9. Effects of confinement, light, and temperature on rate of oxygen consumption of male house flies. 48

Figure 10. Mean hourly oxygen consumption (microlitres/hr) of male house flies at 17 and 27C in the dark. 49

Figure 11. Log of RF vs log of oxygen consumption (both variables unadjusted for size) at 17 and 27C. 50

Figure 12. Log of rf vs log of oxygen consumption (both variables unadjusted for size) at 17 and 27C, regression of combined data. 51

Figure 13. The relationship between wet weight and total oxygen consumed, between 24 and 96 hours of age, for male house flies maintained at 17C. 52

Figure 14. Mean hourly oxygen consumption (microlitres/hr) of male house flies at 17C in the dark. 53

Figure 15. Coefficient of variation of hourly oxygen consumption of male house flies at 17C. 54

Figure 16. Relationship of A) log("above basal" oxygen consumption) and B) log(total oxygen consumption), to the log(HCF), between 24 and 96 hours of age, in male house flies at 17C 55

Chapter 1
INTRODUCTION AND LITERATURE REVIEW

Knowledge of the age structure of insect populations and the ability to determine accurately the age of individual insects would be helpful in studying insect ecology and the epidemiology of insect borne diseases. Consequently there has been a great deal of research on methods for determining the age of insects (reviewed by Tyndale-Biscoe (1984) and Lehane (1985)). A variety of methods are currently used although none are widespread. A recently developed age determination method based on the accumulation of pteridines in the compound eyes of Diptera (Mail *et al.* 1983), has shown promise of being more accurate and more straightforward to use than other methods. The pteridine method of age determination has not yet achieved its initial promise and complements rather than supersedes other methods (Wall, *et al.* 1991; Msangi and Lehane 1991; Lysyk and Krafsur in press) The purpose of the work described in this thesis was to examine the nature of pteridine accumulation in the house fly *Musca domestica* L., in hopes of increasing the usefulness of this method. Because this work grew out of attempts to use this method in population studies of house flies in an agricultural setting, most of the experiments described were conducted on house flies. Before describing the experimental work, I will review other age grading methods used with Diptera, the history and development of the pteridine method of age determination, basic pteridine biology in insects, the relationship of pteridines to lipofuscin and chloroform soluble fluorescent age pigment, and previous studies on ageing in house flies.

AGE GRADING METHODS IN INSECTS

At present all age determination methods used on insects rely on physical wear and tear or physiological changes in the insect. Use of each method is complicated by the fact that insects are poikilotherms and the rate of ageing is correlated with temperature, necessitating conversion of physiological age to chronological age. The simplest method of estimating age in insects is based on the accumulation of cuticular damage sustained by an insect. The most commonly used trait is the amount of wing area lost to fraying. This is an easy but inaccurate method. Mark/release/recapture experiments have demonstrated a large variation in wing fray among individuals (Vale *et al.* 1976) and seasonal variation in the rate of wing fray has been observed in *Glossina palpalis gambiensis* (Ryan *et al.* 1980). Two other mechanical damage indicators of age are mating scars and loss of bristles; both share problems of inaccuracy with the wing fray method.

A fairly new method of age determination is based on the fact that the cuticle of most insects continues to grow for up to 10 days after emergence (reviewed by Neville 1983). Cuticle growth has a threshold temperature below which cuticle is deposited in a helicoidal manner, and above which cuticle is deposited in parallel strands. In situations in which insects experience daily temperature fluctuations above and below this threshold, layering is visible in the cuticle. Layers can be easily observed using light microscopy and are best seen in thin sections of the apodemes or femora. This method can not be

used where the temperature does not regularly fluctuate around the deposition threshold and it does not aid in determining the age of insects that have stopped depositing cuticle. These problems are discussed by Lehane (1985).

Perhaps the most widely used method of age grading insects is the ovarian dissection method. This method relies on structural changes in the ovary during the development and laying of eggs and on changes in the developing eggs. The female insect is dissected and the stage of ovarian development and the number of previous ovarian cycles is determined. Based on this information and on the temperature history of the insect, chronological age is estimated. There are also certain indicators that can be used to distinguish parous from nulliparous insects. For instance, in nulliparous insects the tracheoles supplying the ovary lie in tightly coiled bundles along the exterior of the ovary. During the first ovarian cycle, the growing eggs cause the ovary to increase in size, stretching the adjacent tracheoles out of their coiled arrangement. After oviposition, the ovary contracts but the tracheoles do not regain their original arrangement (Detinova 1962; Anderson 1964). There are some severe limitations to this method, the most general of which is that it can only be applied to females. A further limitation is that in many species there is not a one to one relationship between ovarian cycles and scars or remnants, over the entire reproductive life of the female. When this is the case females can only be divided into broad age classes, such as parous and nulliparous (see Lehane 1985 for a more complete discussion of this topic).

THE PTERIDINE METHOD OF AGE DETERMINATION

The first work published on the use of pteridine accumulation for age determination in insects was on the stable fly *Stomoxys calcitrans* (Mail *et al.* 1983). The relationship between temperature, age and pteridine level was determined for laboratory reared flies and a multiple regression was produced with R^2 values of 0.93 ($p < 0.001$) for males and 0.92 ($p < 0.001$) for females. This regression was used in double blind experiments to estimate the age of laboratory reared flies to within ±1.49 days, this was similar in precision and accuracy to that achieved by the ovarian dissection method. The laboratory regression was then used to develop predictive equations, incorporating sunlight hours and ambient temperature, which were tested in the field. These equations were accurate to ±1.90 days for females and ±1.37 days for males. A final multiple regression of field data was developed and had R^2 values of 0.88 for males ($p < 0.001$) and 0.90 for females ($p < 0.001$). Mail *et al.* (1983) concluded that pteridine accumulation is increased by age and temperature and is greater in males than in females. No tests for effects of size or diet on fluorescence were made.

Lehane *et al.* (1986) fine tuned the field equations from the initial work by measuring the thoracic temperature of basking *S. calcitrans* in the field and by determining the minimum threshold temperature for pteridine accumulation and the rate of pteridine accumulation throughout the normal physiological temperature range of this insect. These refinements improved the predictive accuracy of the equations in the field. In an attempt to further improve the accuracy of the method, Mail and Lehane (1988) examined the age dependence of fluorescent compounds extracted from the head of *S.*

calcitrans, using thin layer chromatography. They found that increases in content of a single pteridine, biopterin, accounted for almost all of the increase in fluorescence level associated with ageing of the stable fly, and unpublished results were cited which extend this result to *Glossina morsitans*. Mail and Lehane concluded that the use of thin layer chromatography for separation and quantification of pteridines offered no predictive advantage over the more straightforward spectrofluorometric method for the two species examined, although the situation may be more complex for other species. In an earlier study examining the distribution of fluorescence between aqueous and organic portions of a chloroform/methanol/water extraction of house flies, Bridges and Sohal (1980) tentatively identified biopterin as the compound contributing most to the age associated increase in fluorescence of the aqueous fraction.

Since 1983 the age dependent increase in head capsule fluorescence (HCF) has been examined in *Stomoxys calcitrans* (Mail *et al.* 1983; Lehane *et al.* 1986; Mail and Lehane 1988; Lysyk and Krafsur in press), *Glossina* spp. (Lehane and Mail 1985; Langley *et al.* 1988; Lehane and Hargrove 1988; Msangi and Lehane 1991), *Cochliomyia hominovorax* (Thomas and Chen 1988), members of the *Simulium damnosum* species complex (Cheke *et al.* 1987, 1990), *Chrysomya bezzania* (Wall *et al.* 1990), *Lucilia sericata* (Wall *et al.* 1991), *Ceritatis capitata* (Camin *et al.* 1991, 1992), and *Haematobia irritans* (Krafsur *et al.* 1992). Most of these studies have followed lines of investigation similar to the initial work of Mail *et al.* (1983). Predictive equations have been developed in the laboratory and then tested in the field in mark/release/recapture experiments. In most studies linear multiple regressions were fitted to the data, producing R^2 values greater than 0.75. There are three exceptions to this method of analysis. Wall *et al.* (1990) fitted a power function of the form $Y = aX^b$ to data for *Chrysomya bezzania*. The curvilinear model accounted for up to 12% more of the variance than did the standard linear model. In the blowfly *Lucilia sericata*, Wall *et al.* (1991) found that a third order polynomial fitted by least squares provided the best fit for the data. Discriminant function analysis was used by Cheke (1990) to distinguish parity of blackflies. Except for a study by Camin *et al.* (1992), none of these studies identified the contributions of individual pteridines to the age dependent increase in fluorescence. Many of these studies did however examine the effect of size, which accounts for a small, but significant, amount of the variation in fluorescence. A single study investigated the effect of exposure to ultra-violet light on fluorescence in *G. m. morsitans*, but found no correlation between light level and fluorescence (Langley *et al.* 1988).

The effect of feeding and diet has also been examined. Starving *G. m. morsitans* for up to 36 hours has no effect on accumulation rate of fluorescent material (Lehane and Mail 1985). The presence or absence of dietary protein does not affect head capsule fluorescence in *Cochliomyia hominovorax* (Thomas and Chen 1988) and *Chrysomya bezzania* (Wall *et al.* 1990). In contrast to these results, the number of blood meals taken by female *G. m. morsitans* during pregnancy, affects the head capsule fluorescence of their offspring at eclosion (Msangi and Lehane 1991). These results indicate that larval nutrition may be important for initial development of pteridines, even though these are unaffected by nutritional status during adult life.

Monitoring programs which require accurate age estimates of individual flies are used extensively in planning and assessing control programs for tsetse flies *Glossina* spp. (Jordan 1986). It is in hopes of benefiting these programs that the greatest amount of field testing of the pteridine age determination method has been conducted. The predictive value of linear regressions developed in the laboratory (Lehane and Mail 1985) were tested in the field by Lehane and Hargrove (1988) and Langley *et al.* (1988) on tsetse flies using mark/release/recapture experiments. Both studies found that the method was not sufficiently accurate for determining ages of individual insects, although it did provide reliable estimates of continuous age distributions for both males and females. An additional problem with the pteridine method is that it is relatively inaccurate for estimating ages of very young tsetse flies, due to the high degree of variability in fluorescence at eclosion. Langley *et al.* (1988) failed to find a correlation between temperature during pupariation and fluorescence at eclosion. However, in a more detailed study, Msangi and Lehane (1991) determined that variability at eclosion is influenced by size, maternal nutritional status, and duration of the larval and puparial stages (these durations are determined primarily by temperature). They showed also that fluorescent material accumulates in the heads of very young flies while being lost from the abdomen, and that the ratio of abdominal fluorescence to head fluorescence is the best estimator of age for young flies. The use of this ratio improved the accuracy of the method, but did not produce age estimates of individuals that were reliable enough for use in control and monitoring programs. Consequently, the ovarian dissection method is still used in tsetse population monitoring programs (Msangi and Lehane 1991).

Although the pteridine method is not useful at present for estimating the ages of individual insects, two recent studies used it to advantage in studying the demography of insect populations. Krafsur *et al.* (1992) improved upon previous estimates (Krafsur and Ernst 1983, 1986) of survival, reproductive success, and age structure in horn fly, *Haematobia irritans,* populations by using pteridine age determination in conjunction with ovarian dissection. In another recent study that examined the relationship between ovarian development, pteridine accumulation, and temperature in *Stomoxys calcitrans* it was shown that pteridine accumulation has a lower threshold temperature than ovarian development and oviposition (Lysyk and Krafsur in press). This is a further indication that the pteridine method could be more accurate and reliable than the ovarian dissection method if the residual variance in age estimates of wild-caught flies can be reduced

In order to improve the pteridine age determination method the factors that lead to the increase in residual variance in pteridine accumulation in field populations of insects, over that of laboratory populations, must be identified. If these factors can be measured or estimated for field populations, the pteridine method will be free of the greatest obstacle to its widespread use. The factors cited most commonly to account for differences in pteridine level are age, temperature, sex, and body size. There are two other variables that are likely to affect pteridine accumulation: activity and genetic variance in pteridine accumulation. It is generally accepted that pteridine accumulation is an indicator of physiological age that is determined by metabolic rate. If this is the case, then differences in activity could account for some or most of the additional variance in HCF observed in field caught flies, since metabolic rate varies with activity

level. In flying insects, metabolic rate can sustain increases of between 60 and 300 fold (Davis and Fraenkel 1940; Hemmingsen 1960, cited in Peters 1983). Thus relatively small differences in time spent flying could cause large differences in HCF, if there is a reasonably high correlation between metabolism and HCF.

Genetic variance in HCF accumulation rate may also account for some of the unexplained variation in HCF. It is likely, since there are so many genes involved in control of eye pigmentation (83 in *Drosophila melanogaster*, Phillips and Forrest 1980), that genetic differences within populations could lead to differences in accumulation patterns. This has never been tested.

THE BIOLOGY OF PTERIDINES IN INSECTS

The present understanding of pteridine biology sheds little light on how and why pteridines accumulate in the eyes of adult Diptera. Pteridines are end products of purine metabolism. The immediate precursor of the pteridines is guanosine triphosphate, which is converted to dihydroneopterin by GTP cyclohydrolase. In *Drosophila*, this enzyme has an activity peak at pupariation, associated with synthesis of isoxanthopterin in the abdomen, and a larger peak at eclosion, associated with drosopterin synthesis in the eyes. Pteridine biosynthesis and transport are not well understood in insects (Ferré *et al.*1986).

All pteridines found in nature are built around the pterine ring and differ in the nature of the side-chains. In general the pteridines found in insects are polar amphoteric molecules, soluble at high or low pH, but insoluble near pH 7. When dissolved and separated from the protein carriers with which they are normally associated *in vivo*, pteridines exhibit auto-fluorescence under UV light. Pteridines vary in colour, photolability, solubility, and reactivity. They are found at some stage during the development of all insects, and are found in most tissues of most insects at most stages of development (Ziegler and Harmsen 1969). Pteridines have been found in all mammalian tissues examined (Nichol *et al.* 1985).

The nature and function of the pteridines in insects are quite variable among tissues, life stages, and species. Many of the physiological roles of pteridines have been elucidated in mammals, but the results generally hold for those insects that have been examined (Summers *et al.* 1982). Tetrahydrobiopterin is a cofactor for phenylalanine hydroxylase, and tryptophan and tyrosine mono-oxygenases, and is involved in pyrimidine synthesis. Pteridines are involved in, or indicative of, a number of metabolic disorders including phenylketonuria and many cancers. They are also involved in the immune response, where they are markers of T-lymphocyte activation and may be synergists for other immune activators. Pteridines do not have a role as pigments in mammals (Nichol *et al.* 1985).

In insects pteridines play two additional roles. They act as pigments and they are thought to be inert storage excretion products of nitrogen metabolism (Ziegler and Harmsen 1969). The accumulation of pteridines in the wings of Lepidoptera, where they were discovered, and in the compound eyes of Diptera is thought to be a combination of these two functions. In Diptera the study of eye colour mutants has led to the description of numerous pteridines in the compound eye, where they and the ommochromes function

as screening pigments (Ferré *et al.* 1987). They are less important to visual function than are the ommochromes (Langer 1975; Summers *et al.* 1982). Pteridines are localized in the pigment cells which surround the receptor cells in each ommatidium and are found throughout the cytosol, bound to carrier proteins that render them soluble at physiological pH and that prevent fluorescence. In *Drosophila* spp., however, pteridines are localized in membrane bound pigment granules (Summers *et al.* 1982).

LIPOFUSCIN AND ITS RELATION TO PTERIDINES

One of the most commonly observed age-dependent changes in animals is an increase in cellular lipofuscin content. Lipofuscin is a complex association of primarily lipid based compounds that are yellowish-brown in appearance, auto-fluorescent, and contained in membrane bound granules that are 1-5 μm in diameter. Proteins and various compounds that are resistant to acid hydrolysis are also present. Lipofuscin granules increase in size and/or number in ageing, post-mitotic cells and can make up more than 40% of the cytoplasmic space of cells of older animals (Brizzee and Ordy 1981). In insects, accumulation of lipofuscin is greatest in midgut, fat body, brain, and Malphigian tubules. The specific composition of lipofuscin granules is heterogeneous within and between cells, tissues and species (Sohal 1985a).

Lipofuscin appears to originate from the auto-oxidation of lipids, especially membrane lipids, and subsequent reactions of these products. This sequence is postulated to begin with reaction of free radicals with polyunsaturated fats (Tappel 1975). It is believed that lipofuscin granules are secondary lysosomes containing the indigestible residues of autophagic engulfment of cytoplasm and organelles. (Miquel *et al.* 1977). These origins provide a satisfactory explanation of why the lipofuscin content of old cells is greater than that of young cells.

The earliest identifiable description of lipofuscin was that of Hannover (1842, cited in Oliver 1981), who reported its presence in human nerve cells. Later Koneff (1886, cited in Oliver 1981) established a relationship between age and lipofuscin accumulation. Since then lipofuscin accumulation has come to be regarded as one of the hallmarks of ageing, especially in the study of vertebrate cardiac muscle and nervous tissue. Lipofuscin has been studied by comparing normally ageing animals with those having a pathological accumulation of lipofuscin due to genetic defects, and by manipulating normal animals through hormone treatment, dietary alteration, and exposure to lipofuscin inducing compounds. Lipofuscin is normally quantified by chemical extraction and measurement of fluorescence intensity, by morphometric analysis of histological preparations, or by measurement of fluorescence intensity in histological preparations using fluorescence microscopy (Oliver 1981; Hammer and Braum 1988).

An extraction procedure for lipofuscin was developed by Fletcher *et al.* (1973). The method consists of homogenizing tissue in a 2:1 chloroform:methanol mixture which is then washed with water to remove polar compounds. Concentrations of these compounds increase with age and are hereinafter referred to as chloroform soluble fluorescent age pigment (CSF). It has not been established unequivocally that these extracted pigments are homologous with those observed histologically. The ranges of

fluorescence excitation and emission peaks are similar for CSF and lipofuscin (CSF: excitation peak-365±12.4 nm, emission peak-445±15.7 nm, n = 29; lipofuscin: excitation peak-361±10.9 nm, emission peak-444±19.2 nm, n=34 (Hammer and Braum 1988)). However, only a small percentage of fluorophores in histological sections can be removed by washing with chloroform, a quantitative correlation between CSF and lipofuscin has never been established, and lipofuscin *in situ* fluoresces yellow, but CSF fluoresces blue (Sheehy and Roberts 1991). Furthermore, several inconsistencies between age associated accumulation patterns of CSF and lipofuscin have been documented (Sohal 1987). Further confusion arises from the fact that extractions of CSF often contain polar compounds due to inadequate volumes of water being used during the wash. Many of the results linking CSF content to age in insects may be better explained by the association of pteridine concentration with age, since the pteridines were not completely washed from the chloroform phase during extraction (Sheehy and Roberts 1991). (Unless otherwise noted, the published reports cited in this thesis used sufficient wash volumes to achieve complete separation of soluble and insoluble fractions.)

Many studies have reported on age dependent accumulation of lipofuscin (see reviews by Dolman and McLeod 1981, and Wolman 1980) and several studies have been conducted on the accumulation of CSF in insects (reviewed by Sohal 1985a). Accumulation rates of CSF in insects increase with temperature (Sheldahl and Tappel 1974; McArthur and Sohal 1982). In house flies the rates of lipofuscin and CSF accumulation are related to physical activity (Sohal and Donato 1979; Sohal 1981). Flies that are maintained under conditions that lead to relatively low levels of activity, live longer and accumulate lipofuscin and CSF less quickly than do flies maintained under conditions leading to high activity. Increased temperature and increased activity lead to higher metabolic rates which could explain the increases in CSF and lipofuscin through increased production of precursor molecules.

AGEING IN HOUSE FLIES

Ageing in the housefly has been extensively studied in recent years by Sohal and coworkers. This investigation was initially conducted with a view towards confirming the rate of living theory and later with the aim of examining the effects and interactions of pro- and anti-oxidants with ageing, life span and age related phenomena.

The rate of living theory, as originally postulated by Pearl (1922, 1928) states that the duration of life is a function of two variables: the genetically determined, inherent vitality, and the rate of energy expenditure during life. This theory was built upon the observation made by Rübner (1908) that the lifetime energy expenditure of five mammals (horse, cow, dog, cat, and guinea pig) was similar, ranging from 170-226 Kcal/gm, while life span varied fivefold. This led Rübner to postulate that duration of life depends upon the rate at which a discrete amount of biological energy, or vitality, available to an organism is expended. Loeb and Northrop (1917) later showed that longevity of *Drosophila melanogaster* is inversely related to temperature, and postulated that duration of life is determined by either the temperature dependent production of a death causing substance, or the destruction of a death preventing substance. After further work on *D.*

melanogaster and cantaloupe seedlings Pearl (1922, 1928) proposed the rate of living theory. One corollary of this theory is that if metabolic potential, or vitality, is the same within a group of organisms, then barring accident or disease, longevity is a function of metabolic rate.

Testing this corollary was the thrust of much of Sohal's early work on ageing in the house fly. His main contribution in support of the rate of living theory was to show that longevity of house flies varies inversely with activity, when activity is modified by altering population density, and that certain age dependent changes occur more rapidly in house flies from active populations than in those from less active populations (Sohal and Allen 1986). Much of Sohal's recent work on house flies considers, more directly, the relationship between pro- and anti-oxidants and ageing and is outside the scope of this thesis.

In order to alter the activity of house flies, Sohal and coworkers altered the housing conditions or removed the wings of the flies. Surgical removal of the wings of one-day-old house flies resulted in decreased activity and increased longevity (Ragland and Sohal 1973; Sohal and Buchan 1981a). In several experiments flies were housed in one of three confinement systems: singly in 150-250 ml vials containing cardboard mazes that prevented flight, singly in one cubic foot cages, or in groups in one cubic foot cages (within this treatment crowding and sex ratios were varied and large glass bottles were used in some experiments). In all confinement methods flies were provided with food and water. Through doppler radar measurement of activity, it was shown that flies housed singly were significantly less active than flies living in groups, and that increased crowding and higher ratios of males to females also increased activity. (Ragland and Sohal 1975; Buchan and Sohal 1981; Sohal and Buchan 1981a). Throughout these experiments the less active fly populations had greater mean longevity than did the more active ones. However, Sohal was unable to demonstrate this relationship among individuals. Significant correlations were not observed between longevity and activity (Sohal and Buchan 1981a) or between longevity and oxygen consumption (Sohal 1982) when measurements were made on individual flies. Thus, although the crucial proofs are lacking, the body of evidence tends to confirm that life span is determined, in part, by metabolic rate.

Further evidence that activity and metabolic rate are associated with longevity is drawn from the study of effects of housing on age related changes in the house fly. Less active flies accumulate lipofuscin (Sohal and Donato 1979; Sohal 1981) and chloroform soluble fluorescent age pigment (Sohal and Donato 1978; Sohal and Buchan 1981b) at a lower rate than do active flies. Interestingly a significant relationship between activity and chloroform soluble fluorescent age pigment content was demonstrated for individual flies (Sohal and Buchan 1981b). Two other indicators of age, the rates of lipid peroxidation and n-pentane production, were positively correlated with temperature and thus negatively correlated with longevity (Sohal *et al.* 1981, 1985).

The work of Sohal is not without its critics. Lints *et al.* (1984) and Le Bourg (1987) failed to find a correlation between spontaneous locomotor activity and longevity in *D. melanogaster*. These workers contend that Sohal's results show that stress through over activity and exhaustion decrease longevity but that at moderate levels of activity an

inverse correlation with longevity does not exist. In response Sohal (1985b:333) suggested that "deleterious effects of physical activity on life span would occur above a certain threshold level of activity." In his view the experiments of Lints and Le Bourg did not expose the test flies to levels above this threshold. Lints *et al.* (1984:385) make a distinction between activity, "the quality or state of producing action or movement" and reactivity "the quality or state of being readily responsive to a stimulus." Much of Sohal's work examines the effects of reactivity on longevity and age related changes. It is possible that activity and reactivity (*sensu* Lints *et al.* 1984) make separate contributions to ageing. If this is so, the rate of living theory requires some reworking.

