INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction..

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

University of Alberta

Maternal Age Effects on Offspring Quality in House Flies

By

Grant Stewart McIntyre

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**

in

Systematics and Evolution

Department of **Biological Sciences**

Edmonton, Alberta

Fall 2000

University of Alberta

Library Release Form

Name of Author: Grant Stewart McIntyre

Title of Thesis: Maternal Age Effects on Offspring Quality in House Flies

Degree: Doctor of Philosophy

Year this Degree Granted: Fall 2000

14524-85 Ave.
Edmonton, AB
Canada
T5R 3Z4

Date: Sept 29/00

Abstract

Maternal age effects are usually considered to be non-adaptive declines in maternal reproductive performance. However, a review of the literature reveals that, except for a widespread decline in clutch size, insect maternal age effects are quite variable around the generally accepted trends of declining egg size, embryonic development rates, hatch rate, and offspring fecundity. This variability suggests that maternal age effects are adaptive more often than is generally thought. To better understand this phenomenon I examined effects of maternal age on egg number, size, hatch rate, and contents and also on larval competitiveness and adult fluctuating asymmetry in house flies (*Musca domestica* L.).

Although egg size, number and hatch rate decreased with increasing maternal age, egg energy content was not related to maternal age or to egg size. In experiments in which early(-born) and late(-born) offspring, from genetically marked strains, competed against each other late larvae were more competitive, at high density, than were early larvae. In most cases, maternal age was positively associated with larval viability, mass, and development rate. In a longitudinal study, maternal age, larval density, and sex had effects on house fly wing fluctuating asymmetry (FA, a putative estimator of organismal quality) but the response of size FA and shape FA differed. Therefore increasing maternal age appears to decreases developmental stability, at least in some traits, but FA may not be a useful indicator of individual quality in this system. By examining a subset of flies from the competition experiments, FA differences were used to develop further an adaptive hypothesis for maternal age effects in house flies.

House flies may exhibit two alternate larval strategies, each successful under certain conditions. Under low larval densities, larvae with good medium conditioning ability and lower activity will be more successful, but at high larval densities, efficient scramble competitors, with high activity but poor medium conditioning ability, will be more successful. If old house fly females typically lay eggs into higher density situations and produce offspring with lower activity and higher medium-conditioning ability than predicted from their genetic make-up, then this is an adaptive maternal age effect. A program for testing this hypothesis is presented, as are implications of this study for further investigation of maternal age effects.

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Maternal Age Effects on Offspring Quality in House Flies** submitted by **Grant Stewart McIntyre** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Evolution and Systematics**.

Dr. R. H. Gooding

Dr. A. R. Palmer

Dr. B. S. Heming

Dr. M. A. Price

Dr. C. W. Fox

Date: Sept 27/00

To those who never have opportunity to follow their interests

I am indebted to: the kind and insightful supervision of Ron Gooding; helpful criticism from Rich Palmer, Bruce Heming, Chuck Fox, and Mick Price; interesting discussions and advice from Christian Klingenberg and numerous others; and to the incredible depths of support from my beloved wife, Ruth McIntyre.

Table of Contents

List of Tables

List of Figures

Chapter 1: Are insect maternal age effects adaptive?

Introduction

Maternal effects, i.e., influences of maternal phenotype on offspring phenotype, have been viewed historically as sources of unwanted variation in population genetics studies, breeding programs, and selection experiments (Falconer 1981). This is because maternal effects 1) conflate genetic and environmental variance, 2) affect the degree of parent-offspring similarity, and 3) can affect the outcome of selection in unexpected ways (Falconer 1981; Kirkpatrick and Lande 1989). However, for precisely these reasons, maternal effects are of evolutionary interest. Given that individual phenotypes are the units upon which natural selection acts (Futuyma 1998), then the non-genetic contribution of parents to the phenotype of their offspring merits study. This is especially so if some maternal effects are adaptive, i.e., they are responses to predictive environmental cues that improve offspring fitness. There have been numerous demonstrations of the adaptive nature of some maternal effects, but although maternal age effects are often strong there has been little attempt to demonstrate that they are adaptive. Rather, they are assumed to be the result of age-related deterioration.

My objective in this thesis is to consider whether maternal age effects on offspring quality might be adaptive in more cases than is usually thought, by testing assumptions and patterns in a detailed case study of the house fly (*Musca domestica* L.). In this introduction I discuss the nature of maternal effects, particularly in insects, and how they can affect the fitness of parents and offspring. I focus on existing explanations for maternal age effects on offspring quality in insects, review the patterns found among empirical observations of maternal age effects in insects, and test the standard

explanations against these patterns. I then consider some of the questions that the mismatch between observation and explanation raise and introduce my approach to the study of maternal age effects on offspring quality in house flies.

The nature of maternal effects

Definition and evolutionary importance

Simple models of inheritance or phenotype determination include only genetic and environmental effects (and their interaction) for the organism(s) under study: phenotype is the response of genotype to environment (e.g., Futuyma 1998). But in most organisms, parents, especially mothers, contribute more than just genetic material to their offspring. They usually contribute nutrients, cellular components and signaling molecules necessary for embryogenesis and sometimes for later development. They often choose the environment in which their offspring will develop and live. They may provide post-natal care and 'education'. All of these contributions are part of the maternal phenotype and can directly affect the offspring phenotype, independent of (but sometimes interacting with) nuclear genes transmitted to the offspring. It is the maternal phenotype, i.e., the interaction of maternal genotype and maternal environment, that determines these contributions to offspring fitness. Therefore, maternal effects are sometimes defined as an effect of the mother's phenotype on the phenotype of the offspring. A more inclusive term is "inherited environmental effects", which includes all aspects of an offspring's phenotype due to the parents, except those from the nuclear genes transmitted to the offspring. For a more extensive discussion of the nature, kinds, and definitions of maternal effects see Rossiter (1996) and references therein.

Maternal, or inherited environmental, effects have important evolutionary consequences that are described in several quantitative genetic models (Riska 1991; Cheverud and Moore 1994). Theoretical and empirical work has shown that maternal effects can influence the outcome of selection in several ways. They can reinforce, reverse, or induce cycles in the selected traits and because of maternal effects the effects of selection can continue after the selective force has ceased to act (Kirkpatrick and Lande 1989). Maternal effects extend also to non-parental kin (Cheverud and Moore 1994; Wolf et al. 1998), are important in cyclical dynamics of some populations (Ginzburg and Taneyhill 1994, 1995; Rossiter 1994), and influence the evolution of sexually selected traits (Wolf et al. 1997, 1999).

Inherited environmental effects include paternal and maternal effects, both of which can be important. Paternal effects are less studied and are probably smaller than maternal effects in most cases (Mousseau and Dingle 1991a,b). In insects, egg materials come disproportionately from the mother, although non-genetic male contributions can come through nuptial feeding, including food gifts, spermatophores and accessory fluids (reviewed in Vahed 1998). Although there are some well documented cases of non-age-related paternal effects on insect offspring quality (Fox et al. 1995a, 1995b; Crill et al. 1996; Weigensberg et al. 1998) there are many other examples where there is no such effect (e.g., Wedell 1993; Oberhauser 1997). Females of some insect species prefer to mate with males of a particular age class; usually older males are preferred (e.g., Zuk 1987, 1988; Simmons 1995; Conner 1989; Simmons and Zuk 1992), but sometimes younger or intermediate aged males are preferred (Ritchie et al. 1995; Jones et al. 2000). However, few studies relate paternal age to offspring traits and the results are somewhat

contradictory (Rogers and Marti 1997; Price and Hansen 1998; Jones et al. 2000). In the following I consider only maternal age effects, while acknowledging that paternal age effects are likely to play a role in at least some cases.

Since the maternal phenotype can affect almost every aspect of offspring biology, the discussion of maternal effects must be limited to those induced by maternal age. Since maternal age effects differ widely among taxa, depending upon modes of development, post-maturity growth patterns, parental behaviours etc., I have further limited my study to insect maternal age effects. I consider primarily metric variation in offspring fitness traits induced by maternal age, rather than all or nothing developmental switches between types of offspring, because it is such metric variation that is usually considered non-adaptive. This means I have excluded maternal age effects on diapause incidence, perhaps the most well studied insect maternal age effect (reviewed in Mousseau and Dingle 1991a,b; Denlinger 1998).

Adaptive or non-adaptive nature of maternal age effects

The study of maternal effects has recently shifted to demonstrations that they are, or can be, adaptive and that they can act as a form of transgenerational phenotypic plasticity (Mousseau and Fox 1998a, b). If a female responds to changes in an environmental parameter, which is predictive of future conditions, by producing offspring that are better suited to the predicted conditions, this is an adaptive maternal effect (Mousseau and Fox 1998a, b). Minimum conditions for the demonstration of adaptive maternal effects are that the environmental cue be a reliable predictor of future conditions, the cue consistently elicits a specific change in the kind of offspring

produced, and the 'changed' offspring outperform 'normal' offspring in the predicted environment (Mousseau and Fox 1998a, b).

Several published examples of other adaptive maternal effects exist (Mousseau and Fox 1998a,b), but most maternal age effects are considered non-adaptive because they are thought to result from physiological constraints and deterioration (e.g., inability to adequately provision eggs at advanced ages [Murphy et al. 1983]). Support for this comes from the frequently observed decrease in reproductive output of older females (Mousseau and Dingle 1991a, b) and from the consequences of life-history models (e.g., Roff 1992). Variation in offspring quality (= maternal investment) has been modeled extensively within the framework of clutch size/egg size models. These models were reviewed elsewhere (Congdon and Gibbons 1990; Wilson and Lessells 1994) and I will limit this to a brief outline of the models' assumptions and a consideration of how these models impact thinking on maternal age effects.

Most clutch size/egg size models are based on a graphical demonstration by Smith and Fretwell (1974) that there is a population-wide optimum investment per offspring, assuming a positive maternal investment/offspring fitness relation and an even distribution of investment between offspring from a limited resource pool. Egg or offspring size is the usual surrogate for maternal investment in empirical studies and in many theoretical treatments as well (a logical recourse since maternal investment is difficult to define or measure precisely and egg size is often positively related to offspring fitness [reviewed in Azevedo et al. 1997]). The primary assumptions of uniform, optimal egg size within clutches (or females) and positive egg size - offspring fitness relations have several extensions and corollaries, including the existence of an egg

size/number trade-off. Assumptions of these models receive detailed consideration elsewhere [Wilson and Lessells 1994; Bernardo 1996].

Decline in resources available for egg production has been invoked to explain age-related declines in egg size and number. An adaptive explanation, from the viewpoint of maternal fitness, has been supplied in cases where females have a resource acquisition phase, followed by an egg production phase (Begon and Parker 1986). When non-oviposition related mortality is high, and egg size is positively related to offspring fitness, then maternal fitness is maximized by the early production of large eggs followed later by smaller eggs. This strategy is adaptive because it improves maternal fitness, but it is a maladaptive maternal effect, from the perspective of the offspring, because later offspring are smaller and therefore of lower fitness, given the model's assumptions. Even though this model does not apply directly to species with iteroparous adults that accumulate egg resources both before and during the oviposition phase, it has been used as general support for the view that maternal age effects on the offspring are maladaptive results of declining resources (Mousseau and Dingle 1991a, b).

However adaptive maternal age effects do exist in insects. For example, the maternal age-related increase in the incidence of diapause in many seasonal populations corresponds well with the increasing need for offspring to enter diapause as the season advances (Mousseau and Dingle 1991a,b; Denlinger 1998). In *Stator limbatus* (Coleoptera: Bruchidae), adaptive egg size plasticity indicates differences in maternal age effects on egg size, in response to quality of oviposition sites (Fox et al. 1997). Increased development rates in late offspring of temperate *Oncopeltus fasciatus* (Heteroptera: Lygaeidae), may be an adaptation to reach maturity and lay diapausing eggs prior to

winter (Phelan and Frumhoff 1991). Such examples suggest that adaptive maternal age effects exist in some species that experience consistent environmental changes during the life of individuals, or in species in which females can, over time, respond to the specific environment in which they live, by altering the kind of offspring they produce. For example, if, due to seasonal changes or resource depletion, density consistently becomes challenging to their offspring as females age, maternal effects could evolve to avoid or otherwise cope with higher larval density.

Given the existence of adaptive maternal age effects, then either the assumptions or the superstructure of the models suggesting maternal age effects are maladaptive for the offspring are incorrect, at least some of the time. A uniform optimal egg size is not observed for most populations and some workers argue that the notion of optimal egg size should be discarded since it depends on environment and maternal phenotype (e.g., Weatherhead et al. 1999). There is, on the other hand, substantial support for the assumption that egg size is positively related to offspring fitness: 63% of 137 studies reported positive associations between egg size and one or more offspring fitness component(s) (Azevedo et al. 1997). However, the absence of such correlations in 37% of the studies reveals that egg size does not fully explain offspring fitness. Failure to find positive egg size/fitness correlations is often explained away by suggesting that the fitness effects may only manifest themselves under adverse conditions (e.g., Parsons 1994; Fox 1997), but eggs can differ in more than size. Other factors sometimes contribute to fitness variation among eggs, for example, egg content does not always accurately track egg size (Williams 1994; Jaeckle 1995; Bernardo 1996). The theoretical support for the notion that maternal age effects are generally non- or maladaptive results of age related female

deterioration is considerably weakened, since there are numerous exceptions to the primary assumptions of optimal egg size within clutches (or females) and positive egg size - offspring fitness used to construct egg size/maternal investment models.

A positive maternal age effect that is accompanied by declining egg size implies that some other change in the eggs/offspring improves fitness. Diapause is one example: in those situations in which fitness of non-diapausing insects is zero, diapausing insects will have the advantage, regardless of egg size. Similarly if behaviour of offspring differs, then late offspring may not have such a disadvantage as it seems from consideration of egg size alone. In addition, if small eggs have equivalent energy content then they may produce smaller hatchlings with higher energy content and, in times of environmental challenge or nutrient stress, these hatchlings may outlast early offspring, with lower reserves. Future research should consider cases of variable maternal age effects, within and between species, and search for adaptive explanations; this has been done only occasionally (e.g., Mousseau 1991; Phelan and Frumhoff 1991; Braby and Jones 1995; Carriere and Roff 1995; Fox et al. 1997). This approach would be a departure from earlier attempts so understand maternal age effects.

Research on maternal age effects

An early focus of research on maternal age effects was stimulated by the finding that rotifer lines propagated from old females at each generation exhibited decreasing life-spans and eventually died out (Lansing 1947, 1948). This phenomenon, the Lansing Effect, apparently also occurred in other organisms including *Drosophila* and house flies (e.g., Callahan 1962; Wattiaux 1968). However the initial rotifer experiments could not be duplicated (Meadow and Barrows 1971) and most other studies are flawed in one

manner or another (reviewed in Rose 1991). Many of these results may have been due to poor culture techniques rather than maternal age effects. Little recent work has examined this aspect of maternal age effects, although maternal age can lead to decreased fecundity (see below).

More recently a series of large, well-replicated selection experiments for early and late reproduction in *Drosophila melanogaster* have provided considerable insight into the biology of aging (most recently reviewed in Rose 1999). Reproductive schedule responds well to selection, and there is a large correlated response of longevity, with late reproducing lines increasing mean life span by as much as 20% in 25 generations (Rose 1984). Unfortunately for the study of maternal age there has been little study of the differences in maternal age effects between long- and short-lived lines.

Most information about insect maternal age effects is a byproduct of investigation of other factors affecting reproduction, such as maternal size, seasonal effects, quality control in mass rearing programs, etc. Little concerted effort has been directed to understanding maternal age effects in and of themselves. To remedy this, I conducted an extensive review of the literature to identify patterns of insect maternal age effects, which I then discuss in relation to the assumption that maternal age effects are non-adaptive.

Review of insect maternal age effects:

Methods

To clarify our understanding of existing patterns of maternal age effects in insects, I compiled data from 89 reports of maternal age effects. Many of these reports mention maternal age only in passing, having been intended as investigations of other sources of variation in offspring traits. Although I believe this review is a relatively

complete capture of the relevant papers, there are doubtless papers containing maternal age effect data that are not included, since investigations of other sources of offspring variation with some mention of maternal age effects do not show up in standard searches for maternal age effects. There is, however, no reason to think that the reports presented here are not a representative sample.

I compiled information on maternal age effects on egg size, development rate, survival, offspring size, offspring fecundity and maternal fecundity (Table 1.1). For development rate, survival (including adult longevity) and offspring size I specified the developmental stage reported. The effects of multiple mating and/or improved nutrition on maternal age effects are also included. In many papers, maternal age-related egg size changes were demonstrated and comparisons were then made between large and small eggs and among the resulting juveniles. Since egg size varies among females and among ages, the maternal age and other maternal sources of egg size variation were confounded and I excluded such reports from the review.

I summarized trends with counts of the number of species with negative, positive, variable, or no maternal age effects on these parameters (Table 1.2). Variable responses are divided into two groups: responses for which a reasonable explanation has been provided and variable responses that are unexplained. The latter group includes species for which the response differs among reports or among developmental stages. Several older reports describe maternal age effects that cause a decrease followed by an increase, or vice versa, in some offspring trait. These are presented as reported in the detailed review (Table 1.1). There is no straightforward way of converting those responses that involve a change in direction, of maternal age effects, to a common measure for

comparison with reports of responses that do not change direction. Therefore, the responses with direction changes are excluded from the summary table if they are the only report for a species. However, if there is another dissimilar report for the species, it is added to the count of those with unexplained variability. Species counts are divided by order and by type of metamorphosis, although there are too few apterygotes to discern any maternal age effect patterns. I compared maternal age effect patterns between groups using continuity adjusted chi-square tests. Since there were several empty cells in some of these comparisons I reduced the data to 2 x 2 tables by pooling the data into species exhibiting negative maternal age effects and species exhibiting some other effect.

Review: Results

The most unambiguous effect of maternal age is that the fecundity of females declines as they age. All 39 species for which this information was tabulated exhibit this decline. Similarly offspring fecundity declines with increasing maternal age in three species, increases in one, and is unaffected in one.

Maternal age effects on egg size differ dramatically between endo- and exopterygotes. Among 33 endopterygote species, 73% exhibit a decline in egg size, 9% show no effect, 6% show variable responses between reports, and 12% exhibited variable responses among seasonal morphs and maternal diets, or in response to maternal egg size plasticity. Among 20 exopterygote species, 30% show a decline in egg size, 15% show no response, 40% show an increase in egg size, 5% show unexplained variability in response and 10% vary (between increase and no change) in response to morph differences or to grand-maternal effects. These trends are significantly different (chi-square = 7.6, df = 1, P = 0.0058) and are also reflected at the ordinal level (Table 1.2).

Thus egg size decreases with maternal age in most endopterygotes but in half of the exopterygote species egg size increases or varies between increase and no effect.

Summaries of maternal age effects on survival and development rate are complicated by effect differences between developmental stages. Ignoring stage differences, of 24 species for which I found data, 50% have decreased survival, 25% show no effect, 8.3% show increased survival, and 16.7% show unexplained variability (Table 1.2). The pattern does not differ between endo- and exopterygotes (chi-square = 0.2, df = 1, P = 0.68), nor does it differ between life stages (chi-square = 1.04, df = 2, P = 0.59). Thus, insects generally show decreased or unchanged survival with increasing maternal age. This effect may be strongest during embryogenesis since, of the species with reports separated into developmental stages, 11 have negative effects on egg hatch, and only 4 have effects on juvenile survival (Table 1.1).

The development rate of offspring generally decreases with or is unaffected by maternal age in insects. Ignoring stage differences, 10 species exhibited decreased development rates at advanced maternal ages, 7 exhibited no effect, 1 exhibited increased development rates, 3 exhibited variable effects explained/confounded by sex or grand-maternal effects, and 4 exhibited unexplained variability (Table 1.2). This pattern does not differ substantially between endo- and exopterygotes (chi-square = 1.7, df = 1, P = 0.20). When reports separated survival from different developmental stages, two of two exopterygote species show increased embryonic development rates and both of these have positive maternal age/egg size relationships. But five of six endopterygote species show decreased embryonic development rates and four of these produce smaller eggs at later ages (chi-square = 1.6, df = 1, P = 0.21 Table 1.1). There were few reports of

juvenile development rates and for egg to adult development rates, the pattern does not differ from that of all stages pooled.

The pattern of maternal age effects on body size is much more scattered. Of 23 species, 13% show decreased body size at some stage, 26% show no change, 22% show increased size, 13% have variable effects due to morph differences or density effects and 17% exhibit unexplained variability (Table 1.2). This scatter does not differ between endo- and exopterygotes (chi-square = 0.0, df = 1, P = 1.0) and is approximately evenly distributed between measurement of juveniles, pupae and adults (Table 1.1).

In both endo- and exopterygotes improved nutrition of females, or increased contributions from males (e.g., nuptial gifts and larger or more spermatophores) sometimes reduce or eliminate the egg size decline. Of 12 species tested, 58% show amelioration of maternal age effects, 33% show no effect and 8% have a variable response (Table 1.1). Both of the exopterygotes species tested show increased, or unchanged egg size and the amelioration of maternal age effects due to improved nutrition is manifested primarily as reduced clutch size declines (Table 1.1).