The free radical theory of ageing may provide a framework for understanding the effects of activity and reactivity as defined by Lints *et al.* (1984). The link between the rate of living theory and the free radical theory of ageing is based on the postulate that animals that use more oxygen per unit mass will die earlier due to increased oxidative stress and damage (Harman 1992; Sohal and Allen 1986). Normal metabolism generates free radicals, especially oxygen free radicals associated with the electron transport chain. Cells have a variety of mechanisms that prevent or repair damage caused by these free radicals. A current interpretation of research in this area is that as cells become more active metabolically, the generation of free radicals outstrips the ability of the cell to cope adequately, and irreversible cell damage occurs, especially to the mitochondria, and this leads to cell death. Lipofuscin is thought to be a product of this oxidative damage. (Harman 1992; Miquel 1992). House flies whose reactivity levels are above normal may generate more free radicals in certain tissues than can be dealt with and thus ageing (i.e. irreparable cell damage) occurs. This is corroborated by the observation that the flight muscles of old house flies and *Drosophila* display serious damage and loss of mitochondrial organization, as would be expected if increased activity of these muscles leads to ageing and/or cellular damage (Turturro and Shafiq 1979; Miquel 1992).

STATEMENT OF PURPOSE

The purpose of this study was to provide a basic understanding of the pattern of pteridine accumulation in *Musca domestica* L., to compare and contrast this pattern with previously studied Diptera, and to examine the relationships of pteridine accumulation with duration of development, activity, and metabolic rate. The results are discussed in relation to the future usefulness of the pteridine age determination method, the role of pteridine accumulation as a form of storage excretion, and the interrelationships of pteridine accumulation, metabolic rate, physiological age, and the ageing process.

Chapter 2
METHODS COMMON TO THE DESCRIBED EXPERIMENTS

Certain methods are common to many of the experiments described in the following chapters and are given in detail here. These common methods include the history and maintenance of the house fly colony used, the pteridine assay, and the measurement of oxygen consumption.

COLONY HISTORY AND MAINTENANCE

The colony used for these experiments was established with approximately 30 adults collected at the University of Alberta Swine Research Centre on December 12, 1991. During the first six months of laboratory maintenance a number of diets and egg densities were tested and two bottle necks of approximately 50 adults occurred. The following is a description of the maintenance schedule used between July, 1992 and August, 1993, during which time the experiments for this thesis were conducted. Eggs laid over an approximately seven hour period, by 7-21-day-old adults, were collected on cotton moistened with condensed milk. Eggs were separated from the cotton by hand and stirred in deionized water until thoroughly mixed. A volume of ~0.3 ml of eggs was placed in 300 g of larval rearing medium that consisted of 2:1:3 bran:condensed milk:water to which a pinch of baker's yeast was added. This gave an approximate density of six eggs/g of medium, and consistently produced large vigorous flies. This is comparable to optimal densities of five eggs/g, using a different medium, reported by Sullivan and Sokal (1963). Batches of inoculated medium were covered with a two cm layer of fine wood chips and incubated at 27C with a 16L:8D photoperiod. Pupariation occurred after six days and adults began to eclose on the tenth or eleventh day after egg collection. Adults were maintained in mixed sex groups in cages 20 x 20 x 20 cm and provided with water and a 2:1 mixture of granulated sugar and powdered skim milk *ad lib*. This rearing and maintenance schedule is referred to throughout the current work as standard conditions. Deviations in housing conditions and temperature are noted in the methods sections of each experiment.

HEAD CAPSULE FLUORESCENCE ASSAY

Individual flies were cold anesthetized, sexed, and head width was measured with an ocular micrometer, mounted in a Wild M5 dissecting microscope, to estimate body size. Head capsule fluorescence (HFC) was then measured on individual heads, using a modified version of the procedure of Mail *et al.* (1983). Each head was homogenized in 0.3 ml of 0.05 M tris/HCl buffer at pH 8.0. The volume of the homogenate (which was protected from light from this stage onwards) was increased to 5.0 ml and centrifuged at 6000g for 5 minutes. Fluorescence of the supernatant was measured immediately, using the fluorescence attachment on a Beckman DU-2 spectrophotometer with excitation at 360 nm and emission at 450 nm. The daily performance of the spectrophotometer was monitored using 2.1 mM riboflavin in the homogenizing buffer and fluorescence values

were adjusted accordingly. Results are generally reported as either relative fluorescence (RF), the reading taken from the spectrophotometer, or as head capsule fluorescence (HCF), the ratio of relative fluorescence and head width.

RESPIROMETRY

A Gilson differential respirometer was placed in a darkened room and covered with a heavy black cloth during the experiments. Two methods to confine the flies were used, depending on the nature of the experiment. For experiments in which flies were restrained in mini-cages (wire mesh cages, approximately 1 cm^3), 0.5 ml of degassed, deionized water and a small cotton wick were placed in the centre well of each 15 ml reaction vessel. It had been previously determined that this arrangement has no effect on gas volume in the vessel over the time periods used in the experiments discussed below. A mini-cage containing a fly was placed on top of the well in each vessel, in contact with the cotton wick, to prevent dehydration of the fly. As a CO_2 absorbent, 0.3 ml of 25% KOH has placed in the bottom of each reaction vessel. For experiments in which the flies were free within the reaction vessel, the KOH was placed in the centre well, which was covered with a mesh screen, and the water and cotton was placed to one side in the bottom of the reaction vessel. Between readings the system was left open to the air; 15 min prior to each reading the system was closed and allowed to stabilize. Each reading was made over either a 30 min or a 60 min period, measured to the nearest 0.5 min. Readings were recorded as µl O_2/hr and converted to volume of dry oxygen at one atmosphere, 0.0C. For this conversion local barometric pressures were obtained from the weather station at the Edmonton Municipal Airport.

Chapter 3
THE PATTERN OF PTERIDINE ACCUMULATION IN HOUSE FLIES

Due to the lack of information concerning pteridine accumulation in the house fly, a preliminary experiment was conducted similar to previous studies of pteridine accumulation in other insects (Mail *et al.* 1983; Lehane and Mail 1985; Wall *et al.* 1990, 1991). The effects of age, temperature, size, sex, and diet were examined, and a multiple regression equation was calculated from the data. The results are discussed in relation to previous studies.

METHODS

One-day-old adults, that had been reared under standard conditions and that emerged over a 24 hour period, were maintained at 17 or 27C under otherwise standard conditions. (This experiment was conducted also at 22C, but the incubator did not maintain a constant temperature and the data are not included in the analysis.) Protein (powdered milk) was removed from the diet of one group of flies, maintained at 27C. On days 1, 5, 10, and 15 after emergence, ten males and ten females were collected from each cage, with three exceptions. Due to high mortality at 27C, the day 15 sample included only two males from the protein diet regime and nine males from the no protein diet. The final sample from the 17C treatment was made on day 17 instead of day 15. Individual flies were assayed for HCF.

RESULTS

The data were examined for differences in HCF due to diet, size, sex, temperature and age. Means are presented in Table 1. An analysis of covariance, with head width as covariate, revealed that the presence or absence of protein in the diet had no effect on relative fluorescence of either males or females (males: $F_{1,68} = 0.52$, $p = 0.473$; females: $F_{1,77} = 0.14$, $p = 0.708$; Figure 1). Covariance was used because it was suspected that head width would have an effect and mean head widths were significantly different between flies maintained on different diets for each sex (males: $F_{1,69} = 108.18$, $p < 0.001$, females: $F_{1,79} = 15.93$, $p < 0.001$). The effect of size on RF was examined by computing regressions of RF on head width for individual samples at 27C. Since diet did not affect RF, protein and no protein samples were pooled to increase sample size. All 17C samples and the sample of 15-day-old males at 27C were excluded from this portion of the analysis because the sample sizes were too small to test reliably the hypothesis that flies with larger heads exhibit greater RF. Of the remaining seven samples, all produced significant regressions ($p < 0.001$) with an average R^2 of 0.62 (sem = 0.043). The intercept was not significantly different from zero ($\alpha = 0.05$) in any of these regressions. On average, head width accounted for 62% of the variation observed in RF within groups of same age flies at 27C. In light of this relationship between head width and relative fluorescence, pteridine content of the head capsule was reported throughout this thesis as

head capsule fluorescence (HCF), the ratio of RF and head width (both in arbitrary units). For the remainder of the analysis of this experiment HCF was the variable of interest.

An analysis of variance indicated that sex, temperature and age had highly significant effects on HCF, as did the interactions between temperature and age, and sex and age (Table 2). Female house flies are generally larger than males, but males have proportionately larger eyes and in the present study males exhibited greater HCF than did females of the same size and age. The rate of HCF accumulation increased with increasing temperature. The exact nature of the temperature dependence cannot be adequately described from the present data since only two temperatures are included in the final analysis. The relationship of adult maintenance temperature with HCF was not examined further, since this effect has been well studied in other insects (Mail *et al.* 1983; Lehane and Mail 1985; Lehane *et al.* 1986; Lysyk and Krafsur in press).

The relationship between age and HCF was nonlinear (Figure 1). Accordingly, HCF and age were log transformed and regressions of log HCF on log age were calculated for each sex at each temperature (Figure 2). Within each sex at each temperature, age accounted for an average of 97.3% of the observed variation in HCF. For the purposes of comparing total residual variance in RF in this experiment with that in published work, a multiple regression of log RF, with log age (days), temperature (C), sex (which was treated as a categorical variable, male = 1, female = 2), and head width (arbitrary units) as regressors, was calculated.

$$\log(RF) = (0.392 \times \log(age)) + (0.009x \text{ temperature}) - (0.047 \times sex)$$

$$+ (0.008 \times \text{head width}) + 2.107 \qquad (R^2 = 0.92,\ p < 0.001)$$

DISCUSSION

The results of this experiment were similar to the results of studies on other insects (Mail *et al.* 1983; Lehane and Mail 1985; Wall *et al.* 1990, 1991). Age accounted for most of the variation between individuals in pteridine content. Temperature, size, and sex also had significant effects, but the presence or absence of protein in the diet had no effect. The results of this investigation deviated from those reported for other insects in the nature of the relationship between age and fluorescence. With the exceptions of reports on *Chrysomys bezzania* (Wall *et al.* 1990) and *Lucilia sericata* (Wall *et al.* 1991) in which slightly curvilinear relationships were reported, all previous reports suggest a linear relationship between HCF or fluorescence and age. This relationship was strongly curvilinear in house flies. This difference is not easily explained. Use of the riboflavin standard and experiments with tsetse fly (Chapter 4) indicated that this difference was not due to measurement techniques. If pteridine accumulation is a form of storage excretion, as is generally thought (Ziegler and Harmsen 1969; Summers *et al.* 1982), then the

decline in accumulation rate with age in the house fly raises interesting questions about the nature of this process. Do ageing house flies produce less precursor to this waste product? If so why? If not, why does accumulation decline? These questions are discussed in the final chapter of this thesis.

The results of this experiment were similar to other work in the amount of unexplained variation (about 8%) in the overall data set. It is likely that, as with other insects, this residual variation would be larger in flies that had been collected from natural habitats where the environmental complexity is greater than in the laboratory. The following chapter presents results of investigations into factors contributing to this residual variance, which have not been considered in previous work.

Table 1 Mean (sem) relative fluorescence for male and female house flies maintained at two temperatures and two diets.

Sex	Age (days)	17C protein and carbohydrate	27C carbohydrate	27C protein and carbohydrate
male	1	9.35(0.329)	10.15(0.302)	8.59(0.351)
	5	16.99(0.467)	23.70(0.362)	22.39(0.316)
	10	19.38(0.523)	26.46(0.358)	26.64(0.322)
	15		27.64(0.430)	30.73(0.286)
	17	24.48(0.453)		
female	1	8.24(0.300)	9.17(0.347)	7.97(0.209)
	5	14.92(0.562)	21.41(0.601)	20.68(0.402)
	10	18.47(0.468)	24.89(0.385)	23.33(0.413)
	15		24.63(0.290)	25.17(0.472)
	17	21.50(0.370)		

Table 2. Anova table of HCF of adult house flies examining effects of sex, temperature, age, and interactions.

Source	Sum of Squares	d.f.	F	p
AGE	9096.94	4	1156.37	< 0.001
SEX	205.133	1	104.30	< 0.001
TEMPERATURE	682.92	1	347.24	< 0.001
AGE*SEX	31.56	4	4.01	< 0.01
AGE*TEMPERATURE	327.67	2	83.31	< 0.001
SEX*TEMPERATURE	0.54	1	0.28	> 0.05
AGE*SEX*TEMPERATURE	6.43	2	1.64	> 0.05
ERROR	372.25	213	--	--

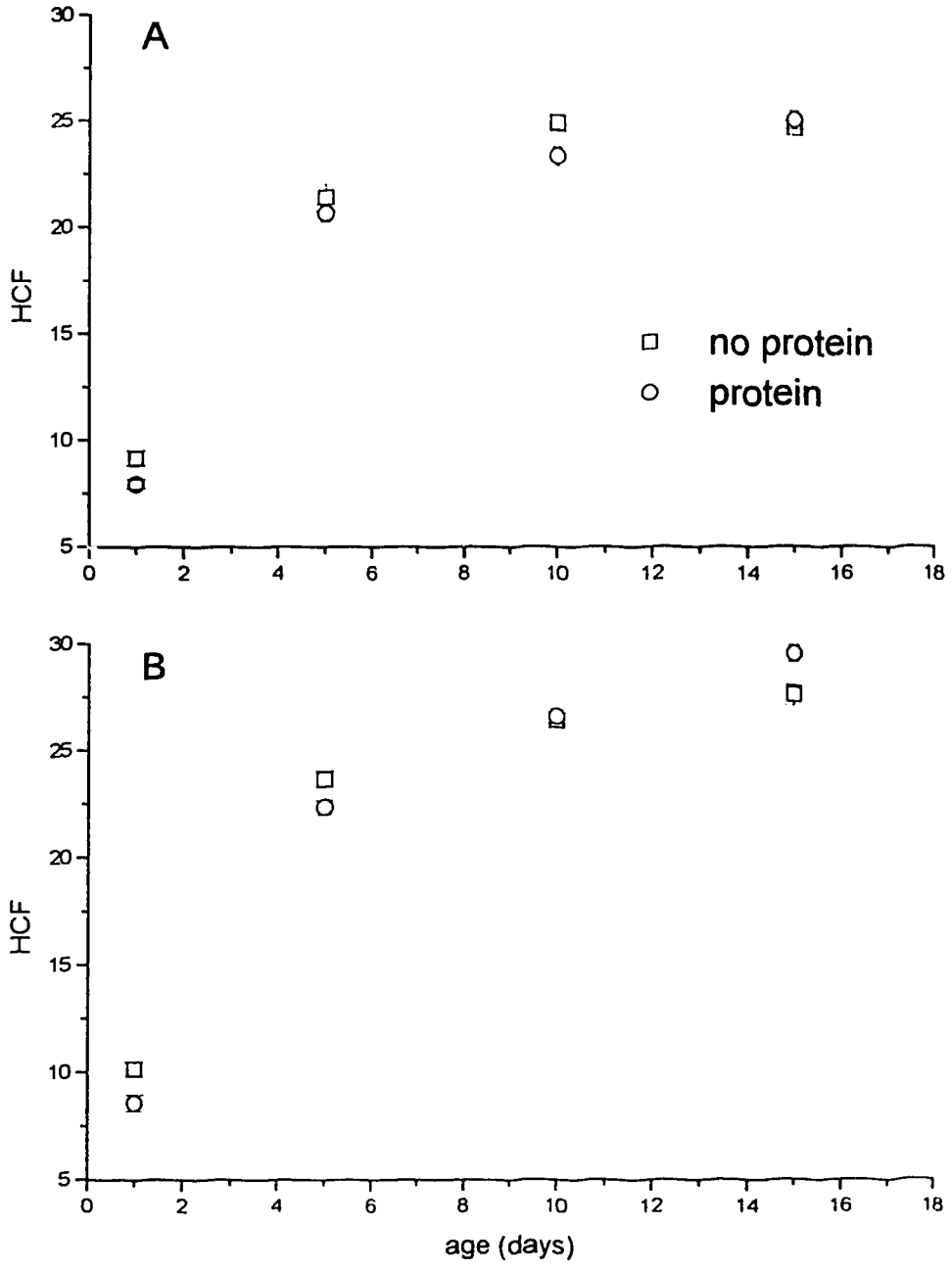


Figure 1. The effect of the presence of dietary protein on the accumulation of pteridines in (A) female and (B) male house flies at 27C. (Vertical bars = standard error)

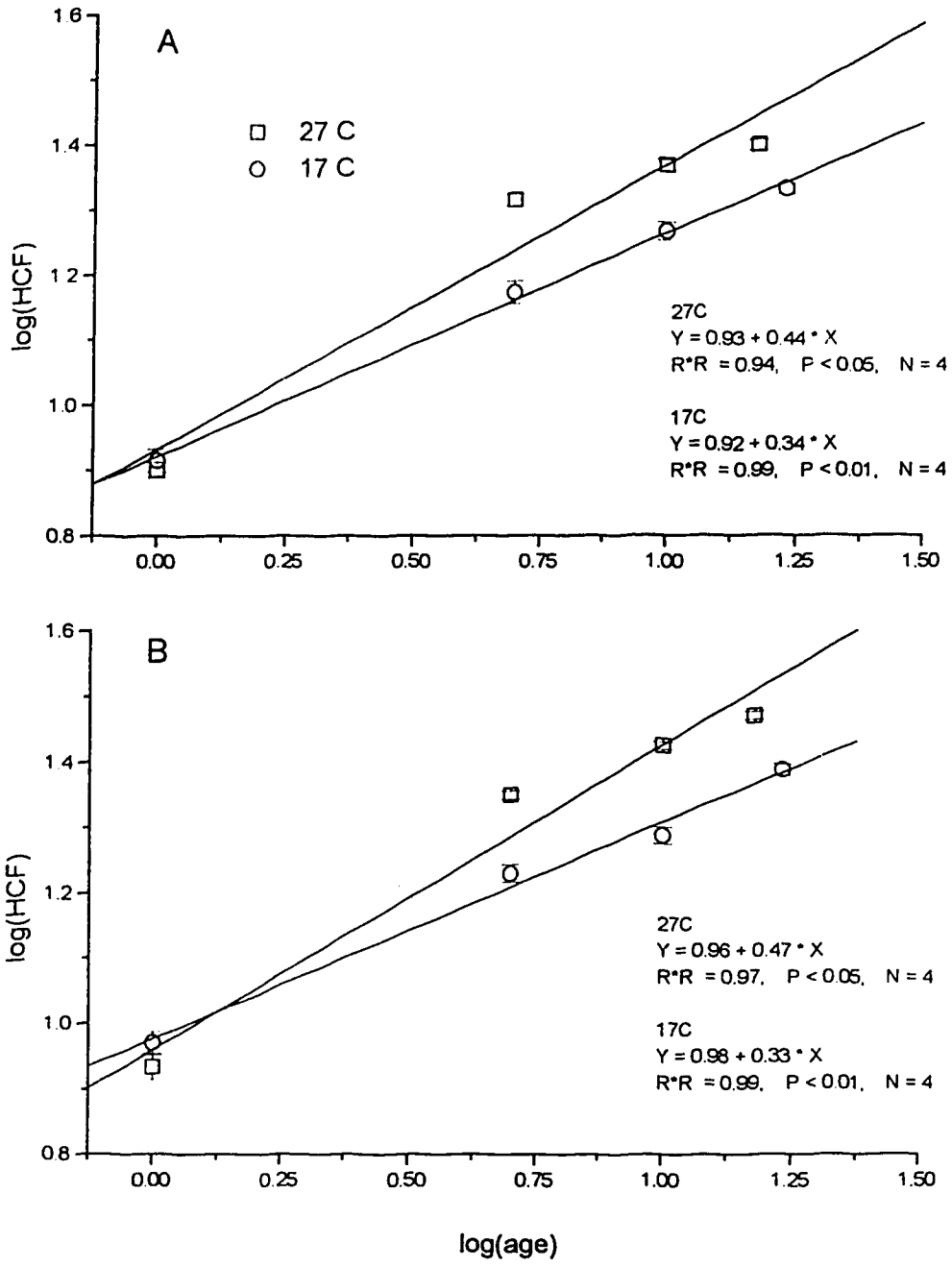


Figure 2. The effect of age on head capsule fluorescence in (A) female and (B) male house flies at 17 and 27C, including regressions of log(HCF) on log(age) (vertical bars = standard error).

Chapter 4
PTERIDINE ACCUMULATION IN *GLOSSINA MORSITANS MORSITANS*

The most unusual result of the investigation into the pattern of pteridine accumulation in house flies was the markedly *curvilinear* nature of the relationship between HCF and age. Previous work reported either linear relationships (Lehane and Mail 1985; Langley *et al.* 1988; Lehane and Hargrove 1988; Lysyk and Krafsur in press) or relationships with a very slight degree of curvilinearity (Wall *et al.* 1990, Wall *et al.* 1991). Although the use of the riboflavin standard indicated that there were no technical problems with the HCF assay procedures used it was decided to confirm this through an examination of the pteridine accumulation pattern in *Glossina morsitans morsitans* Westwood, which has been reported to exhibit linear increases in fluorescence with respect to time (Lehane and Mail 1985). Since the short-lived eye colour mutant, *salmon*, of this species was readily available it was decided to compare the pteridine accumulation of the mutant with wild type flies to address some questions concerning the relationship of the rate of ageing with pteridine accumulation, in addition to determining the degree of curvilinearity present in the age/HCF relationship.

The greatest gains in the understanding of eye pigment biology in insects were made by investigating eye colour mutants, especially in *Drosophila* spp. (Ferré *et al.* 1986). In considering questions of HCF accumulation it was thought that a comparison of eye colour mutants of *G. m. morsitans* with wild type flies would prove fruitful, since the age dependent accumulation of pteridines is relatively well known in this species. In *G. m. morsitans*, *salmon* mutants have shorter longevity than do wild type flies (Gooding 1982). If these short-lived mutants have an increased rate of pteridine accumulation, it might indicate that the shortened longevity is due to increased metabolism. Alternatively the *salmon* mutants may be dying at a younger chronological and physiological age because of events that are not related to ageing *per se*.

METHODS

The flies used in this experiment were from an inbred line (#231) of *G. m. morsitans* that has been selected for four biochemical (arginine phosphokinase, aldehyde oxidase, xanthine oxidase, malic acid dehydrogenase) and two visible (*ocra* body colour and *salmon* eye colour) marker genes. The colony is maintained by crossing *+/sal* females with *sal*/Y males; thus all males have a similar genetic background. Males used in this experiment were marked within 24 hours of eclosion with Testor's enamel paint, to indicate their date of eclosion, and placed in 4 x 8 x 16 cm cages. Each cage contained flies that emerged during up to 10 consecutive days, but never more than a total of 15 flies. For two weeks of the approximately five week puparial period, puparia were maintained at temperatures between 15 and 18C; the remainder of the time was at 24C. Adult flies were maintained at 24C by feeding on rabbits five days per week for up to 12 weeks. Mortality was noted every two or three days. When the oldest fly was 86 days old, all surviving flies were assayed for HCF. To gather more information concerning

HCF in young flies, a second collection of flies was made. These flies were assayed for HCF when the oldest were 14 days of age.

RESULTS

There was no difference between *salmon* and wild type with respect to size (head width), longevity (mean age at death), or numbers which died during the course of the experiment (*salmon*: 8 dead, 36 alive; wild type: 15 dead, 45 alive, $\chi^2 = 0.35$, $p > 0.05$; Table 3). A test for homogeneity of regression coefficients revealed that the regressions of HCF and RF on age differed significantly between *salmon* and wild type flies of up to 84 days of age (HCF: $F_{1,77} = 11.32$, $p < 0.01$; RF: $F_{1,77} = 7.11$, $p < 0.01$; Figure 3). There was a difference, in intercept but not in slope of the regression of HCF on age, between *salmon* and wild type for flies 10 days of age or younger with *salmon* flies exhibiting higher HCF levels. These regressions for flies older than 10 days of age did differ in slope between genotypes. Furthermore, the regressions of HCF on age differed between flies 10 days of age or less and flies greater than 10 days of age, for both genotypes. Younger flies exhibited greater rates of pteridine accumulation than did older flies. Regressions of HCF on age for young and old flies are presented in Figure 4, and statistics for the comparisons made between regressions are in Table 4.