There are several maternal age effects that may or may not be related to offspring performance described in the literature. Fluctuating asymmetry increases (*Drosophila melanogaster* (Diptera: Drosophilidae) Parsons, 1962), does not change (*D. melanogaster*, Wakefield et al. 1994), or decreases with increasing maternal age (*Glossina palpalis gambiensis* (Diptera: Glossinidae), McIntyre and Gooding 1998) and there are strain specific effects on sternopleural chaetae number (*D. melanogaster*, Durrant 1955). Starvation resistance decreases (*Musca domestica* (Diptera: Muscidae), Callahan 1962), as does desiccation resistance (*Eretmapodites chrysogaster* (Diptera:

Culicidae), Hylton 1967). Late *Malacosoma pluviale* (Lepidoptera: Lasiocampidae) larvae are less active (Wellington, 1965). There is variation in egg RNA and DNA content with increasing maternal age (*D. melanogaster*, Tsien and Wattiaux 1971), decreases in yolk protein content (*Hyalaphora cecropia* (Lepidoptera: Saturnidae), Tefler and Rutberg 1960; *Lymantria dispar* (Lepidoptera: Lymantriidae), Rossiter 1991) and decreases in trehalose concentration (*Aulocara elliotti* (Orthoptera: Acrididae), Quickenden and Roemhild 1969). Late female offspring have later onset of pheromone production (*Caryedon serratus* (Coleoptera: Bruchidae), Chaibou et al. 1994) and there are variable effects on recombination frequency (*D. melanogaster*, Valentin 1973). Late *Tribolium castaneum* (Coleoptera: Tenebrionidae) larvae show an increased response to selection for total merit (a combination of pupal and larval weight [Patterson et al. 1983]). In *Tenebrio molitor* (Coleoptera: Tenebrionidae), increased maternal age leads to more rapid decrease in the four of ten amino acids (Ludwig and Jones 1964). Late offspring in this species also exhibit unchanged oxygen consumption, earlier decrease in cytochrome oxidase activity, but higher combined activity of cytochrome oxidase, acid phosphatase and alkaline phosphatase than do early offspring (Ludwig et al. 1962).

Review: Discussion

The most consistent effect of increasing maternal age is a decrease in maternal fecundity (Table 1.2). This observation (coupled with declines in egg size and hatch rate observed in some insects) is the primary basis for the assumption that maternal age effects are non-adaptive results of general physical deterioration and exhaustion of resources, although this strategy can be adaptive for the mother (Begon and Parker 1986).

This explanation is only valid if the eggs differ in size only and if size fully explains offspring fitness (see pages 5-6).

Offspring fecundity also declines with increasing maternal age in most species (Table 1.1). Other maternal age effects can mediate offspring fecundity changes. For example, later offspring of the aphid *Cavariella aegopodii* (Homoptera: Aphiidae) have higher fecundity because they are larger and larger females have higher fecundity (Dixon et al. 1993). The data are insufficient to confirm or reject the Lansing Effect, which is generally discounted (see pages 8-9), but they do suggest that maternal age effects on offspring fecundity should be investigated further.

The most notable variation in maternal age effects on egg size is that endopterygotes usually show decreases in egg size with increasing maternal age, but exopterygotes often produce larger eggs at advanced ages (Table 1.2). Two explanations have been proposed for this difference (Murphy et al. 1983; Solbreck 1986). Degeneration of flight muscles in exopterygotes may free resources for egg development, or adult feeding in exopterygotes provides a continuous source of egg materials which may then become larger as clutch size decreases. Of the endopterygotes examined for egg size response to maternal age, most fly throughout adult life and produce their eggs primarily from resources acquired as larvae. That cessation of flight and muscle degeneration contribute to egg size increases is supported by comparisons of macropterous with brachy- or apterous forms: increase in egg size is only exhibited in the flighted forms (Solbreck 1986). Similarly, in viviparous aphids, alate forms exhibit an increase in progeny size over the first few days of adult life while apterate individuals show no change (Dixon and Wratten 1971; Newton and Dixon 1990). Furthermore,

muscle material contributes to reproduction, not only in exopterygotes (Kobayashi and Ishikawa 1993; Tanaka 1993), but also in endopterygotes (Stjernholm and Karlsson 2000). However, some species retain flight capabilities and exhibit increased egg size as adults (Table 1.1) so flight muscle degeneration is not a complete explanation.

Maternal nutrition is intimately linked to egg production so patterns of egg material accumulation might explain variation in maternal age effect on egg size. In insects, slightly more than half of the species tested show an amelioration of egg size decline with increased access to nutrients. Others however, are better able to maintain egg number while maternal age effects on egg size are unchanged. Further tests of this would be useful, preferably using a variety of species with different feeding and reproductive patterns, and in both stressful and benign conditions (Murphy et al. 1983; Moore and Singer 1987; Karlsson 1987).

If maternal age effects are generally maladaptive then maternal age effects should be correlated within and across life stages, unless maternal age effects disappear early during development. The correlations between maternal age effects are quite scattered (Table 1.1). One exception is that those insects with declining egg size also have declining development rates and vice versa (Table 1.1). This is consistent with the frequently observed positive correlation of egg size with development rate in insects (Azevedo et al. 1997).

This review shows that maternal age effects need not decrease in strength as the offspring develop, as is usually assumed for maternal effects (Mousseau and Dingle 1991a, b; Bernardo 1996). This assumption is made for two reasons: after hatching the role of the offspring's (non-maternal) environment increases and should reduce

differences between larvae, and maternal effects are more often found in eggs and immature stages than in adults. The strongest evidence that maternal age effects decrease with age would be identification of species in which maternal age affects eggs or early immature stages, but not late immature stages or adults. I found no such cases for survival or development rate, possibly because values for these traits are usually not reported separately for different developmental stages, obscuring stage differences that might be present. For egg size 4 species exhibited negative maternal age effects on egg size but no effect on adult or late immature size, but 3 species exhibited effects on size of eggs and of later stages. There are however more reports of maternal age effects on eggs and early immature stages than there are on late immature stages or adults. Whether this is because maternal age effects are truly more common in immature stages or because these stages are examined for such effects more often is unclear. Thus, the literature provides only weak support for the idea that maternal age effects decrease in strength with increasing progeny age.

The main conclusion derivable from this review is that, except for a widespread decline in clutch size, maternal age effects are quite variable around the generally accepted trends of declining egg size, embryonic development rates, hatch rate, and offspring fecundity. This variability, coupled with the cases of variable maternal age effects that can be explained through morph, strain, host or other differences, suggests that maternal age effects are adaptive more often than is generally thought.

Program of Study

One way to gain further insight into maternal age effects is to make a detailed comparison of offspring of young and old females from a single species at several stages

of development and at each stage, to examine appropriate fitness related traits. I have conducted such a study of house flies (*Musca domestica* L.) testing for maternal age effects on egg number, size, hatch rate, and contents and also on larval competitiveness and adult fluctuating asymmetry. I use these data to reexamine assumptions about the non-adaptive nature of maternal age effects.

House flies are excellent organisms for studies of aging and maternal age effects. They are a common cosmopolitan species that is easily maintained in the laboratory. Their life-span of 2-8 weeks varies with environmental conditions and is short enough to allow longitudinal studies of maternal age effects. The general biology, including larval and population biology, is well described (see subsequent chapters for descriptions and references). There are existing age determination methods that allow extension of laboratory work into field situations (Lehane 1985; McIntyre and Gooding 1995). Because they exploit ephemeral resource patches and exhibit seasonal increases in density, offspring of old females will generally experience different conditions than those of young females. Thus it is possible that they will show adaptive maternal age effects, although they fit the pattern of many insects assumed to have only non-adaptive maternal age effects. Below I outline those aspects of the program that are described in Chapters 2 to 5.

Maternal age effect on house fly eggs, Chapter 2

The most important assumption, in the study of patterns of maternal investment, is that egg size is a good estimator of maternal investment and egg quality. In a recent review, egg size was found to be unrelated to offspring fitness in 37% of cases (Azevedo et al. 1997). In many organisms there is significant variation in yolk contents at the population,

individual, and intra-clutch levels, and it has been suggested that yolk provisioning may be a more accurate measure of maternal investment than egg size (reviews in Williams 1994; Jaeckle 1995; Bernardo 1996). Intraspecific variation in insect egg content has only rarely been related to egg quality. In the grasshopper, *Aulocara elliotti*, egg mortality increases with maternal age and trehalose concentration decreases (Quickenden and Roemhild 1969). In the gypsy moth, *Lymantria dispar*, large eggs contain proportionately more yolk protein than do small eggs and large eggs, and the resulting larvae, could be more resistant to environmental stress than are small eggs (Capinera et al. 1977). However, survival of starved gypsy moth hatchlings is correlated with levels of two yolk proteins (vitellin and glycine rich protein) but not with egg weight (Diss et al. 1996). Overall, these studies indicate that egg content may be more important than egg size in insects.

The first step in my study of maternal age effects on offspring quality in house flies was to examine the relationship of egg size and egg content. If egg size is not closely related to egg content then the negative effects of maternal age on offspring quality is called into question.

Maternal age effect on house fly larvae, Chapter 3

When larval competition is important to success, as it is for house flies (Sullivan and Sokal 1963; Bryant and Sokal 1967, 1968; Kence and Jdeidi 1997), one critical test of maternal age effects on larvae is to compare competitive ability of offspring from different maternal age classes. Maternal age related differences between immature individuals are usually measured as performance or fitness components of isolated groups or individuals. There have been few studies of maternal age effects in which offspring of young and old females interact. Therefore the intraspecific competitive fitness of

offspring of different maternal ages is essentially unknown in insects, in spite of the considerable body of research seeming to address this. I tested early and late offspring from genetically marked strains against each other to determine if there are maternal age effects on competitive ability in house flies and used the results to develop an adaptive hypothesis for maternal age effects on house fly offspring.

Maternal age effect on house fly adults, Chapters 4 and 5

The ultimate test that maternal age effects impact surviving adults is that their fitness differs. Testing this proved to be beyond the scope of this study. Instead I used FA as an estimator of fitness/quality differences between offspring of young and old house flies. Fluctuating asymmetry (FA, small random departures from bilateral asymmetry) has been proposed as a sensitive indicator of developmental stability (DS) and organismal quality that may reveal quality differences between groups of organisms, before such differences are detectable using conventional fitness measures (Leary and Allendorf 1989; Graham et al. 1993). Altered FA levels may thus provide indications of maternal age effects on adult offspring quality. Empirical studies provide equivocal evidence for maternal age effects on FA in insects. For example, FA of sternopleural bristle number increased with maternal age in one *Drosophila melanogaster* population (Parsons 1962). However, in another population, maternal age did not affect FA of sternopleural bristle number, wing length or wing area (Wakefield et al. 1994). In tsetse (*Glossina palpalis gambiensis*) wing FA decreased slightly with maternal age (McIntyre and Gooding 1998; Klingenberg and McIntyre 1998). These divergent results suggest that maternal age effects on FA vary among species and populations. This variation may be related to the adaptive nature of some maternal age effects since it seems to depend upon the life

history, particularly the relative contribution of late-born offspring to female fitness (McIntyre and Gooding 1998).

Here I used Procrustes techniques (Klingenberg and McIntyre 1998) to determine the effects of maternal age and larval density upon house fly wing FA, in a longitudinal study and then compared the responses of shape FA and size FA to these stresses. I also compared FA among a subset of survivors of the competition experiments and used FA to gain additional insight into the nature of larval competition in this species.

An adaptive hypothesis for house fly maternal age effects, Chapter 6

By combining the results of these investigations into maternal age effects on eggs, larvae, and adults, I was able to develop a testable hypothesis about the nature of maternal age effects in house flies. A program for testing this hypothesis is presented, as are proposals for how similar studies in other species should be conducted.

Table 1.1. Maternal age effects on fecundity, egg size, survival, development rate, and body size in insects. Unless marked otherwise fecundity is that of the mother, development rate and survival are from egg to adult, and body size is that of the adult progeny. Adult longevity is included under survival, although it is not exactly equivalent to measures of egg and juvenile survival. Mediating factors include the effects of diet and mating on the extent of maternal age effects on offspring characters or maternal fecundity. Symbols: a: egg to adult; al: adult longevity; as: adult size; D: improved diet; e: egg; h: hatchling; j: juvenile (larvae or nymph); p: pupae; m: mother; M: multiple matings; o: offspring; +: increases with maternal age (or increases maternal age related declines for D and M); -: decreases with maternal age (or reduces maternal age related declines for D and M); x: no maternal age effect detected; ∪: decrease followed by increase; ∩: increase followed by decrease.

Taxon	Fecundity	egg size	survival	development rate	body size	mediating factors	References
Apterygota							
Collembola							
Orchesella cincta (Entomobryidae)				- (1),x (2)			1-2
Exopterygota							
Heteroptera							
Horvathiolus gibbicollis (Lygaeidae)	-	+,x [a]					3
Lygaeus equestris (Lygaeidae)	-	x					4
Oncopeltus cingulifer (Lygaeidae)		-	x	x	x		5
Oncopeltus fasciatus (Lygaeidae)		- (5), ∩ (6)	- (5), ∩ (e,6)	+ (5), ∪ (e,6)	+ ♂, x ♀		5-7
Graphosoma lineatum (Pentatomidae)		-			x (j)		7
Nezara viridula (Pentatomidae)	- (m, o)	+	- (e, j, al)	x (j)			8-9
Podisus maculiventris (Pentatomidae)		+,x [b]	x (e, j)	+ (e), - (j)	- (12), x (11)	D: -	10-11
Podisus nigrispinus (Pentatomidae)	-	+	x (e, j)	x	-		12
Dysdercus spp. (Pyrrhocoridae)	-	+					13
Pyhhrocoris apterus (Pyrrhocoridae)	-	-					14

Taxon	Fecundity	egg size	survival	development rate	body size	mediating factors	References
Homoptera							
Acyrthosiphon pisum (Homoptera)	-				+ (j, as)		15
Aphis fabae (Homoptera)	-				+, x (j, as)a		16
Cavariella aegopodii (Homoptera)	-(m), +(o)				+ (j, as)		17
Sitobion avenae (Homoptera)	-				+, x (j, as)a		18
Orthoptera							
Arphia sulphurea (Acrididae)		+					19
Aulocara elliotti (Acrididae)	-	+		e: + (21), ∩ (22)			20-22
Chorthippus brunneus (Acrididae)	-	+				D: -, M: -	23
Chortophaga viridifasciata (Acrididae)		+					19
Dissosteira carolina (Acrididae)		+					19
Locusta migratoria (Acrididae)	-		- (al)	-	+ (h)		24
Schistocerca gregaria (Acrididae			- (e)				25
Gryllus firmus (Gryllidae)		x	x (e)				26
Gryllus veletis (Gryllidae)		x	x (e)				26
Gryllus pennsylvanicus (Gryllidae)		-	+ (e)				26
Scapteriscus acletus (Gryllotalpidae)	-	-	- (e)				27
Scapteriscus vicinus (Gryllotalpidae)	-	-	- (e)				27
Endopterygota							
Coleoptera							

Taxon	Fecundity	egg size	survival	development rate	body size	mediating factors	References
Callosobruchus chinensis (Bruchidae)	- (m, o)	-	- (e, j) - (a), e: - (30), x	∪ (e), - (a)			28
Callosobruchus maculatus (Bruchidae)	-	-	(31)	- (e, a)	-	D: -, M: -	29-31
Caryedon serratus (Bruchidae)				-			32
Stator limbatus (Bruchidae)		-,x,+ [c]					33
Sitophilus granarius (Curculionidae)	-			∪			34-35
Luciola cruciata (Lampyridae)		-	x (e) - (e, j); al: - (39), x				36
Tenebrio molitor (Tenebrionidae)		x	(40)	+			37-42
Tenebrio obscurus (Tenebrionidae)				x	+		43
Tribolium confusum (Tenebrionidae)			- (j), + al	- (e)			44
Tribolium castaneum (Tenebrionidae)					+ (j)		45
Zophobas atratus (Tenebrionidae)	- (o)		- (a, al)	- (j)	-,+ (j) [d]		46
Diptera							
Eretmapodites chrysogaster (Culicidae)			- (e)				47
Drosophila melanogaster (Drosophilidae)	-	-/x [e] (48,49), - (50), ∩ (53)	- (e)	∩ (e)	∪ (52), x (56)		48-57
Glossina morsitans morsitans (Glossinidae)				p: +,x [e,f]	-,x [e] (♂)		58
Glossina palpalis gambiensis (Glossinidae)				p: x	x (♂)		58
Glossina palpalis palpalis (Glossinidae)				p: +,x [f]	x (♂)		58
Musca domestica (Muscidae)			- (al)	x			59
Scathophaga stercoraria (Scathophagidae)		-		-		D: -	60
Lepidoptera							

Taxon	Fecundity	egg size	survival	development rate	body size	mediating factors	References
Tyria jacobiae (Arctiidae)	-	-	- (c)		x (j)		61
Danaus plexippus (Danaidae)	-	-				M: x	62-63
Epirrita autumnata (Geometridae)	x (o)	-		- (c)	x (p)		64
Malacosoma pluviale (Lasiocampidae)*		-		- (j)			65
Jalmenus evagoras (Lycaenidae)	-	-				D: x	66
Lymantria dispar (Lymantriidae)*		-		- (c, j)	-		67-68
Panolis flammea (Noctuidae)	-	-					69
Euphydryas editha (Nymphalidae)	-	- (70), x (71)				D: - (70), x (71)	70-71
Euploea core corinna (Nymphalidae)	-	x, +[g]				D: -	72
Hipparchia semele (Nymphalidae)	-	-					73
Speyeria mormonia (Nymphalidae)	-	-					74
Papilio machaon (Papilionidae)		-					75
Pieris rapae (Pieridae)	-	-				M: -	76-78
Chilo partellus (Pyralidae)	-	x					79
Parapediasia teterrella (Pyralidae)	-	x					80
Hyalaphora cecropia (Saturnidae)*		-					81
Coenonympha pamphilus (Satyridae)	-	-		x (c)			82
Lasiommata maera (Satyridae)	-	-					75
Lasiommata megara (Satyridae)	-	-					75, 83-84
Lasiommata petropolitana (Satyridae)	-	-					75

Taxon	Fecundity	egg size	survival	development rate	body size	mediating factors	References
Lopinga achine (Satyridae)		-					75
Mycalesis perseus (Satyridae)	-	-, +*					85
Mycalesis sirius (Satyridae)	-	-				D: x	85
Mycalesis terminus (Satyridae)	-	-, x*				D: -	85
Pararge aegeria (Satyridae)	-	-	- (c)			M: x	75, 86-87
Choristoneura fumiferana (Tortricidae)	-	-	+	- (c)			88-89

Notes: varies with a) morph; b) grand-maternal effects; c) oviposition host; d) density; e) strain; f) sex; g) diet

* eggs are produced sequentially during pupal period; maternal age refers to the age of the pupa

References: 1) Janssen et al 1988; 2) Stam et al 1998; 3) Solbreck 1986; 4) Solbreck et al 1989; 5) Phelan and Frumhoff 1991; 6) Richards and Kolderie 1957; 7) Larsson 1989; 8) Kiritani and Kimura 1967; 9) McLain and Mallard 1991; 10) Legaspi and O'Neil 1994; 11) Mohaghegh et al 1998a; 12) Mohaghegh et al 1998b; 13) Kasule 1991; 14) Honek 1992; 15) Murdie 1969; 16) Dixon and Wratten 1971; 17) Dixon et al 1993; 18) Newton and Dixon 1990; 19) Landa 1992; 20) Van Horn 1966; 21) Quickenden and Roemhild 1969; 22) Visscher 1971; 23) Butlin et al 1987; 24) Cassier 1967; 25) Injeyan et al 1981; 26) Carriere and Roff 1995; 27) Forrest 1986; 28) Hussain 1994; 29) Wasserman and Asami 1985; 30) Fox 1993; 31) Fox and Dingle 1994; 32) Chaibou et al 1994; 33) Fox et al 1997; 34) Howe 1967; 35) Howe and Hole 1967; 36) Yuma 1984; 37) Ludwig 1956; 38) Tracey 1958; 39) Ludwig and Fiore 1960; 40) Ludwig and Fiore 1961; 41) Ludwig et al 1962; 42) Ludwig and Jones 1964; 43) Fiore 1960; 44) Schneider 1941; 45) Patterson et al 1983; 46) Tschinkel 1993; 47) Hylton 1967; 48) Warren 1924; 49) David 1959; 50) Parsons 1962; 51) Delcour 1968; 52) Delcour and Heuts 1968; 53) Delcour 1969; 54) Tsien and Wattiaux 1971; 55) Valentin 1973; 56) Wakefield et al 1994; 57) Crill et al 1996; 58) McIntyre and Gooding 1998; 59) Callahan 1962; 60) Jann and Ward 1999; 61) Richards and Myers 1980; 62) Svard and Wiklund 1988; 63) Oberhauser 1997; 64) Ruohomaki et al 1993; 65) Wellington 1965; 66) Hill and Pierce 1989; 67) Leonard 1970; 68) Rossiter 1991; 69) Leather and Burnand 1989; 70) Murphy et al 1983; 71) Moore and Singer 1987; 72) Hill 1989; 73) Garcia-Barros 1992; 74) Boggs 1986; 75) Karlsson and Wiklund 1985; 76) Jones et al 1982; 77) Kimura and Tsubaki 1985; 78) Watanabe and Ando 1994 ; 79) Berger 1989; 80) Marshall 1990; 81) Tefler and Rutberg 1960; 82) Wickman and Karlsson 1987; 83) Wiklund and Karlsson 1984; 84) Karlsson and Wiklund 1984; 85) Braby and Jones 1995; 86) Wiklund and Persson 1983; 87) Karlsson 1987; 88) Harvey 1977; 89) Campbell 1962.