DISCUSSION

The difference in slope of HCF on age between young and old tsetse flies revealed a slight curvilinearity in the pattern of pteridine accumulation in tsetse flies that is not easily noticed in the analysis of the entire data set. The apparent linearity in *G. m. morsitans* suggests that the curvilinear pattern of pteridine accumulation observed in house flies was not an artifact of the HCF assay procedure used. Thus, differences between the patterns exhibited by house flies and tsetse flies may be quantitative in nature rather than qualitative.

However, since the rate of accumulation of pteridines in wild type *G. m. morsitans* in this study was much lower than previously reported (Lehane and Mail 1985; Langley *et al.* 1988; Lehane and Hargrove 1988; Msangi and Lehane 1991) the interpretation of age dependent decrease in pteridine accumulation rate is not the only possibility. The doubling time of fluorescence at emergence of 135.9 days in this study, calculated from the regression of HCF on age for wild type flies up to 86-days-old, is more than four times longer then the mean calculated from previously reported data (mean = 32.2 days, sem = 3.16, n = 4). Three of these estimates are from field studies in which temperatures are not reported, so temperature may account for some of this difference. However the difference between the present study and the estimate of 40.4 days doubling time at 24.5C of Lehane and Mail (1985) cannot be due solely to temperature differences during adult life. Msangi and Lehane (1991) demonstrated that temperature during puparial period affects fluorescence at eclosion. It is possible that the two weeks of maintenance of the puparia between 15 and 18C may have led to the lower pteridine accumulation rates in

this population. An additional possibility is that differences in method account for some of this difference.

The unusually long fluorescence doubling time suggests an alternative explanation for the observed curvilinearity. Flies which accumulated pteridines more rapidly may have died at earlier ages in both genotypes, and there was no curvilinearity in the relationship between HCF and age in this species. This possibility is supported by the observation that wild type flies less than 10 days of age had a fluorescence doubling time of 39.5 days. This possibility could be tested by assaying larger numbers of flies and examining the data more closely for changes in pteridine accumulation rate. If the early death of flies that accumulate pteridines more rapidly explains the observed decline in pteridine accumulation rate, older flies should exhibit invariant rates of pteridine accumulation. The small number of old flies examined precluded this type of analysis. A further possibility, is that this highly inbred line has some genetic difference causing lower rates of pteridine accumulation.

The lack of a difference between longevities of wild type and *salmon* mutants made it impossible to use these data to address the questions concerning the rate of living in these genotypes.

Table 3. Comparison of mean (sem) age at death and mean size for *salmon* and wild type male *G. m. morsitans*. Ages are in days, head widths are arbitrary units on an ocular scale in a Wild (M5) microscope.

	salmon	wild type	t	p	d.f.
age at death	33.1(5.17)	33.2(4.90)	0.01	< 0.01	21
size (head width)	60.3(1.42)	59.7(1.46)	1.03	> 0.05	56

Table 4. Comparisons of regression coefficients of head capsule fluorescence on age, for salmon and wild type male *G. m. morsitans* divided into young (up to 10-days-old) and old (more than 10-days-old) age classes. Regressions are presented in Figures 3 and 4.

Comparison	d.f	F	p
salmon versus wild type, young and old pooled	1,77	11.32	< 0.01
young *salmon* versus young wild type	1,21	0.14	> 0.05
old *salmon* versus old wild type	1,52	8.63	< 0.01
young versus old *salmon*	1,32	7.29	< 0.05
young versus old wild type	1,41	5.19	< 0.05

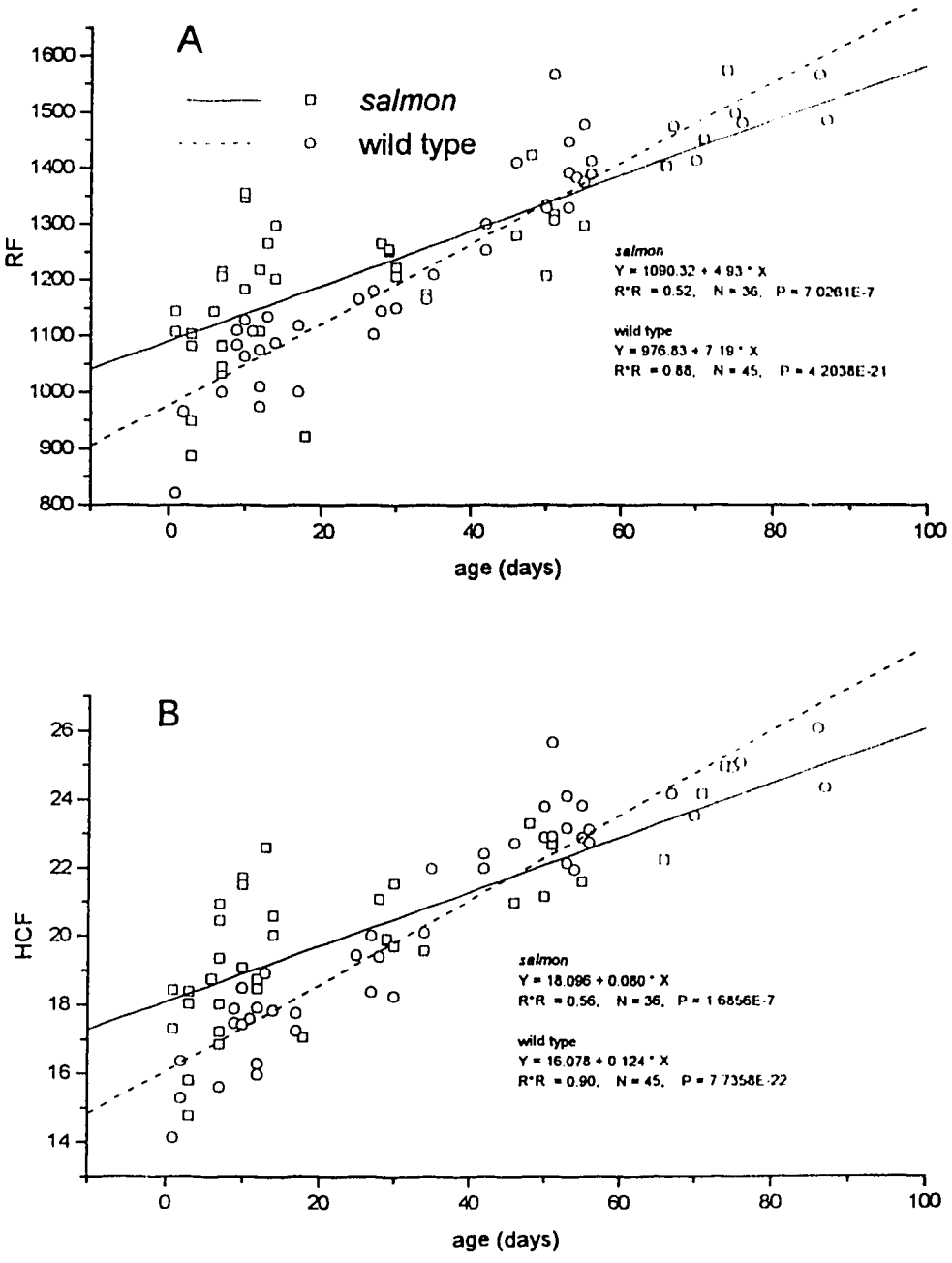


Figure 3. Relationship of A) relative fluorescence and B) head capsule fluorescence with age, for male wild type and *salmon* mutants of *G. m. morsitans*

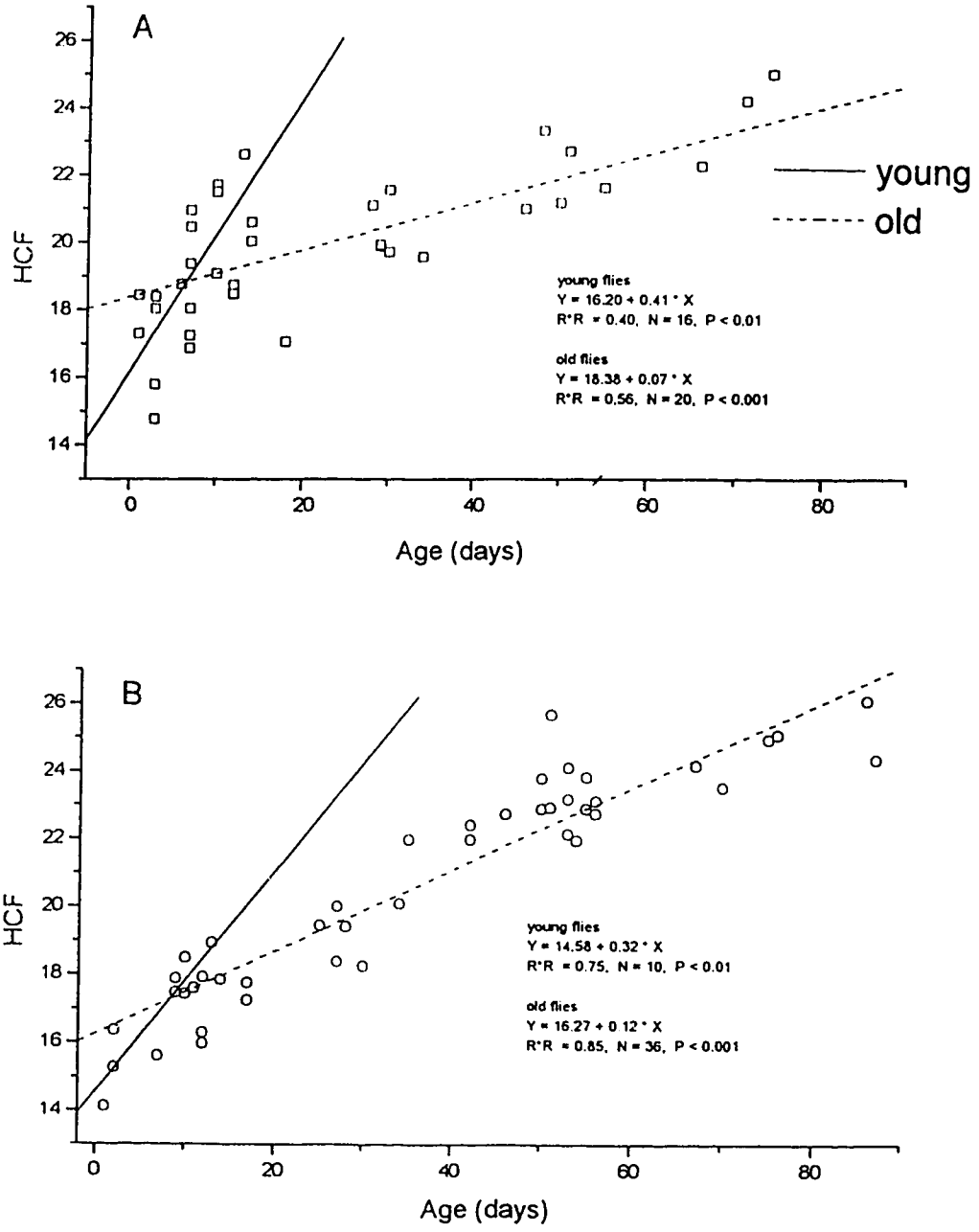


Figure 4. Regressions of HCF on age for young (10 days old or less) and old (greater than 10 days old) male *G. m. morsitans*. A) *salmon* mutants B) wild type.

Chapter 5
EFFECTS OF DURATION OF DEVELOPMENT AND ACTIVITY ON PTERIDINE ACCUMULATION

Any improvement of the pteridine method of age determination for adult Diptera depends upon identifying the remaining factors that contribute to the variation in pteridine accumulation in these insects. Of the factors that contribute to pteridine accumulation, the effects of development temperature and duration on pteridine level have not been examined closely enough to establish their importance. Another factor, which has not been examined, but which is likely to affect pteridine accumulation is activity that affects metabolic rate and physiological age. In this chapter I present an investigation of these aspects of pteridine accumulation.

The duration of larval and puparial periods affect pteridine levels at eclosion of *G. m. morsitans* (Msangi and Lehane 1991). These durations are largely determined by temperature. To determine the variation in pteridine content of house flies that is associated with development duration, two experiments were conducted. The first examined the variation caused by development at different temperatures and is similar to the study conducted by Msangi and Lehane (1991). The second examined the variation caused by differences in duration of development at a single temperature. The second type of experiment has not been reported previously and was prompted by the observation that adult house flies emerging from a single egg batch reared at 27C emerged over a four day period, although the eggs were deposited over a seven hour period. Calculated from the time of hatching, " zero-day-old" adults differ in age by up to four days.

Pteridine accumulation is thought to indicate, and to depend upon, physiological age, which is dependent upon metabolic rate. Metabolic rate in poikilotherms is directly related to ambient temperature. Thermal regulation by the insect and the insect's activity level modify the effect of ambient temperature on metabolic rate in insects. In developing the pteridine age determination method, the behavioral and physiological mechanisms of insects for controlling body temperature have been considered (Mail *et al.* 1983; Lehane *et al.* 1986), but the effect of activity on pteridine accumulation has not been examined.

In house flies activity affects longevity and physiological age, which was estimated through chloroform soluble fluorescent age pigment concentrations (Ragland and Sohal 1975; Sohal and Buchan 1981b). These pigments constitute a lipid fraction that accumulates with age in a wide variety of tissues and organisms, but it is not related to pteridine fluorescence (Sheehy and Roberts 1991). If HCF is a true measure of physiological age, it is likely to be significantly affected by activity. Thus two flies of the same age, experiencing the same ambient temperature, but having different activity histories might have very different physiological ages and very different amounts of head capsule fluorescence.

The effect of activity on pteridine accumulation may be examined by quantitative and semi-quantitative methods. If increased activity leads to increased pteridine accumulation, then high activity flies should have more head capsule fluorescence than less active flies that have the same chronological age and temperature experience. The present chapter deals with semi-quantitative approaches to the problem. A more

quantitative approach, linking oxygen consumption to pteridine levels, is discussed in the following chapter.

In the semi-quantitative investigation of the effect of activity on pteridine accumulation, two approaches involving manipulations that have an effect on activity of male house flies were used. The rationale for the first is that surgical removal of the wings of male house flies reduces activity, and the less active flies experience greater longevity and accumulate chloroform soluble fluorescent age pigments at lower rates than normally active flies (Sohal and Buchan 1981a, 1981b). Therefore surgical removal of the distal half of the wings of male flies may be used to investigate the effect of activity on pteridine accumulation. In the second approach male house flies may be maintained in the dark, since this had been found to decrease their metabolic rate, as estimated through oxygen consumptio. (Chapter 6).

A third semi-quantitative approach for examining activity and pteridine accumulation is based upon the observation that house flies lose the ability to fly before death. This occurs even in the absence of wing damage, and may be taken as an indication of senescence and impending death. It is possible that flies that can no longer fly have been more active than others, since sexually vigorous male house flies loose their flight ability earlier than less sexually active males (Ragland and Sohal 1973). Sohal (1991) showed that crawlers (flightless winged flies) and fliers (winged flies retaining flight abilities), from a single population at 12 days of age, had significantly different longevities. If HCF is related to physiological age rather than chronological age, then crawlers should have higher HCF levels than fliers of similar age.

METHODS

Effect of Development Temperature

To examine the effect of temperature on duration of development of house flies, three batches of medium were inoculated with eggs from the same batch and placed at 17, 22, or 27C. Emergence and sex ratio were monitored. Sex ratio was based on a sub-sample unless the number of flies emerging was small. The temperature at the centre of the larval medium was measured periodically to estimate the relationship between ambient temperature and temperature of the inoculated medium during development.

In a separate experiment to examine the effect of development temperature on HCF, two batches of larval medium, inoculated with eggs from the same batch, were placed at 17 or 27C. On the first day of emergence a sample of flies was taken from each culture and assayed for HCF. Samples consisted of 10 flies of each sex at 27C but samples were smaller than this at 17C because few flies were obtained on the first day of emergence.

Effect of Development Duration

Adults emerging from a double batch of medium inoculated with 0.6 ml of eggs were divided into cohorts that were based upon day of emergence. Groups of 180 (sex ratio approximately 1:1) one-day-old adults from each cohort were placed in cages at 22 and 27C, under standard conditions. For all 5 cohorts, a sample of up to 5 flies of each

sex were collected and assayed for HCF on the day of emergence. Only on the second and third days of emergence were there sufficient flies to conduct the remainder of the experiment. For cohorts from these two days, cohort B and C respectively, at 1 through 10, and 15, days of age as adults, five flies of each sex were collected from each temperature and assayed for HCF.

Effect of Presence and Absence of Light

Two hundred one-day-old males, that were reared under standard conditions at 27C and were placed in the dark prior to emergence, were used. These flies were divided into two treatments, both of which were maintained under standard conditions at 27C with one group being placed in a cardboard box. Mortality was determined on days 4, 7, 10, and 14, and on each occasion 12 flies were collected from each treatment, 10 of which were assayed for HCF. On these days the flies maintained in the dark were exposed to room light for less than ten minutes. Mortality was also recorded at age 37 days.

Effect of Wing Removal

One hundred one-day-old male flies that were reared and maintained under standard conditions at 27C were used. The distal half of each wing was removed from 50 flies, rendering them incapable of flight. These flies were then placed in a 20 x 20 x 20 cm cage with the 50 non-mutilated flies. At 6 and 10 days mortality was recorded and 12 flies were collected from each treatment, 10 of these were assayed for HCF. Mortality was recorded also on day 28.

Effect of Loss of Flight Ability

Twenty male flies, 20 days old, were taken from a large population that had been reared and maintained under standard conditions at 27C. The following scale was used to rate the amount of mechanical damage incurred by each wing.

0 = wing undamaged (or very minor fraying)

1 = more than 2/3 of wing is present

2 = less than 2/3 of wing is present

After giving each fly a combined wing damage rating of from zero to four, the flies were assayed for HCF.

RESULTS

Effect of Development Temperature

When reared at 27C, the first flies eclosed 10 days after the time of egg collection and flies continued to emerge until 13 days after egg collection. At 22C the first flies eclosed at 15 days after egg collection and the last on day 19. At 17C the first flies emerged at 26 days after egg collection and the last on day 38. At all three temperatures the sex ratio favoured females, and males emerged slightly earlier than females (Figure

5). This ratio did not differ between temperatures (χ^2 = 2.00, n.s.) Approximately twice as many flies survived to eclosion at 17C than did at 27C, with an intermediate number of survivors at 22C (Table 5). The latest emergents were 46, 27, and 30% older than the earliest emergents at 17, 22, and 27C respectively. The temperature of the larval medium was between three and four degrees Celsius above ambient during larval development and very close to ambient between pupariation and eclosion at all three temperatures.

In the experiment examining the effect of development temperature on HCF at eclosion, the first emergence occurred 10 days after egg collection at 27C, and 28 days after egg collection at 17C. These development periods were similar to those in the experiment examining the effect of temperature on development. The mean head width of the first emergents at 27C was smaller than that of those at 17C (males t = 7.44, 16 d.f., $p < 0.001$; females t = 6.06, 12 d.f., $p < 0.001$), but the first emergents at 27C had significantly higher HCF than did those at 17C (males t = 5.60, 16 d.f., $p < 0.001$; females t = 4.24, 12 d.f., $p < 0.001$) (Table 6).

Effect of Development Duration

At 27C flies emerged on days 10 through 14 after egg collection (Figure 6), although only one male emerged on day 14. About twice as many flies emerged from the double batch of medium as from the single batch that was used in the experiment on the effect of temperature. A regression analysis of HCF on day of emergence for one-day-old flies revealed a significant trend towards higher HCF with increased duration of development (Figure 7). There was no effect of sex on HCF in this data set so the data were pooled and a single regression was calculated. The regression of HCF upon age at eclosion was highly significant ($p < 0.001$) and 23.3% of the observed variation in HCF at eclosion was explained by duration of development.

A four way analysis of variance of HCF with cohort, age as adults, sex and temperature as predictors (Table 7) revealed that in addition to the expected effects of age as adults, sex, and temperature, cohort had a significant effect, explaining 0.38% of the total variation in the data set. In accord with the trend towards higher HCF in late emerging flies, the mean HCF of cohort C was higher than that of cohort B (cohort B: mean=13.77, sem 0.080; cohort C: mean=14.29, sem 0.080; $F_{1,351}$ = 23.35, $p < 0.001$). However, differences between cohorts were generally undetectable within age as adults, sex, and temperature treatments (Figure 8; Appendix A). The interactions between cohort and age, and between cohort, age, and temperature were also significant (Table 7, Figure 8). This indicates that the cohorts not only differed in HCF at eclosion, but they accumulated HCF at different rates, and there were cohort specific effects of maintenance temperature. Much of the difference between regressions of log(HCF) on log(age), at both temperatures, appeared to be due to differences between cohorts with respect to HCF on day of emergence for both males and females. Accordingly regressions that excluded the measurements of HCF at age 1 day were calculated. The differences in slopes remained, although the overall slopes were less, as would be expected (Appendix B).

Effect of Presence or Absence of Light

Holding flies in the light or in the dark had no significant effect upon HCF accumulation or mortality up to 14 days of age (Table 8). By 37 days of age the mortality was significantly higher among the flies maintained under light conditions (in light: 14 alive, 38 dead; in dark: 39 alive, 13 dead, $\chi^2 = 22.16$, $p < 0.01$).

Effect of Wing Removal

Flies with the distal half of their wings removed did not differ significantly from controls with respect to mortality or HCF for the first 10 days of life at 27C (Table 9). The early deaths of two wing-clipped flies was probably caused by the stress of wing removal, but the large number of flies surviving the experiment indicates that inability to fly was not fatal to male flies during the first 10 days of life at 27C. The mortality of the wing-clipped flies was significantly lower than that of non-mutilated controls at 28 days (wing-clipped: 12 alive, 12 dead; control: 2 alive, 22 dead, $\chi^2 = 8.17$, $p < 0.01$).

Effect of Loss of Flight Ability

There was a significant difference between mean HCF for flies grouped by wing rating ($F_{4,15} = 10.04$, $p < 0.001$, Table 10). However most of the variance was due to the single fly with the wing rating of 1. When this fly was removed from the analysis the anova was not significant ($F_{3,15} = 0.11$, $p = 0.95$). Since there was no general trend in HCF that can be related to wing rating and since virtually all of the between class variance was attributable to a single fly, the data are interpreted as indicating that differences in wing damage, and flight ability, accumulated during the first 20 days of life at 27C do not affect HCF in male house flies.

DISCUSSION

The results indicate that temperature and duration of development had independent effects on pteridine level at eclosion and that the variation at eclosion, due to duration of development, was maintained throughout adult life. The semi-quantitative approach taken in these experiments did not reveal a relationship between activity and pteridine level, and therefore a more quantitative approach will be needed to link of activity to accumulation of head capsule fluorescence.

Development temperature had an effect on size and HCF of the earliest emergents from an egg batch. For flies reared at 27C, the earliest emergents were smaller but had greater HCF than those at 17C. This effect of temperature on size is not unusual, in populations of house flies near Ames, Iowa there is a seasonal fluctuation in adult size, which may be explained by an inverse relationship between size and development temperature or by differential rates of adult survival. In winter, older adult females tend to be larger than young females; this was interpreted as indicating that large females survive longer than small females at low temperatures (Krafsur 1985; Krafsur *et al.* 1985). It is possible that a similar selection for large individuals occurs during the larval and pupal stages. The effect of development temperature on HCF may be due to differential responses of development rate and of HCF accumulation rate to temperature If the rate

of HCF accumulation increases more rapidly with temperature than the rate of development, this may account for the observed difference between temperatures in HCF at eclosion. The present results are similar to observations made on tsetse flies, in which it was found that temperature affected development rate and relative fluorescence (Msangi and Lehane 1991).