Table 1.2. Numbers of apterygote, exopterygote, and endopterygote insect species exhibiting negative (-), absence of (x), positive (+), or variable - and unexplained (V_r) or explained by some measured factor - (V_{ex}) maternal age effects on egg size, development rate, survival, body size, and offspring fecundity. Maternal fecundity is also included although it is not a maternal effect *per se*. Totals for apterygote, exopterygote, and endopterygote are further divided into totals for insect orders. Full details and references in Table 1.1.

Taxon	Egg size					Development rate					Survival					Body Size					Fecundity (offspring)					Fecundity (mother)				
	-	x	+	V_{ex}	V_r	-	x	+	V_{ex}	V_r	-	x	+	V_{ex}	V_r	-	x	+	V_{ex}	V_r	-	x	+	V_{ex}	V_r	-	x	+	V_{ex}	V_r
Apterygota (Collembola)										1																			
Exopterygota	6	3	8	2	1	1	3		1	2	5	5	1		1	1	2	3	2	2	2					15				
Heteroptera	3	1	3	2	1		3		1	1	1	3			1	1	2			2	1					6				
Homoptera																		2	2		1					4				
Orthoptera	3	2	5			1				1	4	2	1					1								5				
Endopterygota	24	3		4	2	9	4	3		1	7	1	1		3	2	4	2	1	2	2	1				24				
Coleoptera	3	1		1		4	1	1		1	2	1			3	1		2	1		2					3				
Diptera	1				1	1	2	2			3						2			2						1				
Lepidoptera	20	2		3	1	4	1				2		1			1	2					1				20				
Total	30	6	8	6	3	10	7	3	1	3	12	6	2		4	3	6	5	3	4	4	1				39				

References:

Azevedo, R.B.R., French, V., and Partridge, L. 1997. Life-history consequences of egg size in *Drosophila melanogaster*. Am. Nat. **150**: 250-282.

Begon, M. and Parker, G. A. 1986. Should egg size and clutch size decrease with age?. Oikos **47**: 293-302.

Berger, A. 1989. Egg weight, batch size and fecundity of the spotted stalk borer, *Chilo partellus* in relation to weight of females and time of oviposition. Entomol. Exp. Eppl. **50**: 199-207.

Bernardo, J. 1996. The particular maternal effect of propagule size, expecially egg size: patterns, models, quality of evidence and interpretations. Amer. Zool. **36**: 216-236.

Boggs, C. L. 1986. Reproductive strategies of female butterflies: variation and constraints on fecundity. Ecol. Entomol. **11**: 7-15.

Braby, M. F. and Jones, R. E. 1995. Reproductive patterns and resource allocation in tropical butterflies: influence of adult diet and seazonal phenotype on fecundity, longevity and egg size. Oikos **72**: 189-204.

Bryant, E. H. and Sokal, R. R. 1967. The fate of immature housefly populations at low and high densities. Res. Popul. Ecol., **9**, 19-44.

Bryant, E. H. and Sokal, R. R. 1968. Genetic differences affecting the fates of immature housefly populations at two densities. Res. Popul. Ecol., **10**, 140-170.

Butlin, R. K., Woodhatch, C. W. and Hewitt, G. M. 1987. Male spermatophore investment increases female fecundity in a grasshopper. Evolution **41**: 221-225.

Callahan, R. F. 1962. Effects of parental age on the life cycle of the house fly, *Musca domestica* Linnaeus (Diptera: Muscidae). J. N. Y. Entomol. Soc. **70**: 150-158.

Campbell, I. M. 1962. Reproductive capacity in the genus *Choristoneura* Led. (Lepidoptera: Tortricidae). I. Quantitative inheritance and genes as controllers of rates. Can. J. Genet. Cytol. **4**: 272-288.

Capinera, J. L., Barbosa, P. and Hagedorn, H. H. 1977. Yolk and yolk depletion of gyspy moth eggs: implications for population quality. Ann. Ent. Soc. Am. **70**: 40-42.

Carriere, Y. and Roff D. A. 1995. The evolution of offspring size and number: a test of the Smith-Fretwell model in three species of crickets. Oecologia **102**: 389-396.

Cassier, P. 1967. Influence du poids a la naissance des larves nouveau-nees sur le determinisme des caracteres phasaires du Criquet migrateur, *Locusta migratoria*

migratorioides (R. et F.), phase *gregaria*.. C. R. Acad. Sc. **264**: 1501-1503.

Chaibou, M., Pouzat, J. and Biemont, J. C. 1994. Variations in sex pheromone production in the female progeny of *Caryedon serratus* from Niger as a function of maternal age (laying rank) and of the duration of development. J. Afri. Zool. **108**:267-278.

Cheverud, J.M. and Moore, A.J. 1994. Quantitative genetcs and the role of the environment provided by relatives in behavioral evolution. In: Boake, C. R. B. (ed). Quantitative Genetic Studies of Behavioral Evolution, pp. 67-100. Univ. Chicago Press, Chicago.

Congdon, J.D., and Gibbons, J. W., 1990. Turtle eggs: their ecology and evolution. In: Gibbons, J.W. (ed). Life History and Ecology of the Slider Turtle, pp. 109-123. Smithsonian Institution Press, Washington D.C.

Conner, J. 1989. Older males have higher insemination success in a beetle. Anim. Behav. **38**: 503-509.

Crill, W. D., Huey, R. B. and Gilchrist, G. W. 1996. Within- and between-generation effects of temperature on the morphology and physiology of *Drosophila melanogaster*. Evolution **50**: 1205-1218.

David, J. 1959. Influence de l'age de la femell sur les dimensions des oeufs de *Drosophila melanogaster*. C. R. Acad. Sc. **249**: 1145-1147.

Delcour, J. 1968. Cell size and cell number in the wing of *Drosophila melanogaster* as related to parental ageing. Exp. Gerontol. **3**:247-255.

Delcour, J. 1969. Influence de l'age parental sur la dimension des oeufs, la duree de developpement, et la taille thoracique des descendants, chez *Drosophila melanogaster*. J. Insect Physiol. **15**:1999-2011.

Delcour, J. and Heuts M. J. 1968. Cyclic variations in wing size related to parental ageing in *Drosophila melanogaster*. Exp. Gerontol. **3**:45-53.

Denlinger, D. L. 1998. Maternal control of fly diapause. In: Mousseau, T. A. and Fox, C. W. (eds) Maternal Effects as Adaptations, pp. 275-287. Oxford Univ. Press, New York.

Diss, A. L., Kunkel, J. G., Montgomery, M. E., and Leonard, D. E. 1996. Effects of maternal nutrition and egg provisioning on parameters of larval hatch, survival and dispersal in the gypsy moth, *Lymantria dispar* L.. Oecologia **106**: 470-477.

Dixon, A. F. G., Kundu, R. and Kindlmann, P. 1993. Reproductive effort and maternal age in iteroparous insects using aphids as a model group. Funct. Ecol. **7**:267-272.

Dixon, A. F. G. and Wratten, S. D. 1971. Laboratory studies on aggregation, size and fecundity in the black bean aphid, *Aphis fabae* Scop.. Bull. ent. Res. **61**: 97-111.

Durrant, A.,1955. Effect of time of embryo formation on quantitative characters in *Drosophila*. Nature **175**: 560-561.

Falconer, D. S. 1981. Introduction to quantitative genetics. Longman, New York.

Fiore, C. 1960. Effects of temperature and parental age on the life cycle of the dark mealworm, *Tenebrio obscurus* Fabricius. Ann. N. Y. Ent. Soc. **68**: 27-35.

Forrest, T. G. 1986. Oviposition and maternal investment in mole crickets (Orthoptera: Gryllotalpidae): Effects of season, size, and senescence. Ann. Entomol. Soc. Am. **79**: 918-924.

Fox, C. W. 1993. The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). Oecologia **96**: 139-146.

Fox, C. W. 1997. Egg-size manipulations in the seed beetle *Stator limbatus*: consequences for progeny growth. Can. J. Zool. **75**: 1465-1473.

Fox, C. W. and Dingle, H. 1994. Dietary mediation of maternal age effects on offspring performance in a seed beetle (Coleoptera: Bruchidae). Funct. Ecol. **8**: 600-606.

Fox, C. W., McLennan, L. A. and Mousseau, T. A. 1995a. Male body size affects female lifetime reproductive success in a seed beetle. Anim. Behav. **50**: 281-284.

Fox, C. W., Waddell, K. J. and Mousseau, T. A. 1995b. Parental host plant affects offspring life histories in a seed beetle. Ecology **76**: 402-411.

Fox, C.W., Thakar, M. S. and Mousseau, T.A. 1997. Egg size plasticity in a seed beetle: An adaptive maternal effect. Am. Nat. **149**: 149-163.

Futuyma, D.J., 1998. Evolutionary Biology. Sinauer Associates, Sunderland, Mass.

Garcia-Barros, E. 1992. Evidence for geographic variation of egg size and fecundity in a Satyrine butterfly, *Hipparchia semele* (L.) (Lepidoptera, Nymphalidae-Satyrinae). Graellsia **48**: 45-52.

Ginzgurg, L. R. and Taneyhill, D. E. 1994. Population cycles of forest Lepidoptera: a maternal effect hypothesis. J. Anim. Ecol. **63**: 79-92.

Ginzgurg, L. R. and Taneyhill, D. E. 1995. Higher growth rate implies shorter cycle, whatever the cause: a reply to Berryman. J. Anim. Ecol. **64**: 294-295.

Graham, J. H., Freeman, D. C. and Emlen, J. M. 1993. Developmental stability: a sensitive indicator of populations under stress. In Landis, W.G., Hughes, J.S., and Lewis, M.A., (eds). Environmental Toxicology and Risk Assessment, pp. 136-158. American Society for Testing and Materials, Philadelphia (PA).

Harvey, G. T. 1977. Mean weight and rearing performance of successive egg clusters of eastern spruce budworm (Lepidoptera: Tortricidae). Can. Ent. **109**: 487-496.

Hill, C. J. 1989. The effect of adult diet on the biology of butterflies 2. The common crow butterfly, *Euploea core corinna*. Oecologia **81**: 258-266.

Hill, C. J. and Pierce, N. E. 1989. The effect of adult diet on the biology of butterflies 1. The common imperial blue, *Jalmenus evagoras*. Oecologia **81**: 249-257.

Honek, A. 1992. Female size, reproduction and progeny size in *Pyrrhocoris apterus* (Heteroptera, Pyrrhocoridae). Acta Entomol. Bohemoslov. **89**: 169-178.

Howe, R. W. 1967. The influence of age of the parents on some characteristics of the offspring of insects bred in the laboratory. J. Stored Prod. Res. **3**: 371-385.

Howe, R. W. and Hole, B. D., 1967. The Yield of cultures of *Sitophilus granarius* at 25C and 70 per cent relative humidity with some observations on rates of oviposition and development. J. Stored Prod. Res. **2**: 257-272.

Hussain, T. 1994. The effect of parental age on progeny of *Callosobruchus chinensis* Linn.. Bangladesh J. Zool. **22**: 55-59.

Hylton, A. R. 1967. Survival of eggs of *Eretmapodites chrysogaster* Graham. J. Med. Ent. **4**: 374-375.

Injeyan, H. S., Dale, J. F. and Tobe, S. S. 1981. Increased juvenile hormone synthesis and decreased fertility in aging *Schistocerca gregaria*. Can. J. Zool. **59**:1744-1748.

Jaeckle, W. B. 1995. Variation in the size, energy conent, and biochemical composition of invertebrate eggs: correlates to the mode of larval development. In: McEdward. L. R. (ed). Ecology of Marine Invertebrate Larvae, pp. 49-77. Discorides Press, Portland.

Jann, P. and Ward, P. I. 1999. Maternal effects and their consequences for offspring fitness in the Yellow Dung Fly. Funct. Ecol. **13**: 51-58.

Janssen, G. M., de Jong, G., Joose, E. N. G. and Scharloo, W. 1988. A negative maternal effect in springtails. Evolution **42**: 828-834.

Jones, R. E., Hart, J. R. and Bull, G. D. 1982. Temperature, size and egg production in the cabbage butterfly, *Pieris rapae* L.. Aust. J. Zool. **30**:223-232.

Jones, T. M., Balmford, A. and Quinnell, R. J. 2000. Adaptive female choice for middle-aged mates in a lekking sandfly. Proc. R. Soc. Lond. B **267**: 68.

Karlsson, B. 1987. Variation in egg weight, oviposition rate and reproductive reserves with female age in a natural population of the speckled wood butterfly, *Pararge aegeria*. Ecol. Entomol. **12**: 473-476.

Karlsson, B. and Wiklund, C. 1984. Egg weight variation and lack of correlation between egg weight and offspring fitness in the wall brown butterfly *Lasiommata megara.* Oikos **43**: 376-385.

Karlsson, B. and Wiklund, C. 1985. Egg weight variation in relation to egg mortality and starvation endurance of newly hatched larvae in some satyrid butterflies. Ecol. Entomol. **10**: 205-211.

Kasule, F. K. 1991. Egg size increases with maternal age in the cotton stainer bugs, *Dysdercus fasciatus* and *D. cardinalis* (Hemiptera: Pyrrhocoridae). Ecol. Entomol. **16**: 345-349.

Kence, M., and Jdeidi, T. 1997. Effect of malathion on larval competition in house fly (Diptera: Muscidae) populations. J. Econ. Entomol. **90**: 59-65.

Kimura, K. and Tsubaki, Y. 1985. Egg weight variation associated with female age in *Pieris rapae crucivora* Boisduval (Lepidoptera: Pieridae). Appl. Ent. Zool. **20**: 500-501.

Kiritani, K. and Kimura, K. 1967. Effects of parental age on the life cycle of the southern green stink bug, *Nezara viridula* L. (Heteroptera: Pentatomidae). Appl. Ent. Zool. **2**: 69-78.

Kirkpatrick, M. and Lande, R. 1989. The evolution of maternal characters. Evolution **43**: 485-503.

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

Kobayashi, M., and Iskikawa, H. 1993. Breakdown of indirect flight muscles of alate aphids (*Acyrthosiphon pisum*) in relation to their flight, feeding and reproductive behavior. J. Insect Physiol. **39**: 549-554.

Landa, K. 1992. Adaptive seasonal variation in grasshopper offspring size. Evolution **46**: 1553-1558.

Lansing, A. I. 1947. A transmissible, cumulative and reversible factor in aging. J. Gerontol. **2**: 228-239.

Lansing, A. I. 1948. Evidence for aging as a consequence of growth cessation. Proc. Natl. Acad. Sci. U.S.A. **34**: 304-310.

Larsson, F. K. 1989. Female longevity and body size as predictors of fecundity and egg length in *Graphosoma lineatum* L. Dtsch. ent. Z. **36**: 329-334.

Leary, R. F. and Allendorf, F. W. 1989. Fluctuating asymmetry as an indicator of stress: Implications for conservation biology. Trends Ecol. Evol. **4**: 214-217.

Leather, S. R. and Burnand, A. C. 1989. Factors affecting life-history parameters of the pine beauty moth, *Panolis flammea* (D&S): the hidden costs of reproduction. Funct. Ecol. 3: 331-338.

Legaspi, J. C., and O'Neil, R. J. 1994. Developmental response of nymphs of *Podisus maculiventris* (Heteroptera: Pentatomidae) reared with low numbers of prey. Environ. Entomol. **23**: 374-380.

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

Leonard, D. E. 1970. Intrinsic factors causing qualitative changes in populations of *Porthetria dispar* (Lepidoptera: Lymantriidae). Can. Ent. **102**: 239-249.

Ludwig, D. 1956. Effects of temperature and parental age on the life cycle of the mealworm, *Tenebrio molitor* Linnaeus (Coleoptera, Tenebrionidae). Ann. Ent. Soc. Am. *49*: 12-15.

Ludwig, D. and Fiore, C. 1960. Further studies on the relationship between parental age and the life cycle of the mealworm, *Tenebrio molitor*. Ann. Ent. Soc. Am. *53*: 595-600.

Ludwig, D. and Fiore, C. 1961. Effects of parental age on offspring from isolated pairs of the mealworm *Tenebrio molitor*. Ann. Ent. Soc. Am. **54**: 463-464.

Ludwig, D. and Jones, C. R. 1964. Changes in the concentration of certain amino acids in homogenates of the yellow mealworm, *Tenebrio molitor*, during aging. Ann. Ent. Soc. Am. **57**: 210-213.

Ludwig, D., Fiore, C. and Jones, C. R. 1962. Physiological comparisons between offspring of the yellow mealworm, *Tenebrio molitor*, obtained from young and old parents. Ann. Ent. Soc. Am. **55**: 439-442.

Marshall, L. D. 1990. Intraspecific variation in reproductive effort by female *Parapediasia teterrella* (Lepidoptera: Pyralidae) and its relation to body size. Can. J. Zool. **68**: 44-48.

McIntyre, G. S. and Gooding, R. H. 1995. Pteridine accumulation in *Musca domestica*. J. Insect Physiol. **41**: 357-368.

McIntyre, G. S. and Gooding, R. H. 1998. Effect of maternal age on offspring quality in tsetse (Diptera: Glossinidae). J. Med. Ent. **35**: 210-215.

McLain, D. K. and Mallard, S. D. 1991. Sources and adaptive consequences of egg size variation in *Nezara viridula* (Hemiptera: Pentatomidae). Psyche **98**: 135-164.

Meadow, N. D. and Barrows, C. H. 1971. Studies on aging in a bdelloid rotifer. II. The effects of various environmental conditions and maternal age on longevity and fecundity. J. Gerontol. **26**: 302-309.

Mohaghegh, J., De Clercq, P.and Tirry, L. 1998a. Effects of maternal age and egg weight on developmental time and body weight of offspring of *Podisus maculiventris* (Heteroptera: Pentatomidae). Ann. Ent. Soc. Am. **91**: 315-322.

Mohaghegh, J., De Clercq, P.and Tirry, L. 1998b. Maternal age and egg weight affect offspring performance in the predatory stink bug *Podisus nigrispinus*. Biocontrol **43**: 163-174.

Moore, R. A. and Singer, M. C. 1987. Effects of maternal age and adult diet on egg weight in the butterfly *Euphydryas editha*. Ecol. Entomol. **12**: 401-408.

Mousseau, T. A. 1991. Geographic variation in maternal-age effects on diapause in a cricket. Evolution **45**:1053-1059.

Mousseau, T. A. and Dingle, H. 1991a. Maternal effects in insect life histories. Annu. Rev. Entomol. **36**: 511-534.

Mousseau, T. A. and Dingle, H. 1991b. Maternal effects in insects: examples, constraints, and geographic variation. In: Dudley, E. C. (ed) The unity of evolutionary biology, vol. II, pp. 745-761. Dioscoridies Press, Portland.

Mousseau, T. A. and Fox, C. W. 1998a. The adaptive significance of maternal effects. Trends Ecol. Evol. **13**: 403-407.

Mousseau, T. A. and Fox, C. W. 1998b. Maternal effects as adaptations. Oxford Univ. Press, New York.

Murdie, G. 1969. The biological consequences of decreased size caused by crowding or rearing temperatures in apterae of the pea aphid, *Acyrthosiphon pisum* Harris. Trans. R. Ent. Soc. Lond **121**: 443-455.

Murphy, D. D., Launer, A. E. and Ehrlich, P. R. 1983. The role of adult feeding in egg production and population dynamics of the checkerspot butterfly *Euphydryas editha*. Oecologia **56**: 257-263.

Newton, C. and Dixon, A. F. G. 1990. Embryonic growth rate and birthweight of the offspring of apterous and alate aphids: a cost of dispersal. Entomol. Exp. Appl. **55**: 223-229.

Oberhauser, K. S. 1997. Fecundity, lifespan and egg mass in butterflies: Effects of male-derived nutrients and female size. Funct. Ecol. **11**: 166-175.

Parsons, P.A., 1962. Maternal age and developmental variability. J. Exp. Biol. **39**: 251-260.

Parsons, P. A. 1994. Developmental variability and the limits of adaptation: interactions with stress. In: Markow, T. A. (ed) Developmental Instability: Its Origins and Evolutionary Implications, pp. 247-255. Kluwer, The Netherlands.