The elevation of temperature of the larval medium in this experiment is typical of fermenting substrates and occurs in manure containing developing house flies in nature (e.g. Lysyk and Axtell 1987). In future investigations of the effect of development temperature on HCF it is recommended that both ambient and medium temperature be considered, or that flies be cultured in small containers where high surface area to volume ratio will limit temperature build up in the larval medium. The ages at emergence in the present study were somewhat greater than those predicted from the data of Lysyk and Axtell (1987). These predictions are 36.8, 25.9, and 14.9 days at 17, 22, and 27C respectively. This difference may be due to selection for rapid development, at the lower temperatures experienced in this area, by the locally obtained strain of house flies.

In addition to the differences caused by development duration due to differences in temperature, there was a significant increase in HCF at eclosion in flies that emerged on consecutive days from the same egg batch. These differences were found to persist through the first two weeks of adult life in cohorts of flies that emerged on consecutive days, and that were maintained at 22 and 27C. There are several possible explanations for the effect of development time. The difference may be due to variation among families. It is possible that most of the offspring of a single female emerge over a relatively narrow time period. Thus the flies emerging on different days would have different genetic backgrounds, resulting in differences in HCF. This explanation requires that the genetics of development time and HCF accumulation be closely, or causally, linked

A more likely explanation is that longer development at the temperatures studied resulted in a greater number of degree days above the HCF accumulation threshold, and thus higher HCF levels at eclosion. In Diptera, the majority of the fluorescent pteridines present at eclosion accumulate in the head capsule during the last half of the puparial period, with peak production occurring shortly before eclosion (Ziegler and Harmsen 1969; Summers *et al.* 1982). In flies with slower development, the period of pteridine accumulation would be lengthened, and since this accumulation is thought to be a form of storage excretion, it is unlikely to be limited to some optimum or maximum level during puparial development.

This raises the question of why the development time of house flies is so variable within a single batch of medium. It is possible that since egg deposition occurred over approximately seven hours, those eggs which were laid first get ahead and stay ahead of the eggs which are laid later during the collection period. There are several possible explanations for why the difference in time of laying is magnified from seven hours to a four day difference between earliest and latest eclosion. Possibly the medium used is less suitable for late hatching larvae and they fall farther behind in development. Since increased larval density slows development (Sullivan and Sokal 1963) it is possible that the larvae that hatch later experience an effectively higher density resulting in slower

development. Alternatively, most of the difference may be due to duration of the puparial stage; those that pupariate first do so while the medium is still warmer than ambient and thus their pupariation is accelerated during the early stages.

The fact that the two cohorts exhibit different accumulation patterns with respect to age and temperature is difficult to explain. For males and females at both temperatures, the rate of accumulation is slightly greater in the cohort that emerged first. It may be that flies that develop rapidly as larvae are metabolically more active than slowly developing flies and higher HCF accumulation rate is one result of increased metabolism. That the cohort with the greater metabolic rate, according to this theory, has lower HCF at emergence may be explained by the shorter puparial period, which may have a greater effect on HCF at eclosion than a small difference in metabolic rate.

The two semi-quantitative experiments, in which fly activity was restricted in order to influence the effects of activity on HCF failed to demonstrate a relationship between activity and HCF. It is possible that despite the manipulations, the activity levels did not differ between the experimental and the control flies, or that the differences in activity over the experimental period produced differences in HCF that were too small to be detected by the HCF assay procedure used. The higher long term mortality in the "high activity" controls indicated that exposure to continual darkness and wing removal significantly increased longevity. Provided that decreased activity increases longevity as postulated by the rate of living theory (see Chapter 1), the results suggest that the failure of these experiments to detect effects of activity on pteridine levels was a technical problem and that a more rigorous, quantitative examination will be required to detect such a relationship, if it exists. Such experiments are described in the following chapter.

Accumulated wing damage and loss of flight ability were not correlated with HCF. These results do not corroborate previous reports that old flies that have lost their ability to fly accumulate age dependent products more quickly than those retaining flight ability (Ragland and Sohal 1973; Sohal and Buchan 1981a). The time course for HCF accumulation in house flies makes it unlikely that differences in flight ability of old flies would be strongly correlated with differences in HCF. The rate of HCF accumulation is greatest during the first week of adult life and declines steadily with age. By age 14 days, differences in mean HCF between consecutive days are less than 2% (data not shown). Very large sample sizes would therefore be needed to detect differences in this age range. Differences in flight ability that occur over a few days in two- or three-week old house flies are unlikely to alter metabolism sufficiently to cause detectable differences in HCF.

Table 5. Emergence data for house flies mass reared at 17, 22, and 27C.

temperature (C)	males	females	total
27	356	381	747
22	459	516	975
17	570	695	1265

Table 6. Mean head width and HCF at eclosion for earliest emergent male and female house flies mass reared at 17 and 27C. Head width is in arbitrary units on an ocular scale iin a Wild (M5) microscope; standard errors in parentheses.

variable	sex	17C	27C	t	d.f.	p
head width	males	59.6(1.06)	49.1(0.94)	7.44	16	0.001
	females	62.5(1.49)	51.8(0.94)	6.06	12	0.001
HCF	males	6.27(0.308)	8.59(0.276)	5.60	16	0.001
	females	5.78(0.436)	7.97(0.276)	4.24	12	0.001

Table 7. Anova table for effects of emergence cohort, age, sex, and temperature on HCF of house flies.

Source	Sum of Squares	d.f.	F	p
COHORT	29.20	1	21.18	< 0.001
AGE	7114.11	10	515.96	< 0.001
COHORT*AGE	30.45	10	2.21	< 0.01
SEX	194.94	1	141.39	< 0.001
COHORT*SEX	2.47	1	1.79	> 0.05
AGE*SEX	43.65	10	3.17	< 0.001
COHORT*AGE*SEX	11.25	10	0.82	> 0.05
TEMP	381.31	1	276.55	< 0.001
COHORT*TEMP	0.51	1	0.37	> 0.05
AGE*TEMP	50.37	10	3.65	< 0.001
SEX*TEMP	0.08	1	0.06	> 0.05
COHORT*AGE*TEMP	30.43	10	2.21	< 0.05
COHORT*SEX*TEMP	1.82	1	1.32	> 0.05
AGE*SEX*TEMP	16.03	10	1.16	> 0.05
COHORT*AGE*SEX*TEMP	6.14	10	0.45	> 0.05
ERROR	483.96	351	---	---
TOTAL	8409.96	438	---	---

Table 8. Mortality and mean (sem) HCF for male house flies maintained in the presence or absence of light.

	light		dark				
age(days)	HCF	mortality*	HCF	mortality*	t	p	d.f.
4	13.7(0.31)	1	13.9(0.31)	1	1.12	> 0.05	18
7	17.3(0.27)	2	17.6(0.36)	1	0.77	> 0.05	18
10	19.0(0.38)	2	20.2(0.50)	1	1.82	> 0.05	18
14	20.0(0.32)	4	19.7(0.34)	2	0.65	> 0.05	18
37	---	38	---	13	---	---	---

*cumulative mortality

Table 9. Mortality and HCF means (sem) for wing-clipped and non-mutilated flies.

	wing-clipped		non-mutilated				
age	HCF	mortality*	HCF	mortality*	t	p	d.f.
1	--	2	--	0	--	--	--
6	15.8(0.28)	2	15.6(0.36)	0	0.45	> 0.05	18
10	19.1(0.34)	2	19.9(0.44)	0	1.43	> 0.05	18
28	---	12	---	22	---	---	---

*cumulative mortality

Table 10. Head capsule fluorescence values for 20-day-old male house flies grouped by wing damage rating.

damage rating	n	mean(sem)
0	1	22.0(--)
1	1	30.6(--)
2	9	22.2(0.27)
3	7	22.5(0.69)
4	2	22.1(0.18)

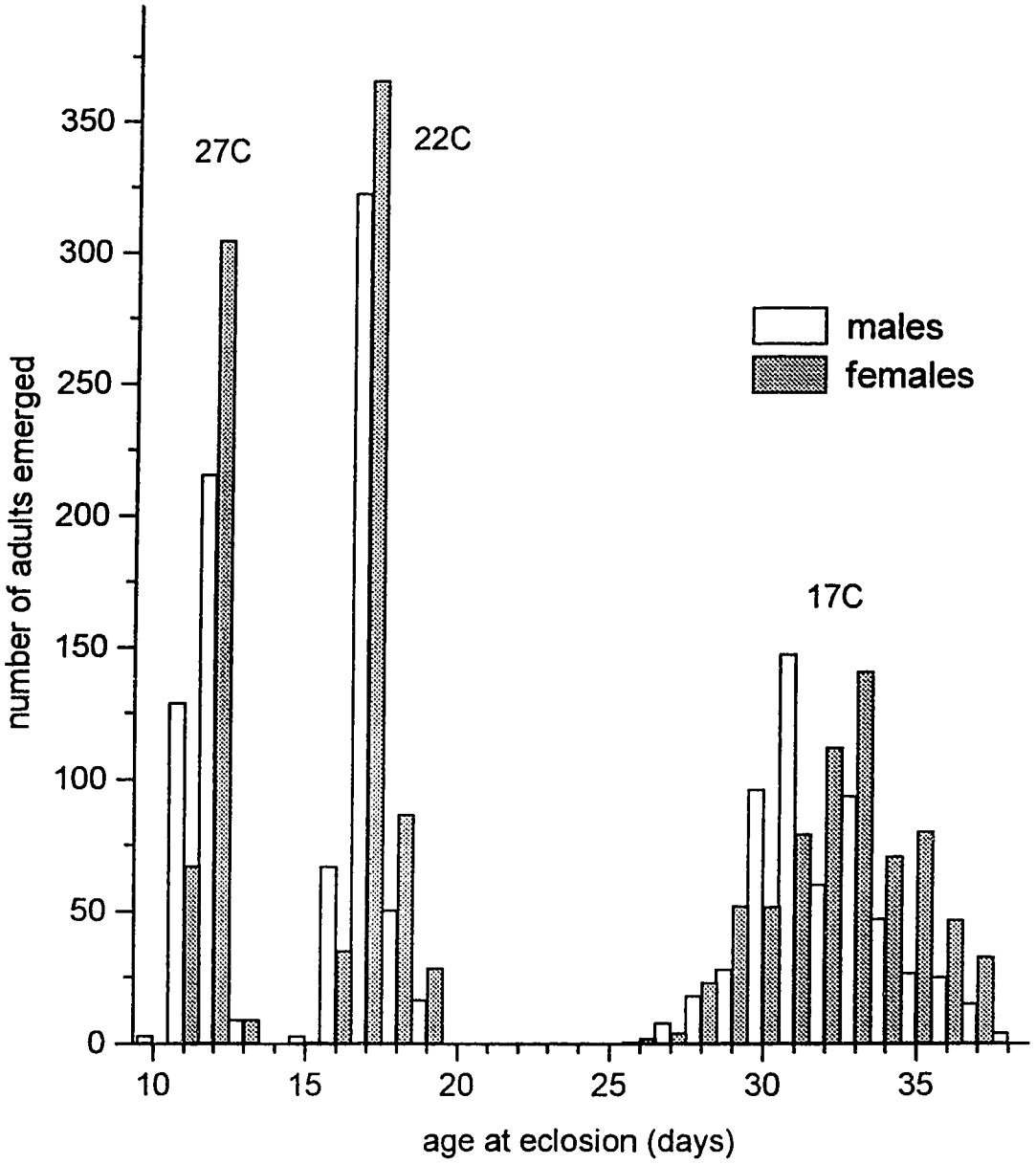


Figure 5. Emergence patterns for male and female house flies mass-reared at 17, 22, and 27C.

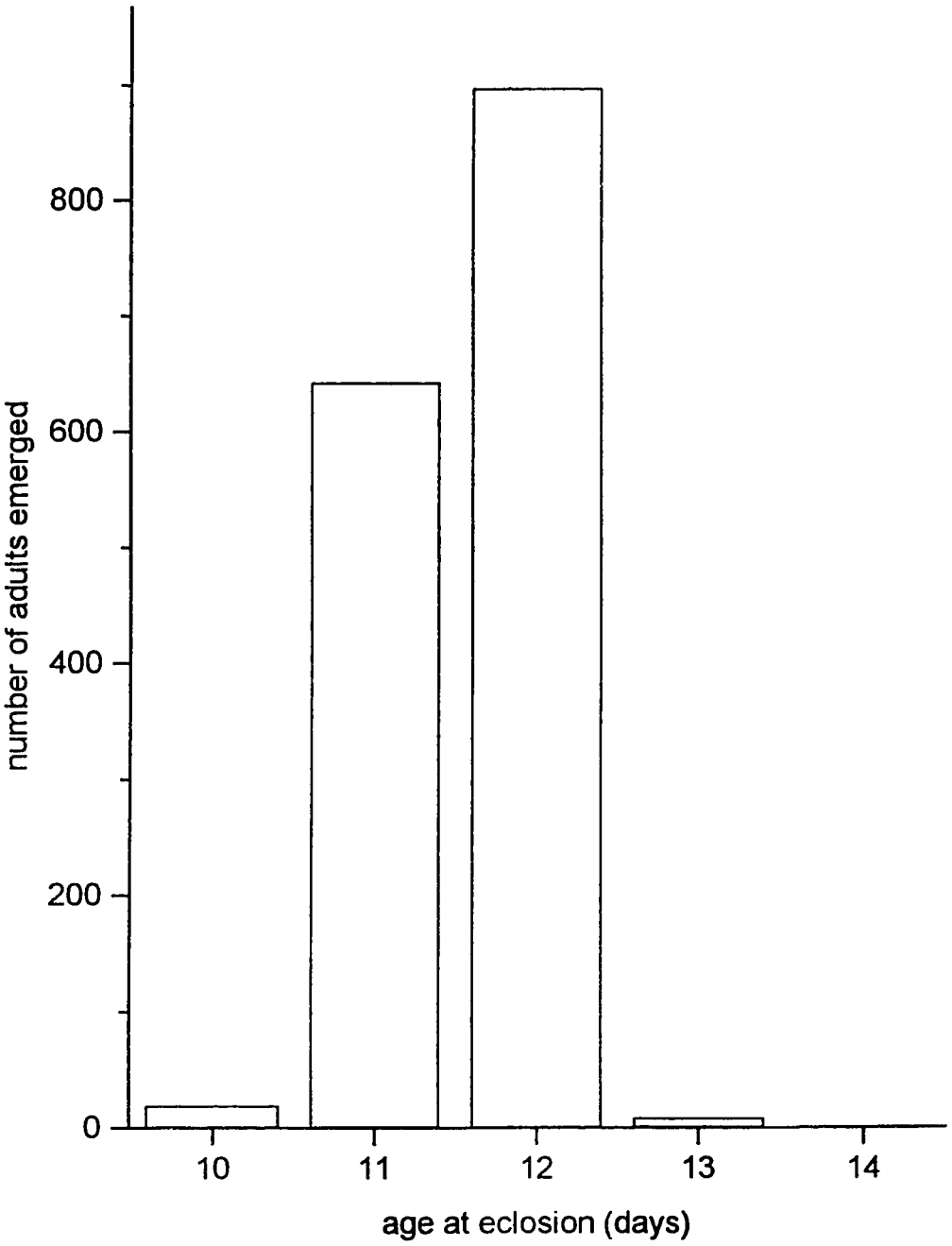


Figure 6. Emergence pattern of house flies mass-reared at 27C.

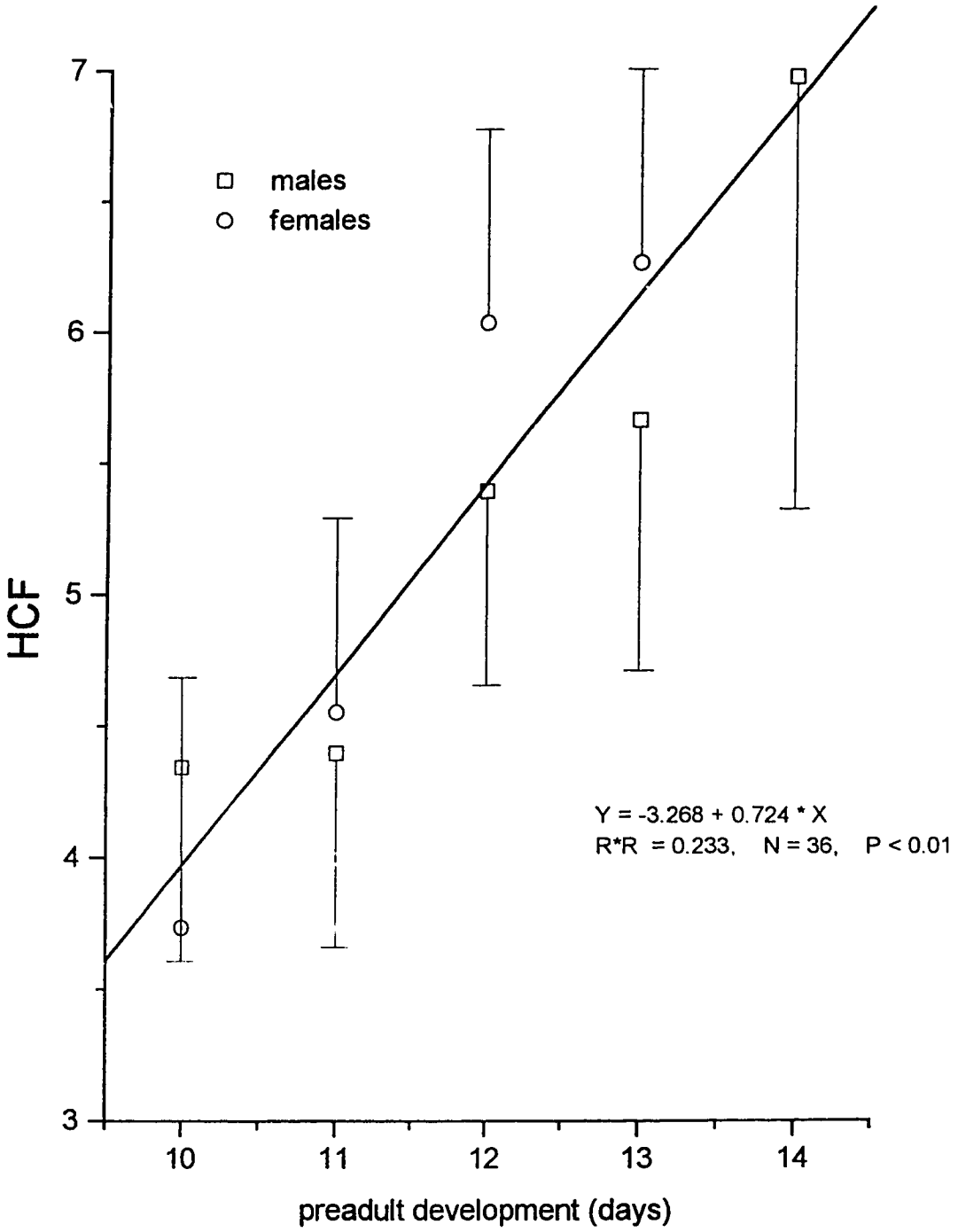


Figure 7. The relationship of HCF, at one day of age, to duration of preadult development for house flies at 27C. Plotted points are means for males and females on each day of emergence. Vertical bars are standard errors. Regression is calculated from the raw data.

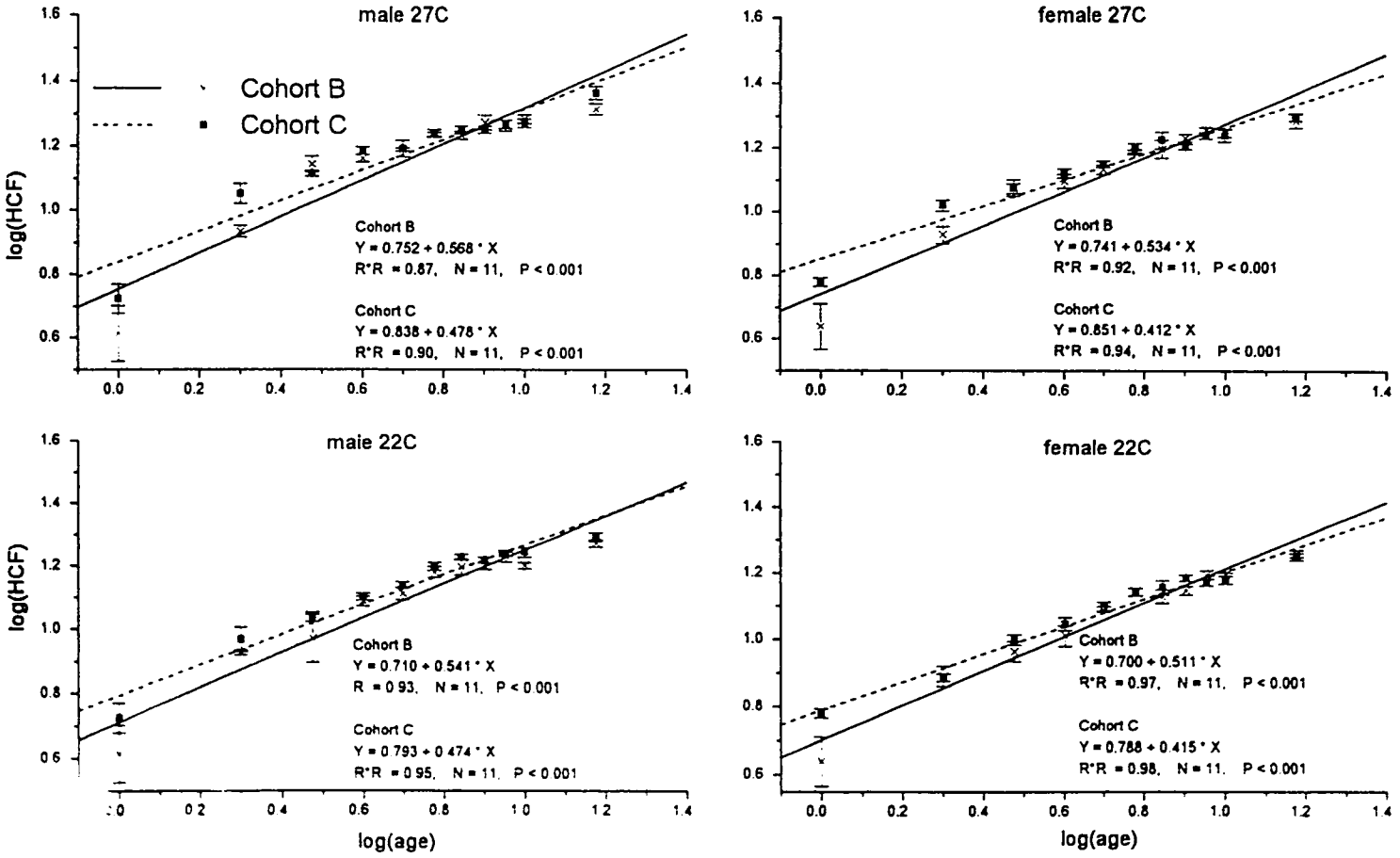


Figure 8. Relationship of log(HCF) and log(age) for house flies emerging on consecutive days from the same egg collection, maintained at two temperatures, vertical bars are standard errors.

Chapter 6
THE RELATIONSHIP BETWEEN HEAD CAPSULE FLUORESCENCE AND OXYGEN CONSUMPTION

Much of the work on the pteridine age determination method is based on the assumption that pteridine levels are affected by physiological age and metabolic rate. A corollary of this is that activity level should have an affect on HCF. Failure to demonstrate this corollary in the house fly through experiments limiting the activity of males by maintenance in the dark and restriction of flight ability, by clipping the wings, demonstrated a need for an experiment that quantifies the effects of confinement, light conditions, and temperature on oxygen consumption. In this chapter I present the results of experiments that demonstrate the effects of these variables upon oxygen consumption and accumulation of HCF in male house flies. I also demonstrate that HCF is linked to metabolic rate. I conclude the chapter with explanations for the failure of the experiments described in the previous chapter to detect a relationship between activity and accumulation of HCF.