Patterson, D. L., Friars, G. W., and McMillan, I. 1983. Effects of parental age and sampling method on response to index selection in *Tribolium castaneum*. Can. J. Genet. Cytol. **25**: 47-52.

Phelan, J. P. and Frumhoff, P. C. 1991. Differences in the effects of parental age on offspring life history between tropical and temperate populations of milkweed bugs (*Oncopeltus* spp.). Evol. Ecol. **5**: 160-172.

Price, D. K. and Hansen, T. F. 1998. How does offspring quality change with age in male *Drosophila melanogaster*? Behav. Genetics. **28**: 395-402.

Quickenden, K. L. and Roemhild, G. R. 1969. Maternal age and density effects on carbohydrate partitioned to eggs of the grasshopper, *Aulocara elliotti*. J. Insect Physiol. **15**: 1215-1223.

Richards, A. G. and Kolderie, M. Q. 1957. Variation in weight, developmental rate, and hatchability of *Oncopeltus* eggs as a function of the mother's age. Entomological News **68**: 57-64.

Richards, L. J. and Myers, J. H. 1980. Maternal influences on size and emergence time of the cinnabar moth. Can. J. Zool. **58**: 1452-1457.

Riska, B., 1991. Introduction to the symposium. In: Dudley, E. C. (ed) The Unity of Evolutionary Biology, vol. II, pp. 719-724. Dioscoridies Press, Portland.

Ritchie, M. G., Couzin, I. D. and Snedden, W. A. 1995. What's in a song? Female bushcrickets discriminate against the song of older males. Proc. R. Soc. Lond. B **262**: 2.

Roff, D. 1992. The evolution of life histories: theory and analysis. Chapman and Hall, New York.

Rogers, C. E. and Marti, O. G. 1997. Once-mated beet armyworm (Lepidoptera: Noctuidae): Effects of age at mating on fecundity, fertility, and longevity. Environ. Entomol. **26**: 585-590.

Rose, M. R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution **38**: 1004-1010.

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

Rose, M. R. 1999. Genetics of aging in *Drosophila*. Exp. Gerontol. **34**: 577-585.

Rossiter, M. C. 1991. Maternal effects generate variation in life history: consequences of egg weight plasticity in the gypsy moth. Funct. Ecol. **5**:386-393.

Rossiter, M. C. 1994. Maternal effects hypothesis of herbivore outbreak. Bioscience **44**: 752-763.

Rossiter, M. C. 1996. Incidence and consequences of inherited environmental effects. Annu. Rev. Ecol. Syst. **27**: 451-476.

Ruohomaki, K., Hanhimaki, S. and Haukioja, E. 1993. Effects of egg size, laying order and larval density on performance of *Epirrita autumnata* (Lep., Geometridae). Oikos **68**: 61-66.

Schneider, B. A. 1941. The nutritional requirements of *Tribolium confusum* Duval. Biological Bulletin **80**: 208-227.

Simmons, L. W. 1995. Correlates of male quality in the field cricket, *Gryllus campestris* L: Age, size, and symmetry determine pairing success in field populations. Behav Ecol. **6**: 376-381.

Simmons, L. W. and Zuk, M. 1992. Variability in call structure and pairing success of male field crickets, *Gryllus bimaculatus*: the effects of age, size and parasite load. Anim. Behav. **44**: 1145-1152.

Smith, C. C. and Fretwell, S. D. 1974. The optimal balance between size and number of offspring. Am. Nat. **108**: 499-506.

Solbreck, C. 1986. Wing and flight muscle polymorphism in a lygaeid bug, *Horvathiolus gibbicollis*: determinants and life history consequences. Ecol. Entomol. **11**: 435-444.

Solbreck, C., Olsson, R., Anderson, D. B. and Forare, J. 1989. Size, life history and responses to food shortage in two geographical strains of a seed bug *Lygaeus equestris*. Oikos **55**: 387-396.

Stam, E., Isaaks, A. and Ernsting, G. 1998. Negative maternal effect revisited: A test on two populations of *Orchesella cincta* L. (Collembola : Entomobryidae). Evolution **52**: 1839-1843.

Stjernholm, F. and Karlsson, B. 2000. Nuptial gifts and the use of body resources for reproduction in the green-veined white butterfly *Pieris napi*. Proc. R. Soc. Lond. B **267**: 807-811.

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

Svard, L. and Wiklund, C. 1988. Fecundity, egg weight and longevity in relation to multiple matings in females of the monarch butterfly. Behav. Ecol. Sociobiol. **23**: 39-43.

Tanaka, S. 1993. Allocation of resources to egg production and flight muscle development in a wing dimorphic cricket, *Modicogryllus confirmatus*. J. Insect Physiol. **39**: 493-498.

Tefler, W. H. and Rutberg, L. D. 1960. The effects of blood protein depletion on the growth of the oocytes in the *Cecropia* moth. Biol. Bull. **118**: 352-366.

Tracey, K. M. 1958. Effects of parental age on the life cycle of the mealworm, *Tenebrio molitor* Linnaeus. Ann. Ent. Soc. Am. **51**: 429-432.

Tschinkel, W.R. 1993. Crowding, maternal age, age at pupation, and life history of *Zophobas atratus* (Coleoptera: Tenebrionidae). Ann. Entomol. Soc. Am. **86**: 278-297.

Tsien, H. C. and Wattiaux, J. M. 1971. Effect of maternal age on DNA and RNA content of *Drosophila* eggs. Nature, New Biol. **230**:147-148.

Vahed, K. 1998. The function of nuptial feeding in insects: a review of empirical studies. Biol. Rev. **73**: 43-78.

Valentin, J. 1973. Selection for altered recombination frequency in *Drosophila melanogaster*. Hereditas **74**:295-297.

Van Horn, S. N. 1966. Studies on the embryogenesis of *Aulocara elliotti* (Thomas) (Orthoptera: Acrididae). II. Developmental variability and the effects of maternal age and environment. J. Morph. **120**:115-134.

Visscher, S. N. 1971. Studies on the embryogenesis of *Aulocara elliotti* (Orthoptera: Acrididae). III. Influence of maternal environment and aging on development of the progeny. Ann. Entomol. Soc. Am. **64**:1057-1074.

Wakefield, J., Harris, K. and Markow, T. A. 1994. Parental age and developmental stability in *Drosophila melanogaster*. In: Markow, T. A. (ed) Developmental Instability: Its Origins and Evolutionary Implications, pp. 237-246. Kluwer, The Netherlands.

Warren, D.C. 1924. Inheritance of egg size in *Drosophila melanogaster*. Genetics **9**:41-69.

Wasserman, S. S. and Asami, T. 1985. The effect of maternal age upon fitness of progeny in the southern cowpea weevil, *Callosobruchus maculatus*. Oikos **45**:191-196.

Watanabe, M. and Ando, S. 1994. Egg load in wild females of the small white *Pieris rapae crucivora* (Lepidoptera, Pieridae) in relation to mating frequency. Jpn. J. Ent. **62**: 293-297.

Wattiaux, J. M. 1968. Cumulative parental age effects in *Drosophila subobscura*. Evolution **22**: 406-421.

Weatherhead, P. J., Brown, G. P., Prosser, M. R. and Kissner, K. J. 1999. Factors affecting neonate size variation in northern water snakes, *Nerodia sipedon*. J. Herpetol. **33**: 577-589.

Wedell, N. 1993. Mating effort or paternal investment? Incorporation rate and cost of male donations in the wartbiter. Behav. Ecol. Sociobiol. **32**:239-246.

Weigensberg, I., Carriere, Y. and Roff, D. A. 1998. Effects of male genetic contribution and paternal investment to egg and hatchling size in the cricket, *Gryllus firmus*. J. Evol. Biol. **11**: 135-146.

Wellington, W. G. 1965. Some maternal influences on progeny quality in the western tent caterpillar, *Malacosoma pluviale* (Dyar). Can. Ent. **97**: 1-14.

Wickman, P. and Karlsson, B. 1987. Changes in egg colour, egg weight and oviposition rate with the number of eggs laid by wild females of the small heath butterfly, *Coenonympha pamphilus*. Ecol. Entomol. **12**: 109-114.

Wiklund, C. and Karlsson, B. 1984. Egg size variation in satyrid butterflies: adaptive vs historical "Bauplan", and mechanistic explanations. Oikos **43**: 391-400.

Wiklund, C. and Persson, A. 1983. Fecundity, and the relation of egg weight variation to offspring fitness in the speckled wood butterfly *Pararge aegeria*, or why don't butterfly females lay more eggs? Oikos **40**: 53-63.

Williams, T. D. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. Biol. Rev. **68**: 35-59.

Wilson, K. and Lessells, C. M. 1994. Evolution of clutch size in insects. I. A review of static optimality models. J. evol. Biol. 7: 339-363.

Wolf, J. B., Brodie, E. D., Cheverud, J. M., Moore, A. J. and Wade, M. J. 1998. Evolutionary consequences of indirect genetic effects. Trends Ecol. Evol. **13**: 64-69.

Wolf, J. B., Brodie, E. D. and Moore, A. J. 1999. The role of maternal and paternal effects in the evolution of parental quality by sexual selection. J. Evol. Biol. **12**: 1157-1167.

Wolf, J. B., Moore, A. J. and Brodie, E. D. 1997. The evolution of indicator traits for parental quality: The role of maternal and paternal effects. Am. Nat. **150**: 639-649.

Yuma, M. 1984. Egg size and viability of the Firefly, *Luciola cruciata* (Coleoptera, Lampyridae). Kontyu **52**: 615-629.

Zuk, M. 1987. Variability in attractiveness of male field crickets (Orthoptera: Gryllidae) to females. Anim. Behav. **35**: 1240-1248.

Zuk, M. 1988. Parasite load, body size, and age of wild-caught male field crickets (Orthoptera: Gryllidae): effects on sexual selection. Evolution **42**: 969-976.

Chapter 2: Egg size, contents, and quality: Maternal age and size effects on house fly eggs[1]

Introduction

A mother's contribution to her offspring is extremely important to the fitness of both offspring and mother (Roff 1992; Stearns 1992). For oviparous animals, most theories addressing variation in maternal investment deal with the egg size/number trade-off, and model females' optimal egg size without considering variation in investment between or within females (reviewed in Bernardo 1996; but see McGinley and Charnov 1988). The most widely used estimator of egg quality, and thus maternal investment, is egg size, under the assumptions that large eggs produce offspring with higher fitness and that large eggs are more expensive to produce. There is substantial support for the first assumption: 63% of 137 studies reported positive associations between egg size and offspring fitness component(s) (Azevedo et al. 1997). However, the absence of a correlation in 37% of the studies reveals that egg size does not fully predict offspring fitness. Failure to find egg size/fitness correlations is often explained away by suggesting that the fitness effects may only manifest themselves under adverse conditions (e.g. Parsons 1994; Fox 1997). It is likely also that other factors contribute to fitness variation among eggs. For example, if egg contents do not accurately track egg size then both of the above assumptions may be violated.