METHODS

Effects of confinement, temperature and light on oxygen consumption

A random block experiment with three replications was conducted to examine the effects of confinement, temperature, and light on the oxygen consumption of male house flies. All flies were two days old at the beginning of the experiment and had been reared and maintained under standard conditions. The two confinement methods described in chapter 2 were used. Control reaction vessels that lacked a fly, but contained amounts of water, cotton, and 25% KOH similar to those in the experimental vessels were also measured under the various conditions of light and temperature. The light conditions were normal room light or darkness (the respirometer was placed in a room with no windows, the lights were turned off, and the respirometer was covered with a heavy black cloth). Readings were taken at 17 and 27C. Each replicate began at the same time of day in order to minimize effects of the circadian rhythm of the house flies. After changing light conditions or temperature, one hour was allowed for the system to equilibrate and for the flies to become accustomed to the new conditions, before measurement was resumed.

Periodic measurement of oxygen consumption

Males from a stock population, raised under standard conditions, were isolated in wire mesh cages of approximately 1 cm^3 (mini-cages), 24 hours after the midpoint of a 12 hour emergence period. Thus, on average the flies were 24 hours old at the beginning of the experiment. The unused portion of the stock population was maintained under standard conditions at 27C as a control. The mini-cages containing flies were divided into three groups. Two of these groups were placed individually upon a layer of moist cotton in shell vials, and maintained in closed cardboard boxes in incubators at 17 or 27C. The remaining group was placed directly in the respirometer at 27C. On each day of the

experiment all flies were provided with a small amount of granulated sugar. For the group in the respirometer, oxygen consumption was measured for half hour periods at four hour intervals for 72 hours. After 24 and 48 hours in the respirometer this group of flies was provided with sugar and placed on moistened cotton for 3.5 hours at 27C in the dark. At this time the water and KOH in the reaction vessels were replaced. At the end of the three day measurement period flies from all three groups and a sample from the stock population were assayed for HCF. The experiment was repeated with flies from a new batch of adults that had been raised under standard conditions. In this run, the respirometer was kept at 17C and the stock population was moved to an incubator at 17C at the time flies in the other groups were placed in mini-cages. Again there were control groups of flies in mini-cages maintained in the dark, as described above.

Hourly measurement of oxygen consumption

A large number of one day old flies were collected from a population that had been raised under standard conditions at 27C. In order to develop a regression for estimating the relative fluorescence of the test flies at the beginning of the experiment, 20 males were weighed to the nearest mg and assayed for HCF. Fifteen males were weighed and placed singly in reaction vessels following the setup for unrestrained flies described in chapter 2, with the additional provision of a small amount of granulated sucrose in the bottom of each vessel. The flies were kept in the respirometer at 17C for 73 hours. In a previous experiment this arrangement did not cause undue mortality in the flies, and the KOH remained active for this period. The system was allowed to equilibrate for one hour before measurements began, and readings of oxygen consumed were taken every hour. The apparatus was opened to the atmosphere six times during the experiment, for approximately 10 min each time, in order to reset the calibrated manometer dials, and to allow the partial pressure of oxygen to return to ambient. During each of these adjustment periods an estimated 20 min recording time was effectively lost, due to re-equilibration of the system after closing. No correction was made for this lost measurement time since it was common to all flies. At the end of 72 hours the flies were removed from the apparatus, chilled, and assayed for HCF.

RESULTS

Effects of confinement, temperature and light on oxygen consumption

In examining the effects of confinement method, light and temperature it was found that at both temperatures and under both light conditions the mean oxygen consumption of the controls was not significantly different from zero (Table 11). The mean weight of the flies did not differ significantly between blocks in this experiment ($F_{2,32} = 3.01$, $p < 0.05$), and in an analysis of covariance with wet weight as covariate, the relationship between oxygen consumption and weight in the light and temperature treatments, did not differ between replicates. Therefore, the data were analyzed ignoring these two elements to simplify interpretation. Confinement, light, and temperature all had significant effects that were independent of each other (Table 12, Figure 9). Light had the smallest effect explaining only 2.9% of the variation, with conditions of darkness

decreasing oxygen consumption. Confinement in mini-cages also decreased oxygen consumption, with this effect explaining 6.4% of the observed variation. Temperature was positively correlated with oxygen consumption and had the largest effect, explaining 18.7% of the variation in rate of oxygen consumption.

Periodic Measurement of Oxygen consumption

Ranges and means for oxygen consumption are presented in Table 13. At 27C, eight of fifteen flies survived for three days in the respirometer. Among the survivors there was a twofold variation in mean oxygen consumption. The overall mean at 27C was 1.66 µl/mg/hr (sem 0.050). At 17C, of the original 15 flies, two escaped during transfer to sugar solution, and one manometer leaked, leading to the elimination of three flies from the analysis. Among the 12 remaining flies there was nearly a twofold variation in mean oxygen consumption. The overall mean was 0.76 µl/mg/hr (SEM 0.026). The mean oxygen consumption at 17 and 27C are significantly different ($t = 8.27$, $p < 0.001$, d.f. = 18) and provide an estimate of 2.2 for the Q_{10} value for oxygen consumption of male house flies between 17 and 27C.

At both temperatures there was a pronounced circadian rhythm of oxygen consumption (Figure 10). Oxygen consumption was greatest in synchrony with the photophase experienced by the flies prior to the experiment. At 17C, the rhythm was maintained for the three days of the experiment, but at 27C, the rhythm decayed during the morning of the third day with many of the flies failing to exhibit increased respiration. This was likely due to a general loss of vigour as the flies approached death, but it could indicate that the circadian rhythm was extinguished after 72 hours in the dark. Due to the four hour interval between measurements it is not possible to make more definitive statements about the nature of the observed rhythm.

Mean relative fluorescence for flies in each treatment is presented in Table 14; statistics and probabilities for the comparisons described below are presented in Table 15. There was no difference in mean relative fluorescence, at either temperature, between flies maintained in the respirometer and those in the mini-cages at the same temperature (comparisons 1 and 2), but there was a large difference between means for different temperatures (comparison 3). Surprisingly there was no difference in relative fluorescence within runs, between flies maintained under standard conditions and flies maintained in mini-cages in the dark at the same temperature (comparisons 4 and 5). There was no difference between the 17C mini-cage controls in either run (comparison 6), but the 27C mini-cage controls differed significantly between runs (comparison 7). Using the same flies that were used to calculate the Q_{10} for oxygen consumption, the mean head capsule fluorescence of flies at 27 and 17C indicated a Q_{10} of 1.3 for HCF accumulation in male house flies between ages 24 and 96 hours.

Regressions of the log of relative fluorescence (RF) on the log of average hourly oxygen consumption (µl/hr), unadjusted for head width or wet weight, at 17 and 27C are presented in Figure 11. At 27C the regression was significant ($p < 0.05$) and explained 54% of the variation in the data set. At 17C the regression was not significant. A regression of the combined temperatures was highly significant ($p < 0.001$, $R^2 = 0.69$, Figure 12).

Continuous Measurement of Oxygen consumption

Data from the 20 flies assayed at the beginning of the experiment were analyzed for correlations between head width, wet weight and fluorescence. These correlations were not significant (Table 16) and no reasonable prediction of initial RF of the test flies was possible.

All 15 test flies that were placed in the respirometer survived the experiment and appeared quite vigorous when removed from the apparatus. The total oxygen consumption varied among the flies by a factor of three, ranging from 508.32 µl to 1714.11 µl over the 72 hours; the mean was 1175.0 µl (SEM 76.92) and was significantly correlated with wet weight ($p < 0.01$; Figure 13). A distinct circadian rhythm in oxygen consumption occurred throughout the entire three day period with lower consumption during the period that the flies had experienced as the scotophase prior to the experiment (Figure 14). Interestingly the first two readings were significantly higher than the other readings that were taken during the normal photophase of the flies ($F_{1,718} = 28.63$, $p < 0.001$), indicating that the one hour equilibration was insufficient for the flies to recover from handling, adjust to the temperature change from 27 to 17C, and become accustomed to the reaction vessels. It is apparent also that the increase in oxygen consumption during the photophase was not exhibited to the same extent by all flies. The coefficient of variation of the hourly means was greater during photophase than during scotophase on each day. This fact, and the examination of oxygen consumption versus time plots for each fly, revealed that some flies increased their oxygen consumption during photophase considerably more than did others. The coefficient of variation of the mean of hourly oxygen consumption showed a nonsignificant trend ($p = 0.12$, Figure 15) to increase with time, providing weak evidence that the circadian rhythm may have begun to decay during the experiment.

A regression of the log of RF on the log of total oxygen consumed was not significant (Figure 16B). Oxygen consumption was divided into "basal" and "above basal" consumption for each fly. "Basal" consumption was the oxygen consumed per hour during the three scotophase periods during the experiment. "Above basal" consumption was the oxygen consumed during the photophase period of the experiment, corrected for mean hourly "basal" consumption. There was a significant relationship between "above basal" oxygen consumption and RF ($R^2 = 0.308$, $p < 0.05$, Figure 16A) but not between "basal" oxygen consumption and RF ($R^2 = 0.06$, $p = 0.38$). A single fly had a slightly negative value, -9.4 µl, for "above basal" consumption (this fly exhibited the lowest relative fluorescence). Since analysis was conducted on log transformed values, this fly was arbitrarily given an "above basal" consumption value of 1.0 µl.

DISCUSSION

Light, temperature, and confinement method each had an affect on oxygen consumption with close confinement, lower temperature, and darkness all leading to lower metabolic rate. Some caution is required when interpreting these results since a subsequent experiment in which oxygen consumption was measured hourly over a 72 hour period indicated that it took flies at least three hours to recover from handling and to

become accustomed to the respirometer. In the present experiment, conditions were changed every two hours and therefore the flies may not have settled down to normal levels of activity and oxygen consumption during the experiment. However, the treatments were randomized over three replications, so the effects of light, temperature, and confinement on oxygen consumption in house flies are not in doubt, although the values of hourly oxygen consumption recorded are probably higher than normal. The results of the experiment on the relationship of HCF to oxygen consumption, based on periodic measurements, raises further uncertainty concerning the effects of confinement in mini-cages. Very high mortality occurred in the mini-cages at 27C. This may have been due to inappropriate presentation of food and consequent starvation, to excessive humidity, or to stress due to confinement.

It is surprising that there was no difference in HCF between flies maintained in the dark in mini-cages and flies maintained under standard conditions at 17C or at 27C in the experiments in which periodic measurements of oxygen consumption were made. According to the results of the experiment on effects of light and confinement on oxygen consumption, one would expect activity and HCF to be higher in flies that were unrestrained in 20 x 20 x 20 cm^3 cages and exposed to light for 16 hours per day, than in flies enclosed in the mini-cages in the dark. A possible explanation for the result obtained is that the method of confinement caused the flies to divert more resources to metabolic pathways leading to production of fluorescent compounds in the head capsule. This explanation leads to problems which are beyond the scope of this project. Alternatively, light deprivation may lead to increased HCF. To test the effect of light on HCF the effects of light and activity must be separated. This should be possible by maintaining flies at a temperature below the threshold for activity but above that for pteridine accumulation. (I have estimated that the threshold for pteridine accumulation in male house flies is 7.8C (Appendix C) and Buchan and Sohal (1981) reported that house flies do not walk at temperatures below 15.5C.)

Results of the experiments in which oxygen consumption was measured for 30 min every four hours indicated that there was a relationship between oxygen consumption and RF at 27C, but not at 17C. I felt that a more complete investigation would reveal a similar relationship at 17C. The experiment in which total oxygen consumption of male house flies was measured at hourly intervals at 17C did reveal the presence of such a relationship, but only after separating "above basal" from "basal" oxygen consumption. If HCF accumulation was a product of "above basal" metabolism, this would suggest explanations for the results of earlier experiments. In the experiment in which oxygen consumption was measured every four hours, a relationship between RF and oxygen consumption was detected at 27C but not at 17C. It is possible that at the higher temperature "above basal" oxygen consumption was a greater portion of total consumption than it was at the lower temperature, and thus the relationship between estimated total oxygen consumption and RF was more easily discerned at 27C. In the experiment on the effect of presence or absence of light on pteridine accumulation, conducted at 27C, the differences in activity may have been obscured or limited, by a temperature induced increase in overall metabolism. The same explanation would apply to the experiment on the effect of wing removal on pteridine accumulation. A related explanation lies in

observations made on adult *Glossina morsitans morsitans*. These flies increase their metabolic rate as digestion of the blood meal occurs. At higher temperatures this response is reduced in magnitude (Rajagopal and Bursell 1966). If house flies also respond to higher temperatures, by exhibiting smaller changes in metabolic rate in response to external stimuli, it may be that the experiments on limitation of activity would have quite different results if conducted at a lower temperature. In addition, investigation of effects of activity on HCF may be more fruitful in a species such as *G. m. morsitans*, in which detection of effects of activity on HCF will not be complicated by rapidly changing rates of pteridine accumulation during early adult life.

The data from the experiment in which oxygen consumption was measured periodically were reanalysed for the effect of "basal" and "above basal" oxygen consumption levels on HCF (Appendix D). Except for "basal" oxygen consumption at 27C, no significant relationship was found between either component of metabolism, and HCF at 17 or 27C. The failure to detect a relationship between "above basal" metabolism and HCF at the higher temperature, while detecting a relationship between "basal" metabolism and HCF may be due to a poor estimate of "basal" oxygen consumption. Elevated overall metabolic rates at 27C may have obscured the actual levels of basal metabolism. It is possible that the improvements, especially the more regular readings, made in the current experiment were necessary to detect this effect at 17C.

A circadian rhythm in oxygen consumption was maintained in the dark at 17C for up to three days, although there was some indication that it may have begun to decay towards the end of this time. It is noteworthy that the circadian rhythm was more pronounced in some flies than in others; an explanation would be purely speculative. These results agree with published reports on the nature of the circadian rhythm in Muscoid flies. In *Musca domestica* (Parker 1962) and *Phormia regina* (Green 1964) the circadian rhythm of activity of starved flies that were maintained in the dark continued until death, with peak activity occurring in the second half of the photophase. In starved flies activity increases with time until a rapid decrease occurs shortly prior to death. This increase in activity was not observed in the present investigation, which is evidence that even those flies confined in mini-cages received sufficient food to eliminate this response to starvation. The fact that the flies had not become fully accustomed to the apparatus after one hour of equilibration casts doubt on some aspects of the results of the experiments concerning the effects of confinement and light on oxygen consumption, since a one hour equilibration period was used. It casts doubt also on much of the published work on oxygen consumption in flies, as this equilibration period is the norm in many studies

Table 11. Mean change in oxygen volume of control vessels (µl/hr).

temperature (C)	light conditions	mean (sem)	Prob |mean| > 0.0
17	light	-1.50(2.297)	0.53
17	dark	1.84(0.972)	0.09
27	light	0.44(0.260)	0.13
27	dark	0.07(0.072)	0.35

Table 12. Anova of oxygen consumption of male house flies with confinement method, light conditions, and temperature as variables.

source	d.f.	S.S.	F	p
block	2	769	0.28	> 0.05
cage	1	16267	12.01	< 0.001
light	1	7284	5.38	< 0.05
cage*light	1	2886	2.13	> 0.05
temperature	1	47195	34.84	< 0.001
cage*temperature	1	567	0.42	> 0.05
light*temperature	1	3227	2.38	> 0.05
cage*light*temperature	1	0.04	0.0	> 0.05
error	130	176077	---	---

Table 13. Mean and range of oxygen consumption (µl/mg/hr), measured over three days, for male house flies at two temperatures, based on 1/2 hour measurement periods every four hours.

temperature(C)	mean (sem)	range	n	t	p
17	0.72 (0.016)	0.50-0.98	12	8.27	< 0.001
27	1.66 (0.050)	1.03-2.09	8		

Table 14. Head capsule fluorescence of *M. domestica* males maintained under different light and temperature treatments.

Temperature	Treatment	Conditions	Mean (sem)	n
27C	1	17C dark	10.3 (0.37)	12
	2	27C respirometer	14.2 (0.47)	8
	3	27C dark	14.5 (0.38)	12
	4	27C 16L:8D*	13.9 (0.42)	10
17C	11	17C dark	10.4 (0.38)	12
	12	17C respirometer	10.7 (0.38)	12
	13	27C dark	16.3 (0.42)	10
	14	17C 16L:8D*	10.2 (0.38)	12

*Males maintained under crowded, mixed sex conditions in 20 x 20 x 20 cm cage.

Table 15. Comparisons made between head capsule fluorescence of *M. domestica* males for light and temperature treatments. Data analyzed in this table are the same as presented in Table 14.

Comparison	Description	F	p
1	respirometer vs mini-cage controls (27C)	0.22	0.64
2	respirometer vs mini-cage controls (17C)	0.37	0.54
3	17C vs 27C (both runs)	235.35	0.001
4	mini-cages vs large cage (27C)	0.69	0.40
5	mini-cages vs large cage (17C)	0.48	0.49
6	17C mini-cage controls (between runs)	0.01	0.94
7	27C mini-cage controls (between runs)	10.01	0.002

Table 16. Pearson correlation coefficients between weight, head width, and RF for one-day-old male house flies, numbers above the diagonal are correlation coefficient, those below the diagonal are probabilities of a greater correlation coefficient, n = 20.

	weight	head width	RF
weight	---	0.707	0.215
head width	0.0005	---	0.063
RF	0.362	0.839	---

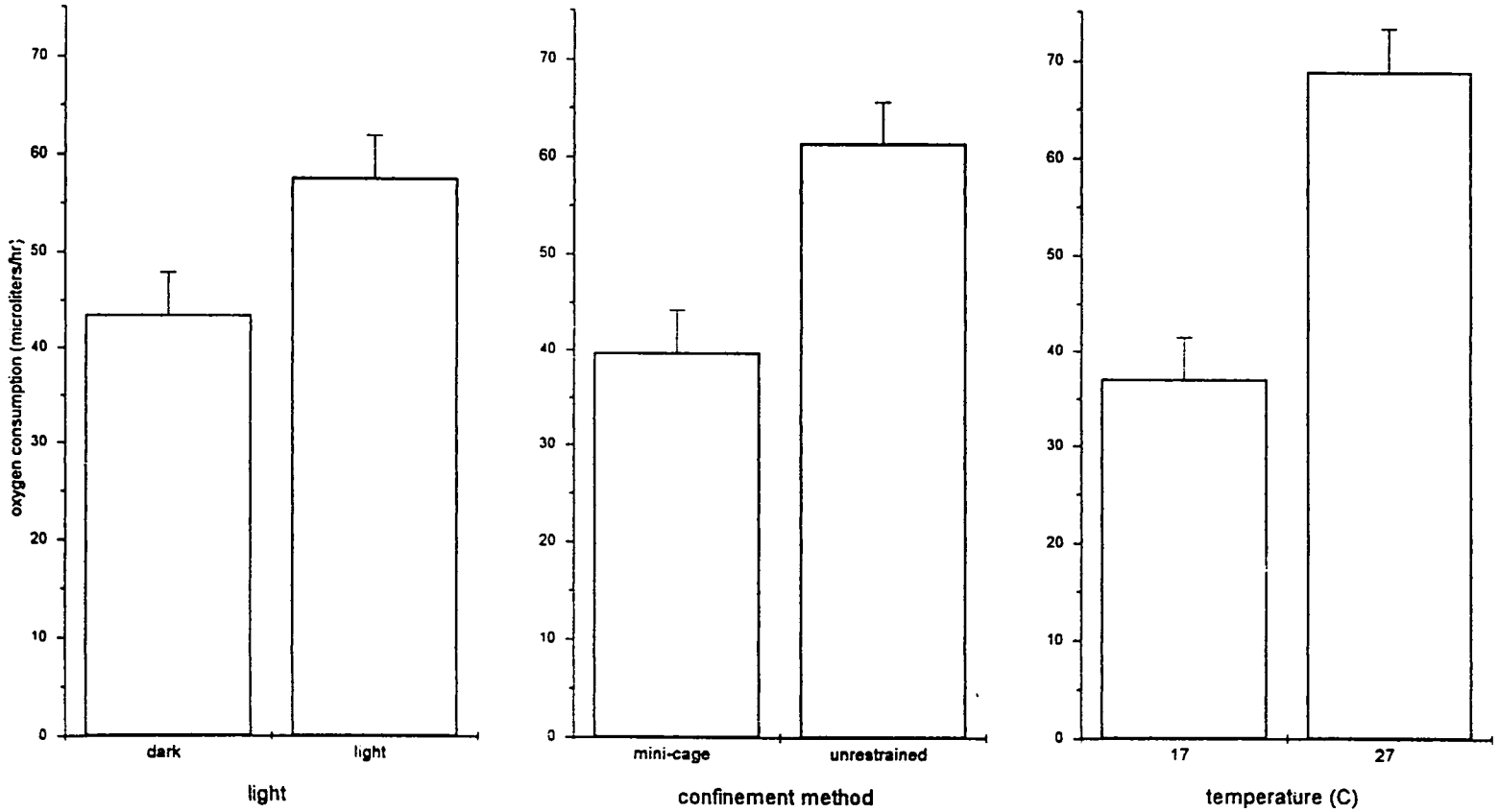


Figure 9. Effects of confinement, light, and temperature on rate of oxygen consumption of male house flies. Confinement was either in a 1 ml wire mesh cage or unrestrained in a 15 ml reaction vessel. Light was either normal room light or darkness. Vertical bars are standard errors.

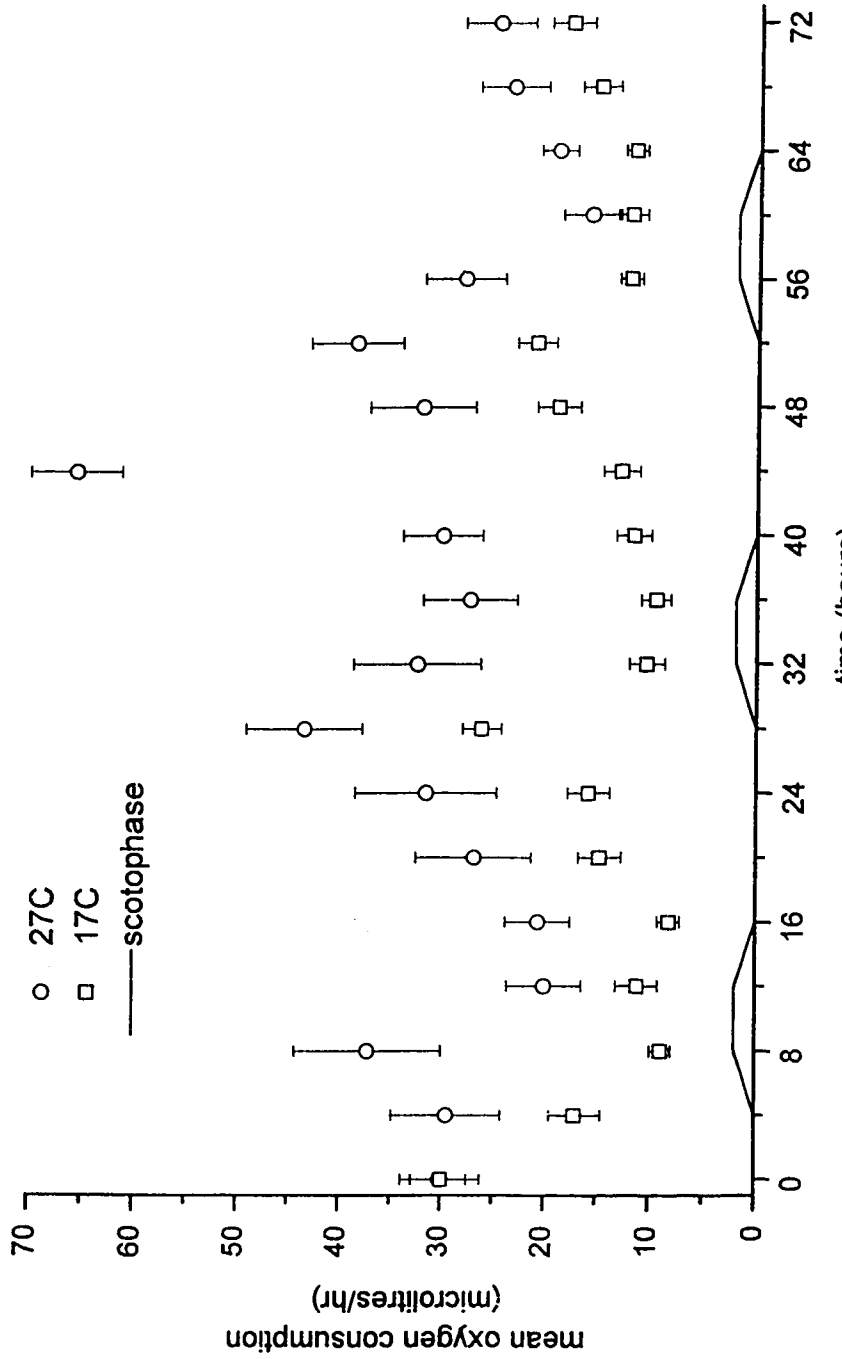


Figure 10. Mean hourly oxygen consumption (microlitres/hr) of male house flies at 17 and 27C in the dark. (Vertical bars are standard errors.)

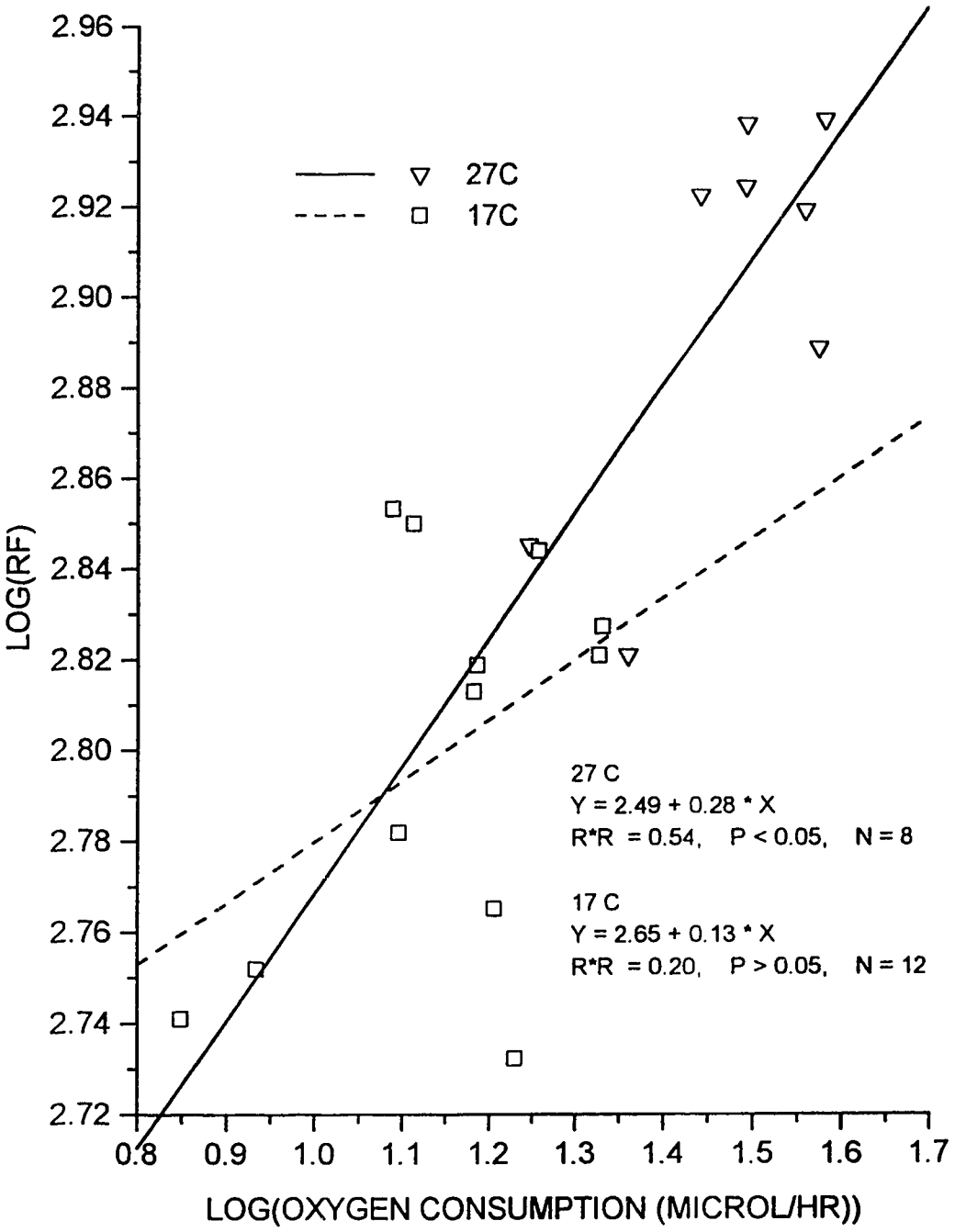


FIGURE 11. LOG OF RF VS LOG OF OXYGEN CONSUMPTION (BOTH VARIABLES UNADJUSTED FOR SIZE) AT 17 AND 27 C

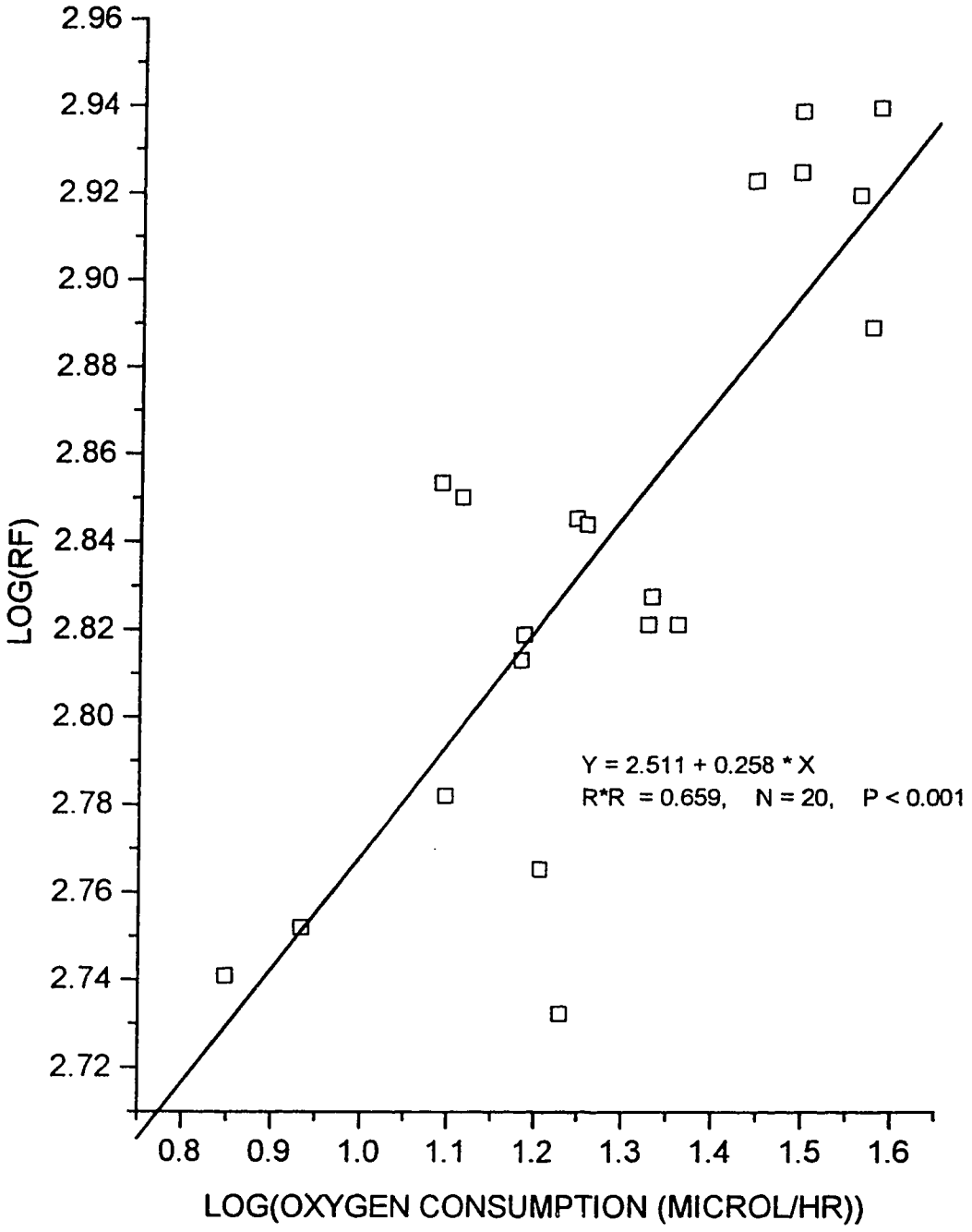


FIGURE 12. LOG OF RF VS LOG OF OXYGEN CONSUMPTION (BOTH VARIABLES UNADJUSTED FOR SIZE) AT 17 AND 27 C, REGRESSION OF COMBINED DATA.

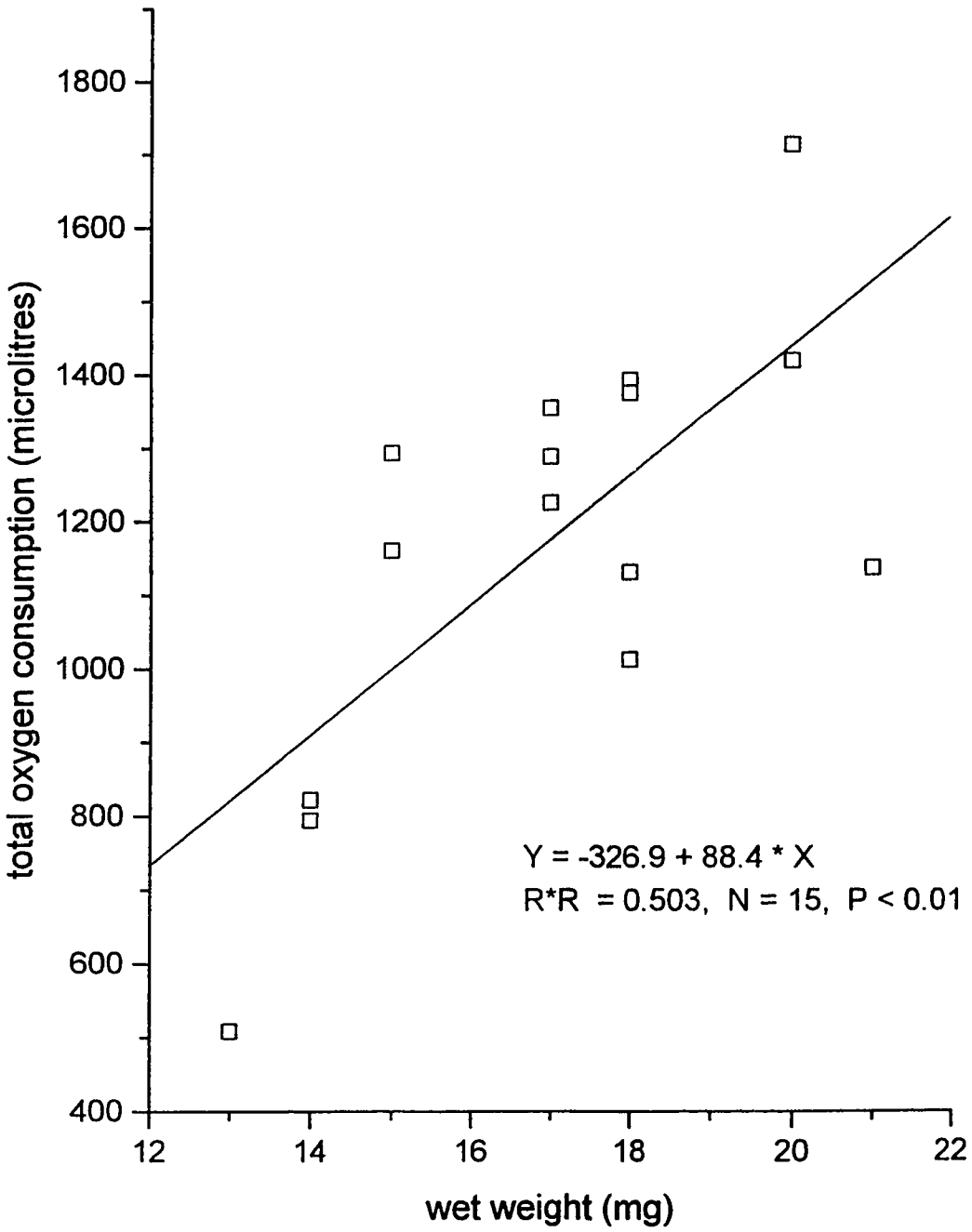


Figure 13. The relationship between wet weight and total oxygen consumed, between 24 and 96 hours of age, for male house flies maintained at 17C.

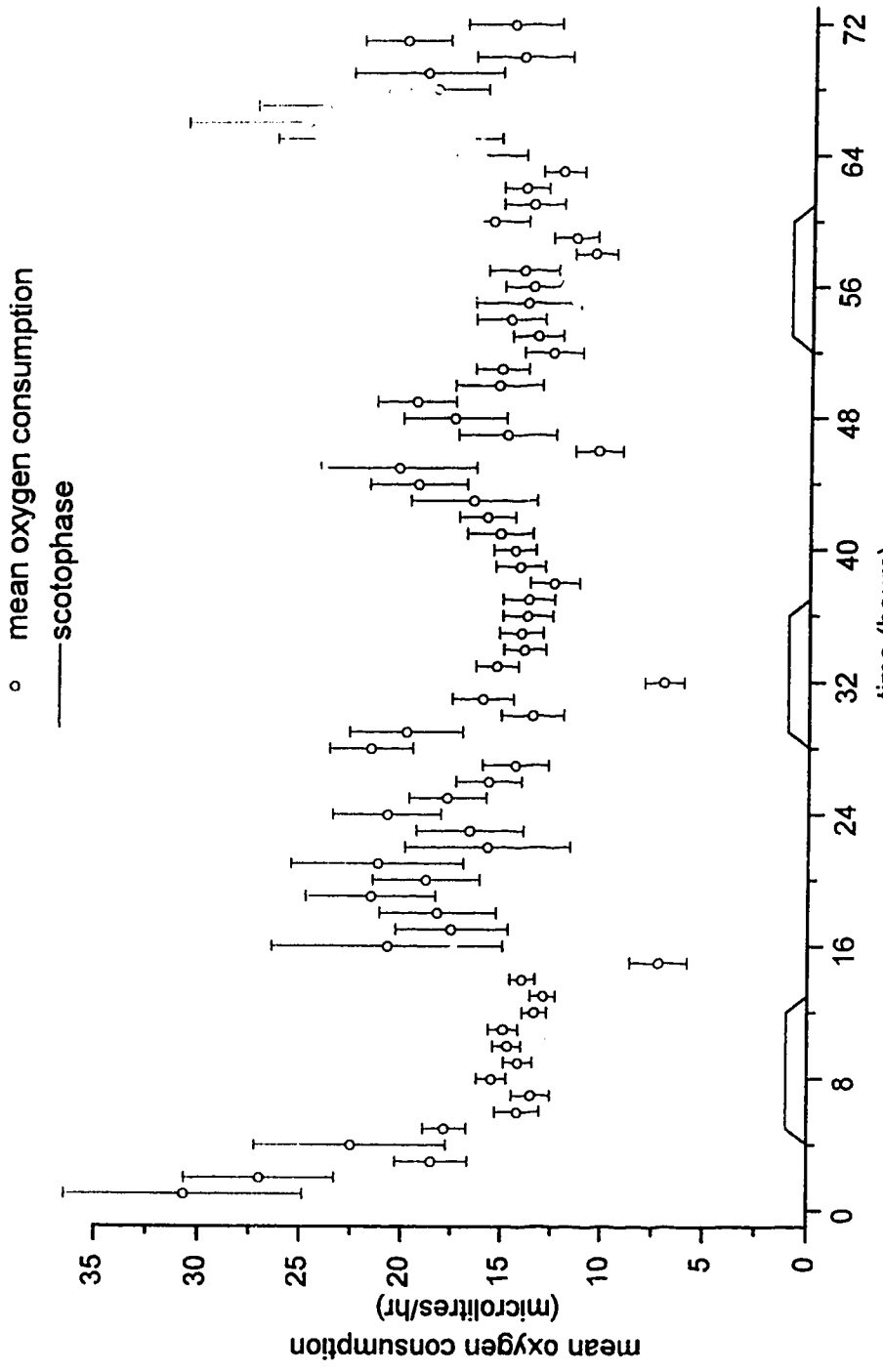


Figure 14. Mean hourly oxygen consumption (microlitres/hr) of male house flies at 17C in the dark. (n = 15, vertical bars are standard errors.)

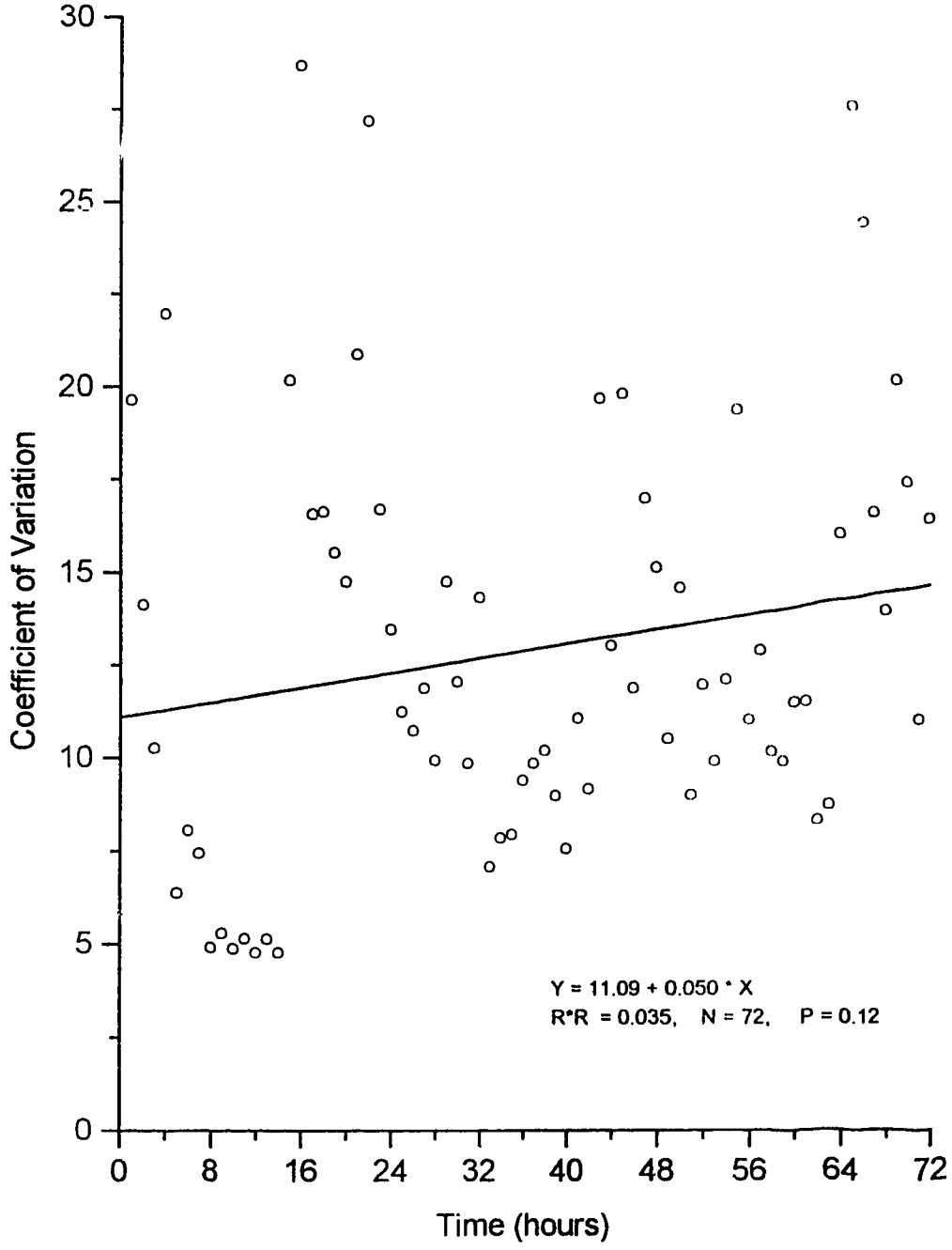


Figure 15. Coefficient of variation of hourly oxygen consumption of male house flies at 17C. (n = 15.)

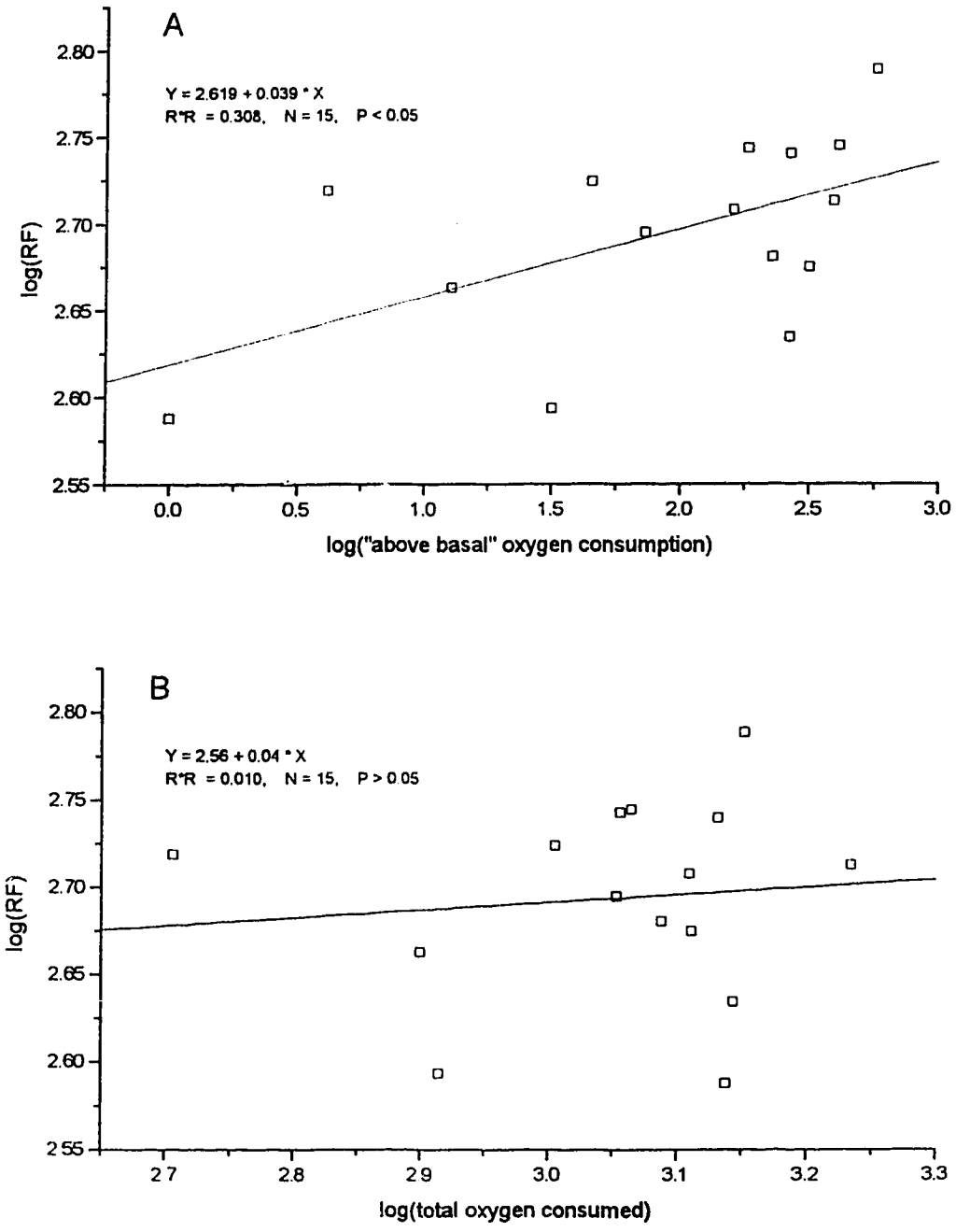


Figure 16. Relationship of A) log("above basal" oxygen consumption) and B) log(total oxygen consumption), to the log(HCF), between 24 and 96 hours of age, in male house flies at 17C.

Chapter 7
GENERAL DISCUSSION

This study presents several new findings concerning pteridine accumulation in the compound eyes of Diptera and confirms, in most respects, previously reported findings. In *Musca domestica* the rate of pteridine accumulation varied inversely with age, unlike many previously examined Diptera in which the rate of accumulation was reported not to vary (Mail *et al.* 1983; Lehane and Mail 1985; Thomas and Chen 1989). In wild type and *salmon* mutants of *Glossina morsitans morsitans* there was a slight curvilinearity in the accumulation pattern of HCF; this species had previously been reported to exhibit a linear relationship between HCF and age (Lehane and Mail 1985). Pteridine accumulation in house flies was affected by variation in the duration of development at each temperature. Head capsule fluorescence and metabolic rate were directly related in house flies, although the observed correlation was between HCF and that portion of metabolism that occurred over and above the level estimated as basal metabolism. These results have implications for the future development of the pteridine method of age determination, for understanding the role of pteridine accumulation in the compound eyes of Diptera, and for understanding the relationship between metabolism and physiological age.

Previous investigations have accounted for the effects of temperature, size, sex, and dietary protein on the rate of pteridine accumulation in flies (Mail *et al.* 1983; Lehane and Mail 1985; Thomas and Chen 1989). These effects are thought to be mediated through their impact on metabolic rate; increased ambient temperature increases metabolic rates in insects, and large insects have correspondingly greater overall metabolism and greater rates of pteridine accumulation than do small insects. Although it is possible that sex-specific metabolic rates determine the sex-specific rate of pteridine accumulation observed in many insects, much of this difference is probably due to differences in total body size and relative size of the compound eyes.

In this study pteridine accumulation depended upon metabolic rate in male house flies, but not in a simple one-to-one manner. Pteridine accumulation was correlated with metabolism that occurred above a threshold metabolic rate. Metabolism below this threshold was not correlated with pteridine level, nor was overall metabolism. This result supports the current understanding of pteridine accumulation, and it implies that any factor which affects metabolism, above the metabolic threshold for pteridine accumulation, will also affect pteridine accumulation. Of the factors known to affect metabolic rate, temperature, size, and sex have been incorporated into existing pteridine age determination models. Two other factors, activity and genetic variation, may be important in determining pteridine accumulation rates but have not yet been incorporated into the pteridine method of age determination.

The possible effect of genetic variation on rates of pteridine accumulation within populations may be direct, through variation in control of pteridine synthesis, transport or deposition; or it may be indirect through effects on metabolic rate. The genetics of eye pigmentation has been well studied, but never with a view to determining levels of variation in the rates of pteridine accumulation in wild populations. Considering the complexity of the genetic control of pteridine pigmentation in the compound eyes of

Diptera (Ferré *et al.* 1986), it would be surprising if there was no genetic variation directly affecting accumulation rates. How important this might be to pteridine age accumulation models has not been determined.

There is considerable genetic variation in activity and metabolic rate in insects. Trout and Kaplan (1970) compared shaker mutants, which exhibit abnormal activity patterns, and wild type *D. melanogaster*. There was a difference of 65% in metabolic rate (μl O_2 consumed/mg/hr) between the normal strain and the most active mutant. Lints *et al.* (1984), in examining spontaneous locomotor activity, noted differences of up to 54% in this variable between strains of wild type *D. melanogaster*. In the present study there was a twofold variation in mass-specific metabolic rate of individual male house flies. How much of this variation is under genetic control is unknown, but the variation in *D. melanogaster* supports the view that some portion of this variability is heritable. In view of the correlation between pteridine accumulation and metabolic rate, genetic variation in the control of metabolic rate will have an effect on pteridine accumulation and may be a source of unexplained error in estimates of age based upon pteridine content.

The correlation between metabolic rate and pteridine accumulation indicates that activity has an effect on pteridine accumulation. However, in the experiments designed to examine this relationship there was no significant effect of activity. As discussed in Chapter 6, this may be because maintenance at a high temperature increased activity and metabolism of both "active" and "inactive" flies to such an extent that the effects of activity were obscured. In spite of the failure of these experiments, the relationship between HCF and oxygen consumption indicates that differences in activity will have an effect on HCF if they affect "above basal" metabolism. This warrants further investigation of activity as a source of variation in HCF.

The effect of variation in duration of development, at a constant temperature, on HCF was a small but significant source of variation in HCF. The results of this study indicate that these differences were greatest at eclosion, and although they were maintained throughout life, their impact decreased with age. This source of variation will not be easily accounted for in models of pteridine accumulation, unless a method of estimating this factor in wild populations is developed.

This investigation offers little hope that the pteridine age determination method will be improved past its present usefulness. Neither duration of development nor metabolic rate are presently estimable in field populations except for the portion of the variation that has already been accounted for through incorporation of temperature into existing models. Although possible genetic contributions to variation in pteridine accumulation are yet to be identified, it is unlikely that the effect of this factor will be estimable in wild populations. The pteridine method remains a useful method for studying age structure of populations, however it is unlikely to become useful for providing accurate age estimates of individual insects. One improvement that can be credited to this study is the demonstration that a closer examination of changes in rates of accumulation of pteridines at different ages may provide more accurate estimates of age. For *G. m. morsitans*, the present models do not take into account the slight curvilinearity of the age/HCF relationship and tend to overestimate the age of very young and very old flies and to underestimate the age of flies of intermediate ages.

The results of this study raise two questions concerning the storage excretion role of pteridine accumulation in the compound eyes of house flies. 1) What is the nature of the metabolic rate threshold of pteridine accumulation? 2) Why do rates of pteridine accumulation decrease so sharply with age? The relationship of HCF with "above basal" metabolism, and the absence of such a relationship with "basal" metabolism, suggests the existence of a threshold metabolic rate, below which HCF does not accumulate in house flies. It is possible that below this threshold energetically efficient, non-pteridine pathways can adequately dispose of the nitrogenous wastes produced. When metabolic rate exceeds the threshold, these systems may be unable to dispose of the increased levels of waste product and these accumulate in the eyes as pteridines. Some support for this is derived from the observation that pierid butterflies, which produce large amounts of pteridines, do not excrete more pteridines than other butterflies producing normal amounts of pteridine (Ziegler and Harmsen 1969). The observation that pteridines accumulate in house flies at temperatures as low as 10C (Appendix C), a temperature at which the metabolic rate is very low, suggests that some small amount of pteridine deposition in the eyes may occur below the threshold, the effect of which may be obscured by other sources of variation in HCF

The curvilinearity of the relationship between HCF and age in house flies may be explained through consideration of the time course of metabolic rate during the life of adult flies. Metabolic rates of house flies increase during early adult life, reach a peak, and then decline for the latter part of the normal life span. At 25C peak metabolic rate is reached at six days of age, and metabolic rate decreases rapidly until the flies are 10-days-old after which the decrease is more gradual (Sohal 1982). Since HCF was correlated with "above basal" metabolism, it is possible that late in life there is a smaller amount of "above basal" metabolism than there is early in life, accounting for the decline in HCF accumulation rate. The only data concerning metabolic rates available for adult tsetse flies, compares metabolic rates during the first (2-4 days of age) and fourth (~10-12 days of age) hunger cycles in *G. m. morsitans*. Older flies exhibit slightly greater metabolic rates than do young flies (Rajagopal and Bursell 1966). This does not contradict the present explanation of decreases in pteridine accumulation rate; adult tsetse flies are expected to have a more constant metabolic rate over their normal life-span, since the curvilinearity observed in this species was less extreme than that of house flies. One possible test of this explanation for the curvilinear relationship between age and HCF is that differences in HCF among activity-limited flies should be less than that among active flies, as limitation of activity should decrease "above basal" metabolism without affecting "basal" metabolism. This may have contributed in part to the difficulty experienced in linking metabolism with HCF in the experiments measuring metabolic rates in confined flies.

This investigation of the age dependent change in pteridine levels in the compound eyes of house flies has implications for two modern theories of ageing. This example of the decrease in a normal physiological function with increasing age may be related to the overall decrease in metabolism of adult house flies (Sohal 1982). One hypothesis of the evolution of ageing holds that natural selection promotes survival and maintenance of physiological function only until post-reproductive age is reached (Rose 1991). Decrease

in metabolism and the consequent decrease in rate of accumulation of HCF may be evidence of such a post-reproductive ageing process, since rates decrease most sharply after female house flies would normally have produced at least one egg batch and males would have mated. Evidence from field studies indicate that relatively few females survive to lay a second egg batch (Krafsur *et al.* 1985), so at this age selection for longevity would be relaxed, concurrent with the observed decrease in metabolism and HCF accumulation. It is interesting that tsetse flies exhibit more sustained levels of deposition of pteridines since their reproductive life is much longer than that of house flies. It would be interesting to follow the oxygen consumption patterns of tsetse flies through their life to see if decline in metabolism is concurrent with change in reproductive status and decline in rate of pteridine accumulation. If this is the case, a decline in the rate of HCF accumulation may be an indicator of the onset of post-reproductive ageing, which could be used in the study of ageing in Diptera. This will require a closer investigation for curvilinearity in pteridine accumulation patterns than has been the norm.

The relationship of "above basal" and "basal" metabolism to HCF implies that the age dependent changes of metabolic rate and pteridine accumulation rate are not related to "basal" metabolism, and with respect to these two factors, the animal is not "ageing" except when it enters the "above basal" metabolic range. This may explain, in part, the discrepancy between the results of Sohal and coworkers (reviewed in Sohal and Allen 1986) who demonstrated an effect of activity on ageing, and those of Lints *et al.* (1984), who observed no effect of spontaneous locomotor activity on ageing. According to the current definitions of "above basal" and "basal" metabolism, the increases in activity observed in house flies due to crowding, probably cause increases in "above basal" metabolism, but the differences in spontaneous locomotor activity are probably related to differences in "basal" metabolism, that may have little effect on ageing and longevity. This bears a similarity to the free radical theory of ageing, which posits that at a cellular level, there is a threshold level of metabolism below which cells sustain little or no irreparable cellular damage and/or ageing, but above this threshold irreparable damage and/or ageing occurs relatively rapidly (Harman 1992; Miquel 1992). Indeed it was noted long ago by Pearl (1924, cited in Lints *et al.* 1984), that physical activity above certain levels has a deleterious effect on longevity.

There are many avenues for further investigation which are suggested by this study, especially into the genetic variation in HCF accumulation, the nature of the metabolic threshold, the effect of duration of development, and the reasons for the decline in the rate of accumulation of HCF with increasing age. To determine what effect genetic variation has on pteridine accumulation three lines of inquiry could be undertaken. The examination of activity mutants for anomalous pteridine accumulation may provide an indication of an effect of this variation upon HCF. A comparison of pteridine accumulation of genetically dissimilar strains of a single species would show whether or not genetic differences are important in determining HCF within normal members of a species. A quantitative genetic investigation of variation within populations would provide information on the importance of genetic variation within these populations in determining pteridine levels. In the case of tsetse flies, an investigation of the effects of maternal age on HCF at eclosion, through measurement of HCF at eclosion of each

offspring of individual females, would provide insight into the effects of maternal age and variation within families and populations for HCF.

Investigation into the duration of development could be conducted best in conjunction with the quantitative genetic study of HCF in house flies. Examination of variation within and between families of house flies for duration of development and HCF at eclosion would address several questions. It would provide information on the genetic variation of development rate, the impact of this on HCF, and on the genetic variation in HCF attached to, and separate from, the genetic variation in development rate. This investigation would require rearing flies in relatively small numbers, which would solve the problems of increased temperature of larval medium due to surface to volume ratio considerations. I suggest also that in such a study HCF determinations should be made closer to the time of eclosion than one day of age.

The nature of the metabolic rate threshold for pteridine accumulation may be approached from several directions, but not easily from any. Since it is likely that biopterin is the major contributor to changes in HCF (Bridges and Sohal 1990; Mail and Lehane 1988), an investigation of the enzymes involved in synthesis of this compound should prove fruitful. Unfortunately for this line of investigation, biopterin is the end product of a pathway beginning with guanosine triphosphate and involving at least three and possibly seven enzymes (Ferré *et al.* 1986). However, if a rate limiting enzyme could be identified, studying the temperature activity curve of this enzyme and the time course of its activity during the insect's life would be useful. It is possible also that the HCF accumulation threshold is actually one of excretion, in which case introduction of exogenous pteridines should have an effect on HCF. Further investigation of the relationship between oxygen consumption, HCF and temperature are in order also. Determination of the metabolic rate at the temperature threshold of HCF accumulation should provide an initial estimate of the metabolic rate threshold for accumulation.

REFERENCES

Anderson, J.R. 1964. Methods for distinguishing nulliparous from parous flies and for estimating ages of *Fannia canicularis* and some other cyclorraphous Diptera. Ann. Ent. Soc. Am. 57:226-236.

Bridges, R.G. and R.S. Sohal 1980. Relationship between age-associated fluorescence and linoleic acid in the housefly *Musca domestica*. Insect Biochem. 10:557-562.

Brizzee, K.R. and J.M. Ordy 1981. Cellular features, regional accumulation and prospects of modificationof age pigments in mammals. P. 101-154. In Sohal, R.S. (ed) Age Pigments. Elsevier North Holland, Amsterdam.

Buchan, P.B. and R.S. Sohal 1981. Effect of temperature and different sex ratios on physical activity and life span in the adult housefly, *Musca domestica*. Exp. Gerontol. 16:223-228

Camin, V., Baker, P., Carey, J., Valenzuela, J. and R. Arredondo-Peter 1991. Biochemical age determination for adult Mediterranean fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 84:1283-1288.

Camin, C.V., Baker, P.S., Valenzuela, J. and R. Arredondo-Peter 1992. Separation and spectrophotometric characterization of some fluorescent pigments from *Ceratitis capitata*, W. (Diptera: Tephritidae) head capsule. Insect Biochem. Molec. Biol. 22:505-509.

Cheke, R.A., Duton, M. Avissey, H.S.K. and M.J. Lehane 1990. Increase with age and fly size of pteridine concentrations in different members of the *Simulium damnosum* species complex. Acta Leiden. 59:307-314.

Cheke, R.A., Garms, R., Howe, M.A. and M.J. Lehane 1987. Possible use of pteridine concentrations for determining the age of *Simulium damnosum* s.l.. Trop. Med. Parasit. 38 346.

Davis, R.A. and G. Fraenkel 1940. The oxygen consumption of flies during flight. J. Exp Biol. 17:402-407.

Detinova, T.S. 1962. Age-grouping methods in Diptera of medical importance with special reference to some vectors of malaria. Monograph Series: W.H.O. no. 47, 216 pp..

Dolman, C.L. and P.M. MacLeod 1981. Lipofuscin and its relation to aging. P. 205-247. In Fedoroff , S. and Hertz, L. (eds) Advances in Cellular Neurobiology vol 2. Academic Press, New York.

Ferré, J., Silva, F.J., Real, M.D. and J.L. Mensua 1986. Pigment patterns in mutants affecting the biosynthesis of pteridines and xanthommatin in *Drosophila melanogaster*. Biochem. Gen. 24:545-569.

Fletcher, B.L., Dillard, C.J. and A.L. Tappel 1973. Measurement of fluorescent lipid peroxidation products in biological systems and tissues. Analyt. Biochem. 52:1-9.

Gooding, R.H. 1982. Laboratory evaluation of the lethal allele *salmon* for genetic control of the tsetse fly, *Glossina morsitans morsitans*. P. 267-278. Sterile Insect Technique and Radiation in Insect Control, IAEA-SM-225, I.A.E.A., Vienna.

Green, G.W. 1964. The control of spontaneous locomotor activity in *Phormia regina* Meigen--I.* Locomotor activity patterns of intact flies. J. Insect Physiol. 10:711-726.

Hammer, C. and E. Braum 1988. Quantification of age pigments (lipofuscin). Comp. Biochem. Physiol. B 90B:7-17.

Hannover, A. 1842. Microskopiske undersogelser af nerve-systemer. K. Dan. Vidensk. Selsk. Skr. Naturvidensk Math. Afd. 10:1-112.

Harman, D. 1992. Free radical theory of aging. Mutat. Res. 275:257-266.

Hemmingsen, A.M. 1960. Energy metabolism as related to body size and respiratory surfaces, and its evolution. Reports of the Steno Memorial Hospital and Nordinsk Insulin Laboratorium 9:6-110.

Jordan, A.M. 1986. Trypanosomiasis control and African rural development. London and New York, Longman.

Koneff, H. 1886. Beitrage zur kenntniss der nervonzellen in den peripheren ganglien. Mitth. d. Naturf. Gesellsch. Bern 44:13-14.

Krafsur, E.S. 1985. Age composition and seasonal phenology of house fly (Diptera: Muscidae) populations. J. Med. Ent. 22:515-523.

Krafsur, E.S. and C.M. Ernst 1983. Physiological age composition and reproductive biology of horn fly populations, *Haematobia irritans irritans* (Diptera: Muscidae), in Iowa, USA. J. Med. Ent. 20:664-669.

Krafsur, E.S. and C.M. Ernst 1986. Phenology of horn fly populations (Diptera: Muscidae), in Iowa, USA. J. Med. Ent. 23:188-195.

Krafsur E.S., Black, W.C. IV, Church, C.J. and D.A. Barnes 1985. Age structure and reproductive biology of a natural house fly (Diptera: Muscidae) population. Environ. Entomol. 14:159-164.

Krafsur, E.S., Rosales, A.L., Robison-Cox, J.F. and J.P. Turner 1992. Age structure of horn fly (Diptera: Muscidae) populations estimated by pterin concentrations. J. Med. Ent. 29:678-686.

Langer, H. 1975. Properties and functions of screening pigments in insect eyes. P. 429-455. In Snyder, A.W. and R. Menzel (eds) Photoreceptor Optics, Springer-Verlag, Berlin.

Langley, P.A., Hall, M.J.R., Felton, T. and M. Ceesay 1988. Determining the age of tsetse flies, *Glossina* spp. (Diptera: Glossinidae): an appraisal of the pteridine fluorescence technique. Bull. Ent. Res. 78:387-395.

Le Bourg, E. 1987. The rate of living theory, spontaneous locomotor activity, aging and longevity in *Drosophila melanogaster*. Exp. Gerontol. 22:359-369.

Lehane, M.J. 1985. Determining the age of an insect. Parasitology Today 1:81-85.

Lehane, M.J. and J. Hargrove 1988. Field experiments on a new method for determining age in tsetse flies (Diptera: Glossinidae). Ecol. Entomol. 13:319-322.

Lehane, M.J. and T.S. Mail 1985. Determining the age of adult male and female *Glossina morsitans morsitans* using a new technique. Ecol. Entomol. 10:219-224.

Lehane, M.J., Chadwick, J., Howe, M.A. and T.S. Mail 1986. Improvements in the pteridine method for determining age in adult male and female *Stomoxys calcitrans* (Diptera: Muscidae). J. Econ. Entomol. 79:1714-1719.

Lints, F.A., Le Bourg, E. and C.V. Lints 1984. Spontaneous locomotor activity and life span: A test of the rate of living theory in *Drosophila melanogaster*. Gerontology 30:376-387.

Loeb, J. and J.H. Northrop 1917. On the influence of food and temperature upon the duration of life. J. Biol. Chem. 32:10.-121.

Lysyk, T.J. and R.C. Axtell 1987. A simulation model of house fly (Diptera: Muscidae) development in poultry manure. Can. Ent. 119:427-437.

Lysyk, T.J. and E.S. Krafsur (in press) Relationship between pterin accumulation and ovarian development in the stable fly Stomoxys calcitrans (L.) (Diptera: Muscidae).

Mail, T.S. and M.J. Lehane 1988. Characterisation of pigments in the head capsule of the adult stablefly *Stomoxys calcitrans* Ent. Exp. Appl. 46:125-131.

Mail, T.S., J. Chadwick and M.J. Lehane 1983. Determining the age of adults of *Stomoxys calcitrans* (L.) (Diptera: Muscidae). Bull. Ent. Res. 73:501-525.

McArthur, M.C. and R.S. Sohal 1982. Relationship between metabolic rate, aging, lipid peroxidation and fluorescent age pigment in milkweed bug, *Oncopeltus fasciatus* (Hemiptera). J. Gerontol. 37:268-274.

Miquel, J. 1992. An update on the mitochondrial-DNA mutation hypothesis of cell aging. Mutat. Res. 275:209-216.

Miquel, J., Oro, J., Bensch, I. and J. Johnson 1977. Lipofuscin: fine structural and biochemical studies. P. 133-182. In Pryor, W.A. (ed) Free Radicals in Biology Vol. 3. Academic Press, New York.

Msangi, A. and M.J. Lehane 1991. A method for determining the age of very young tsetse (Diptera: Glossinidae) and an investigation of the factors determining head fluorescent levels in newly emerged adults. Bull. Ent. Res. 81:185-188.

Neville, A.C. 1983. Daily cuticular growth layers and the teneral stage in adult insects: a review. J. Insect Physiol. 29:211-219.

Nichol, C.A., Smith, G.K. and D.S. Duch 1985. Biosynthesis and metabolism of tetrahydrobiopterin and molybdopterin. Annu. Rev. Biochem. 54:729-764.

Oliver, C. 1981 Lipofuscin and ceroid accumulation in experimental animals. P. 335-353. in Sohal, R.S. (ed), Age Pigments. Elsevier, Amsterdam.

Parker, A.H. 1962. Studies on the diurnal rhythms of the housefly, *Musca domestica* L., in a dry tropical environment. Acta Trop. 19:97-119.

Pearl, R. 1922. The Biology of Death. Lippincott, Philadelphia.

Pearl, R. 1924. Studies in Human Biology. Williams & Wilkins, Baltimore.

Pearl, R. 1928. The Rate of Living. University of London Press, London.

Peters, H.R. 1983. The Ecological Implications of Body Size. Cambridge University Press, Cambridge.

Phillips, J.P. and H.S. Forrest 1980. Ommochromes and Pteridines. P. 541-623. In Ashburner, M. and T.R.F. Wright (eds) The Genetics and Biology of *Drosophila* Vol. 2. Academic Press, New York.

Ragland, S.S. and R.S. Sohal 1973. Mating behavior, physical activity and aging in the housefly, *Musca domestica.* Exp. Gerontol. 8:135-145.

Ragland, S.S and R.S. Sohal 1975. Ambient temperature, physical activity and aging in the housefly, *Musca domestica.* Exp. Gerontol. 10:279-289.

Rajagopal, P.K. and E. Bursell 1966. The respiratory metabolism of resting tsetse flies. J. Insect Physiol. 12:287-297.

Rose, M.R. 1991. Evolutionary Biology of Aging. Oxford University Press, New York & Oxford.

Rubner, M. 1908. Das Problem der Lebensdauer. Berlin.

Ryan, L., Molyneux, D.H. and F.A.S. Kuzoe 1980. Differences in rate of wing fray between *Glossina* species. Tropenmed. Parasitol. 31:111-116.

Sheehy, M.R.J. and B.E. Roberts 1991. An alternative explanation for anomalies in "soluble lipofuscin" fluorescence data from insects, crustaceans, and other aquatic species. Exp. Gerontol. 26:495-509.

Sheldahl, J.A. and A.L. Tappel 1974. Fluorescent products from aging *Drosophila melanogaster*: an indicator of free radical lipid peroxidation. Exp. Gerontol. 9:33-41.

Sohal, R.S. 1981. Relationship between metabolic rate, lipofuscin accumulation and lysosomal enzyme activity during aging in the adult housefly, *Musca domestica.* Exp. Gerontol. 16:347-355.

Sohal, R.S. 1982. Oxygen consumption and life span in the adult male housefly, *Musca domestica.* Age 5:21-24.

Sohal, R.S. 1985a. Ageing in insects. P. 595-631. In Kerkut G.A. and L.I. Gilbert (eds) Comprehensive Insect Physiology, Biochemistry, and Pharmacology. Vol. 10. Pergamon Press, Oxford.

Sohal, R.S. 1985b. Spontaneous locomotor activity as a test for the rate of living theory. Gerontology 31:332-334.

Sohal, R.S. 1987. Quantification of lipofuscin: a critique of the current methodology. Adv. Biosci. 64:83-91.

Sohal, R.S. 1991. Hydrogen peroxide production by mitochondria may be a biomarker of aging. Mech. Age. Dev. 60:189-198.

Sohal, R.S. and R.G. Allen 1986. Relationship between oxygen metabolism, aging and development. Adv. Free Rad. Biol. Med. 2:117-160.

Sohal, R.S. and P.B. Buchan 1981a. Relationship between physical activity and life span in the adult housefly, *Musca domestica*. Exp. Gerontol. 16:157-162.

Sohal, R.S. and P.B. Buchan 1981b. Relationship between fluorescent age pigment, physiological age and physical activity in the housefly, *Musca domestica*. Mech. Age. Dev. 15:243-249.

Sohal, R.S. and H. Donato 1978. Effects of experimentally altered life spans on the accumulation of fluorescent age pigment in the housefly, *Musca domestica*. Exp. Gerontol. 13:335-341.

Sohal, R.S. and H. Donato 1979. Effect of experimental prolongation of life span on lipofuscin content and lysosomal enzyme activity in the brain of the housefly, *Musca domestica*. J. Gerontol. 34:489-496.

Sohal, R.S., Donato, H. and E.R. Biehl 1981. Effect of age and metabolic rate on lipid peroxidation in the housefly, *Musca domestica* L.. Mech. Age. Dev. 16:159-167.

Sohal, R.S., Muller, A., Koletzo, B. and H. Sies 1985. Effect of age and ambient temperature on n-pentane production in adult housefly, *Musca domestica*. Mech. Age. Dev. 29:317-326.

Sullivan, R.L. and R.R. Sokal 1963. The effects of larval density on several strains of the house fly. Ecology 44:120-130.

Summers, K.M., Howells, A.J. and N.A. Pyliotis 1982. Biology of eye pigmentation in insects. Adv. Insect Physiol. 16:119-166.

Tappel, A.L. 1975. Lipid peroxidation and fluorescent molecular damage to membranes. P. 145-170. In [illegible]mps, B.F. and A.V. Artsila (eds) Pathobiology of Cell Membranes Vol 1 Academic Press, New York.

Thomas, D.B. and A.C. Chen 1989. Age determination in the adult screwworm (Diptera: Calliphoridae) by pteridine levels. J. Econ. Entomol. 82:1140-1144.

Trout, W.E. and W.D. Kaplan 1970. A relation between longevity, metabolic rate, and activity in shaker mutants of *Drosophila melanogaster*. Exp. Gerontol. 5:83-92.

Turturro, A. and S.A. Shafiq 1979. Quantitative morphological analysis of age-related changes in flight muscle of *Musca domestica* L.. J. Gerontol. 34:823-833.

Tyndale-Biscoe, M. 1984. Age-grading methods in adult insects: a review. Bull. Ent. Res. 74:341-377.

Vale, G.A., Hargrove, J.W., Jordan, A.M., Langley, P.A. and A.R. Mews 1976. Survival and behaviour of tsetse flies (Diptera: Glossinidae) released in the field: a comparison between wild flies and animal fed and in vitro fed laboratory reared flies. Bull. Ent. Res. 66:731-744.

Wall, R., Langley, P.A. and K.L. Morgan 1991. Ovarian development and pteridine accumulation for age determination in the blowfly *Lucilia sericata*. J. Insect Physiol. 37:863-868.

Wall, W., Langley, P.A., Stevens, J. and G.M. Clarke 1990. Age-determination in the Old-world screw-worm fly *Chrysomya bezzania* by pteridine fluorescence. J. Insect Physiol. 36:213-218.

Wolman, M. 1980. Lipid pigments (chromolipids): their origin, nature and significance. P. 253-267. In Ioachim, H.L. (ed) Pathobiology Annual. Raven Press, New York.

Ziegler, I. and R. Harmsen 1969. The biology of pteridines in insects. Advances in Insect Physiology 6:139-203.

APPENDIX A

Mean (sem) and comparisons of HCF of A) male and B) female (following page) house flies that emerged, on two consecutive days, from an egg batch incubated at 27C. Adults were maintained at 22 and 27C.

A) males

Temperature	Age	Cohort B	Cohort C	t	d.f.	p
	1	4.39(0.808)	5.39(0.480)	1.27	8	> 0.10
	2	8.45(0.125)	9.39(0.680)	1.19	8	> 0.10
	3	9.77(1.334)	10.96(0.326)	1.53	8	> 0.10
	4	12.27(0.393)	12.66(0.295)	0.51	8	> 0.10
	5	13.00(0.560)	13.78(0.285)	0.99	8	> 0.10
22C	6	15.19(0.560)	15.79(0.423)	0.77	8	> 0.10
	7	15.80(0.780)	16.92(0.331)	1.42	8	> 0.10
	8	15.84(0.330)	16.55(0.308)	0.91	8	> 0.10
	9	17.07(0.632)	17.30(0.237)	0.30	8	> 0.10
	10	15.93(0.340)	17.58(0.560)	2.11	8	< 0.05
	15	18.77(0.456)	19.66(0.546)	1.14	8	> 0.10
	1	4.39(0.808)	5.39(0.480)	1.23	8	> 0.10
	2	8.62(0.315)	11.35(0.682)	3.29	8	< 0.01
	3	14.01(0.702)	13.04(0.232)	1.20	8	< 0.10
	4	14.54(0.358)	15.36(0.369)	1.02	8	> 0.10
	5	15.47(0.141)	15.68(0.810)	0.26	8	> 0.10
27C	6	17.43(0.462)	17.31(0.205)	0.50	8	> 0.10
	7	17.20(0.449)	17.91(0.453)	0.87	8	> 0.10
	8	18.94(0.912)	17.93(0.413)	1.26	8	> 0.10
	9	18.65(0.470)	18.47(0.623)	0.21	8	> 0.10
	10	19.34(0.509)	18.80(0.522)	0.67	8	> 0.10
	15	20.86(0.760)	23.37(0.495)	3.11	8	< 0.01

B) females

Temperature	Age	Cohort B	Cohort C	t	d.f.	p
	1	4.55(0.682)	6.03(0.166)	2.49	8	< 0.05
	2	7.82(0.470)	7.67(0.187)	0.24	8	> 0.10
	3	9.25(0.557)	9.95(0.314)	1.17	8	> 0.10
	4	10.31(0.653)	11.12(0.434)	1.37	8	> 0.10
	5	12.35(0.172)	12.52(0.385)	0.30	8	> 0.10
22C	6	13.91(0.323)	16.83(0.324)	0.14	8	> 0.10
	7	13.47(0.559)	14.35(0.601)	1.48	8	> 0.10
	8	14.01(0.373)	15.30(0.321)	2.07	8	< 0.05
	9	15.67(0.460)	14.85(0.321)	1.37	8	> 0.10
	10	15.68(0.169)	15.10(0.395)	0.97	8	> 0.10
	15	18.16(0.387)	17.82(0.391)	0.58	7	> 0.10
	1	4.55(0.682)	6.03(0.166)	1.92	8	< 0.10
	2	8.53(0.486)	10.50(0.383)	2.56	8	< 0.05
	3	11.86(0.383)	11.97(0.656)	0.15	8	> 0.10
	4	12.52(0.591)	13.22(0.377)	0.91	8	> 0.10
	5	13.61(0.421)	14.13(0.277)	0.68	8	> 0.10
27C	6	15.41(0.240)	15.87(0.496)	1.66	8	< 0.10
	7	15.87(1.020)	16.90(0.938)	1.34	8	> 0.10
	8	17.09(0.438)	16.04(0.258)	1.37	8	> 0.10
	9	18.12(0.331)	17.40(0.355)	0.95	8	> 0.10
	10	17.78(0.481)	17.45(0.708)	0.43	8	> 0.10
	15	19.39(0.942)	19.91(0.495)	0.64	7	> 0.10

APPENDIX B

Regressions of log(HCF) on log(age) for adult house flies, aged 2-10 and 15 days post-eclosion, that emerged from the same egg batch on two consecutive days, and were maintained at 22 and 27C. (Data from one day of age is excluded, see chapter 5.)

Sex	Temperature	Cohort	Intercept (sem)	Slope (sem)	R^2	p
male	22C	B	0.81(0.027)	0.42(0.034)	0.77	< 0.001
		C	0.87(0.016)	0.39(0.020)	0.88	< 0.001
	27C	B	0.90(0.023)	0.39(0.028)	0.81	< 0.001
		C	0.96(0.016)	0.33(0.020)	0.86	< 0.001
female	22C	B	0.77(0.019)	0.43(0.023)	0.88	< 0.001
		C	0.80(0.016)	0.40(0.019)	0.90	< 0.001
	27C	B	0.85(0.019)	0.41(0.024)	0.86	< 0.001
		C	0.93(0.015)	0.32(0.019)	0.86	< 0.001

APPENDIX C

An experiment was conducted to estimate the threshold temperature of pteridine accumulation in house flies.

METHODS

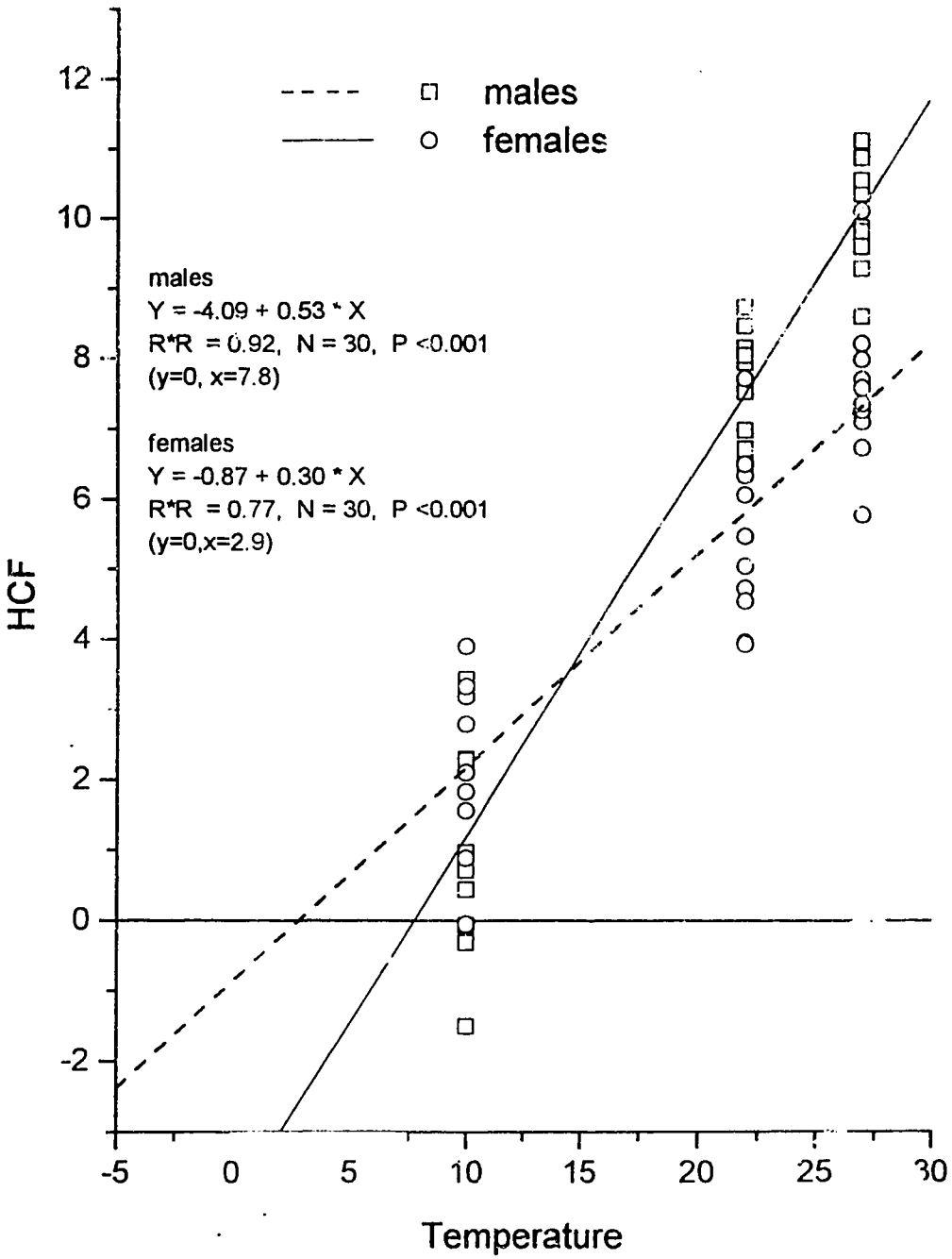
One-day-old flies, that had been reared under standard conditions at 27C were transferred to 10, 22, or 27C and maintained for a further 3 days under standard conditions. The flies at 10C were from a different egg batch than those maintained at 22 and 27C. Prior to transfer between temperatures and at the end of the experiment, 10 flies of each sex from each treatment were assayed for HCF. The mean HCF at one day of age for each treatment was subtracted from the HCF value at four days of age to provide an estimate of accumulation between one and four days of age. Regressions of accumulated HCF on temperature were calculated for each sex.

RESULTS

HCF at one day of age differed between batches for males ($F_{1,18} = 4.76$, $p = 0.04$) but did not differ between batches for females at this age ($F_{1,17} = 0.71$, $p = 0.41$). The regression of HCF, accumulated between 1 and 4 days of age, on temperature was highly significant for both sexes ($p = 0.001$, Appendix C: Graphic). From these regressions, the thresholds of pteridine accumulation were estimated as 7.8C for males and 2.9C for females.

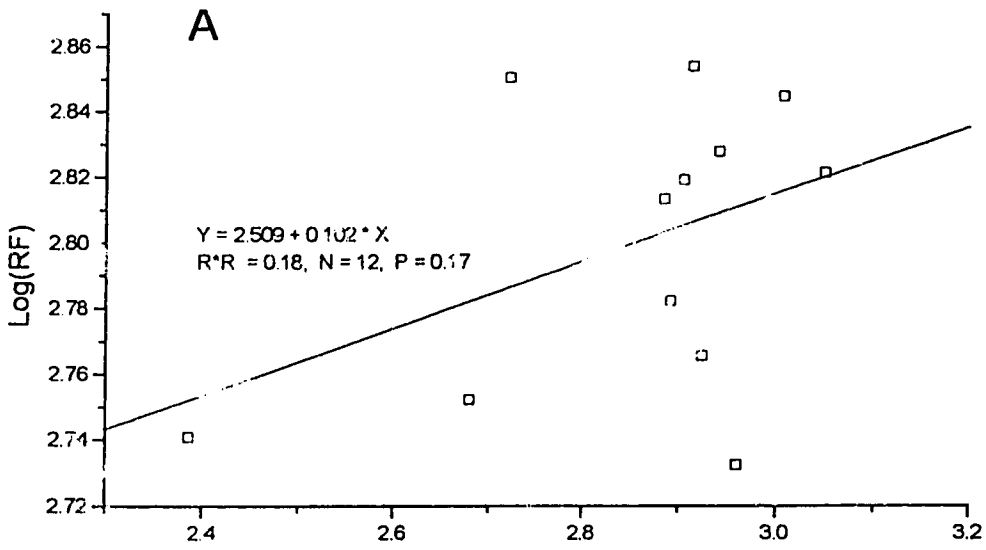
DISCUSSION

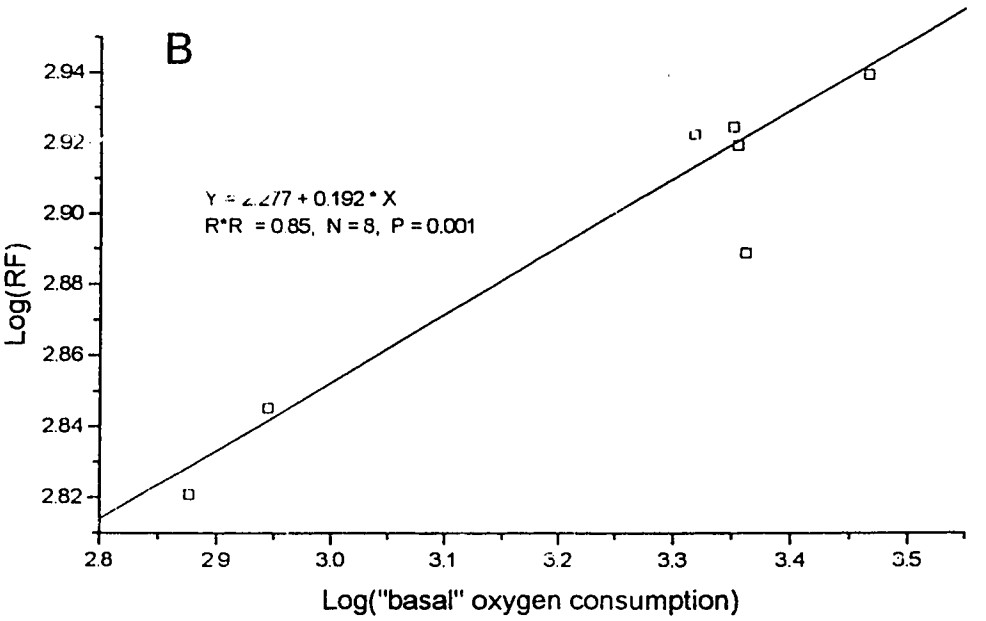
The reason(s) for the difference in HCF between males at one day of age from different egg batches is unknown, as is(are) the reason(s) for the difference between sexes in this respect. Difference in age at eclosion was not controlled in this experiment and may have contributed to the observed difference. The estimate of 7.8C as the threshold temperature for pteridine accumulation in males is similar to estimates reported for male *Stomoxys calcitrans* (7.50C, Lehane *et al.* 1986; 6.5C, Lysyk and Krafsur in press). However the estimate of 2.9C for females is much lower than reported estimates of 8.76C and 6.5C for *S. calcitrans*. Although it is possible that female house flies of this strain have an unusually low pteridine accumulation threshold, before seeking explanations for this it would be useful to confirm this result.



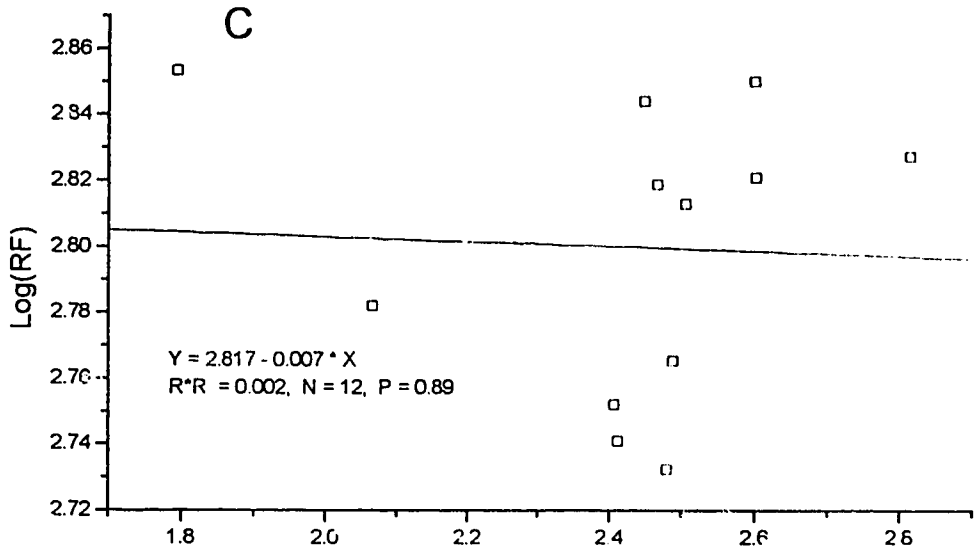
Appendix C; Graphic. Regression of HCF accumulated between 1 and 4 days of age on temperature of male and female house flies. Plotted values are HCF at age four days less an estimate of HCF at one day of age.

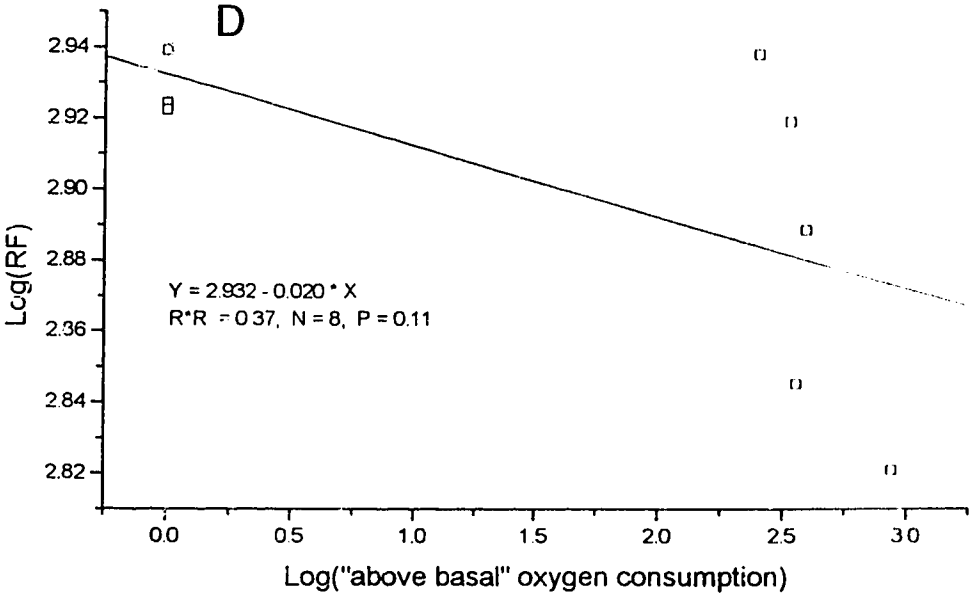
APPENDIX D





Relationship of log(RF) to log("basal" oxygen consumption) and to log("above basal" oxygen consumption) for male house flies maintained at 17C and 27C. Oxygen consumption is estimated from 30 min measurements at 4 hour intervals between 24 an 96 hours of age. A) "basal" oxygen consumption 17C B) "basal" oxygen consumption 27C C) "above basal" oxygen consumption 17C D) "above basal" oxygen consumption 27C.


C
2.86
2.84
2.82
2.80
2.78
2.76
2.74
2.72
Log(RF)
Y = 2.817 - 0.007 * X
R*R = 0.002, N = 12, P = 0.89
1.8
2.0
2.2
2.4
2.6
2.8


D
2.94
2.92
2.90
2.88
2.86
2.84
2.82
Log(RF)
Y = 2.932 - 0.020 * X
R*R = 0.37, N = 8, P = 0.11
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Log("above basal" oxygen consumption)