In many organisms there is significant variation in yolk contents at the population, individual, and intra-clutch levels, and it has been suggested that yolk provisioning may be a more accurate measure of maternal investment than egg size (reviews in Williams

[1] A version of this chapter has been accepted for publication. McIntyre, G. S. and Gooding, R. H. *In press.* Egg size, contents, and quality: Maternal age and size effects on house fly eggs. Can. J. Zool.

1994; Jaeckle 1995; Bernardo 1996). Egg contents and size can vary independently. In echinoderms, egg size can be uncorrelated with energy content among species, between populations, between individuals, and between eggs within single broods (reviewed in Jaeckle 1995). In birds, large eggs can contain disproportionately more albumin or yolk than do small eggs (reviewed in Williams 1994). In the snail *Arianta arbustorum*, egg nitrogen and carbon concentrations vary independently, and the pattern of variation differs among populations and is not related to egg size in successive egg batches (Baur and Baur 1997).

Intraspecific variation in insect egg content has only rarely been related to egg quality. In the grasshopper *Aulocara elliotti*, egg mortality increases with maternal age and trehalose concentration decreases (Quickenden and Roemhild 1969). In the gypsy moth, *Lymantria dispar*, heavy eggs contain disproportionately more yolk protein than do light eggs; heavy eggs, and the resulting larvae, could be more resistant to environmental stress than are light eggs (Capinera et al. 1977). However, survival of starved gypsy moth hatchlings is correlated with levels of two yolk proteins (vitellin and glycine rich protein) but not with egg weight (Diss et al. 1996). Overall, these studies indicate that egg contents may be more important than egg size in insects.

As part of a larger study on maternal effects on offspring quality in house flies, *Musca domestica* L., I examined the relationship of egg size and egg contents. Egg size was positively correlated with maternal size and negatively correlated with maternal age in house flies. I collected large eggs laid by large young females and compared them to small eggs laid by small young females and by large old females. By comparing egg size, contents and hatch rate between egg sizes and maternal phenotypes I addressed two main

questions: 1) Do large and small house fly eggs differ in more than size? and 2) Are small eggs produced by different maternal phenotypes similar in more than size?

Materials and methods

Insects and their maintenance

The culturing methods and early history of my house fly, *Musca domestica* L., colony were described previously (McIntyre and Gooding 1995). Approximately 60 wild caught flies from the original collection site were added to the colony (then at ~500 individuals) in March 1996. The experiments reported here were conducted between March 1996 and February 1998 using flies reared from eggs produced by 10 day old females that were maintained in 8.0 L cages containing 80 females and 40 males at 25C with a 16L:8D photoperiod, referred to below as standard conditions.

Maternal age effects: individual females

Flies were separated from the opposite sex within 12 hours of eclosion to ensure virginity (Keiding and Rosenkilde 1990). Fifteen one-day-old females were randomly paired with two-day-old males from the same rearing container, to ensure earliest possible mating. Each pair was placed in a 250 ml container and provided with food and water *ad lib*; water was changed every 3 days to avoid oviposition on the moistened cotton wick, and food was added every 7 days. Eggs were collected, between 09:00-14:00 on filter paper soaked with 2.5 % ammonium bicarbonate, every three days beginning when females were four-days-old; egg batches were numbered sequentially for each female.

Clutch size was recorded for each egg batch and 10 eggs were measured for length and width (at the mid-length) using a dissecting scope with camera lucida and an FX graphics tablet (Summasketch, Seymour, CT, USA). House fly eggs are approximately ellipsoid and egg volume was estimated as $V = (\pi \times \text{length} \times \text{width}^2)/6$. A separate sample of 10 eggs was sealed in a 1ml microcentrifuge tube at 25C for 24 hours to determine hatch rate. It was not always possible to measure all eggs on the day they were laid, and some were frozen at -20C in 0.85% saline. House fly eggs can be stored in this way with no change in egg length, width, or protein, carbohydrate or lipid content, and house fly eggs do not change size during development (Appendices 1 and 2). After death, wings of males and females were mounted in Euparol and measured to estimate teneral dry mass. I used regressions of daily means (across all females) to relate egg parameters to maternal age, using common data transformations where they were indicated by decreased dependence of residuals on maternal age. Because there were many missing data points (i.e., egg collections during which one or more females did not oviposit) I was unable to use repeated measures analysis of variance. Thus I analyzed average changes across female life span but not individual differences in maternal age effects.

Maternal age effects: population cages

To examine further the effects of maternal age on eggs and to control for possible effects of aging males, four cages were set up under standard conditions. Mortality was recorded at least every three days and dead flies were removed. Cages were maintained until oviposition ceased. In two cages, termed replacement cages (R), 25% of the males were replaced with 3-4-day old males on day 14 and 21, while maintaining the initial 2:1

sex ratio. I chose to replace 25% of the males because the average male can inseminate a minimum of 5 females in a week (McIntyre, unpublished data), thus most receptive females would have been able to mate with a young male. Adding too many males causes negative effects on females, since young males will harass females through frequent mating attempts (Ragland and Sohal 1973). The remaining two cages (N) contained females with only the original males. Eggs were collected twice per week beginning when females were four days old. Egg hatch was checked on days 10 and 24. Eggs were frozen in 0.85% saline for size measurements and biochemical determinations. Since the treatments did not differ until after the first set of analyses (day 10) a comparison of treatment means does not adequately test for effects of young males on eggs produced by old females. I used a two-way analysis of variance (ANOVA) with maternal age and male replacement as main terms. In this analysis the presence of a significant age X replacement interaction indicates an effect of male replacement on female maternal age effects.

Maternal size effects: individual females

Eggs from a single egg collection were seeded into larval medium at 2, 6, 12, 18, and 24 eggs/g. Six eggs/g is near optimal for houseflies (Sullivan and Sokal 1963; McIntyre and Gooding 1995). For each density, on the first day that seven flies of each sex emerged, seven randomly chosen pairs were isolated at 25C with food and water *ad lib*. Daily egg collections were made beginning on day four and continuing until all females had deposited their first egg batch. Clutch size, egg size, and hatch rate were determined for the first egg batch. Females' wings were mounted in Euparol.

Maternal size effects: population cages

Eggs were seeded into two replicate cups at 0.03 and 0.09 ml eggs/30g medium. These densities approximate 10 and 30 eggs/g. Emergents from replicates were pooled and placed in cages under standard conditions. Flies were provided with oviposition medium daily, beginning on day 4 of adult life and continuing until the first day on which enough eggs were laid to indicate that more than a few flies had oviposited. Hatch rate was determined using 50 eggs from the first large batch. Size and biochemical determinations were done on eggs that were stored frozen in 0.85% saline. After the eggs were collected, the wings of several females were slide mounted to estimate teneral mass.

Estimation of teneral dry mass

Four unfed teneral flies of each sex, from each larval density in the experiment on maternal size effects (individual females), were killed by freezing, oven dried at 60C for 72 hrs, and weighed to the nearest 0.1 mg. The right wing of each fly was slide-mounted and the locations of 13 vein intersections were digitized with the equipment used for egg measurements. All possible interlandmark distances were calculated and log log regressions were computed for each sex. The distance between the intersection of vein R_{4+5} with the wing margin and the intersection of the medial vein with the posterior (discal medial-cubital) crossvein (Landmarks 11 and 13, Figure 4.1), gave the most accurate regression for females (data and analyses not presented); teneral mass was estimated according to the formula mass = exp((ln(length) - 1.887)/0.224), ($R^2 = 0.957$, $p < 0.0001$, $n = 20$). This regression was used to estimate teneral dry weight of the flies from all experiments.

Egg content determinations

Carbohydrate and lipid content were determined by modifications of the methods of Van Handel (1985a, 1985b), and protein content by a modification of the method of Bradford (1976), as follows. Carbohydrate and protein determinations were made on the same eggs. Samples of five eggs were homogenized in 100µl 0.01N NaOH and centrifuged at 5000g for 10 minutes. For protein determination, 50µl of supernatant was added to 1ml of Bradford reagent (100mg Coomassie Blue G-250, 50ml 95% ethanol, 100ml 85% phosphoric acid, H_2O to make 1L). Five minutes after mixing, A_{595} was read. Insect egg proteins are often extracted in strong saline solutions, but the quantity of protein extracted from house fly eggs by NaOH was not significantly different from that extracted by 0.5 N NaCl (0.01 N NaOH: 5.3 ± 0.23 µg/egg; 0.5 N NaCl: 5.4 ± 0.17 µg/egg; $t_{15} = 0.60$, $P = 0.56$). For carbohydrate determination 1ml of anthrone reagent (750mg anthrone, 380ml concentrated H_2SO_4, 150ml H_2O) was mixed with the remaining sample and heated at 100C for 17 minutes. After five minutes at room temperature, A_{625} was read. For lipid determination, a separate sample of 5 eggs was homogenized in 100µl 1:1 chloroform:methanol. The solvent was evaporated and the residue was heated for 10 minutes at 100C with 40µl of concentrated H_2SO_4. After removal from heat, 1ml vanillin reagent (600mg vanillin, 100ml H_2O, 400ml 85% phosphoric acid) was added to the mixture. After developing for 25 minutes at room temperature, A_{525} was read. Contents, in µg, were calculated from standard curves for protein, carbohydrate, and lipid derived from solutions of BSA or oyster glycogen in 0.01 N NaOH, and a chloroform solution of olive oil. Biochemical contents were converted to energy content using the following

conversion for metabolizable energy: protein=16.0 kJ/g, carbohydrate=16.0 kJ/g and lipid=37.5 kJ/g (Adrian et al. 1988).

Results:

Maternal age effects: individual females

Maternal age was negatively correlated with egg volume, hatch rate and clutch size for individually housed females (Figure 2.1). Batch number (i.e., the number of clutches laid) was highly correlated with maternal age (Figure 2.1D) but females did not oviposit every time ammonium bicarbonate was presented to them, and oviposition became less frequent as females aged. In general maternal age was a better predictor of clutch size, volume, and hatch rate than was batch number so I present only the maternal age analyses. Untransformed standard errors of egg volume, hatch rate, and clutch size, were generally positively correlated with maternal age (Pearson Correlation Coefficients [n = 103]: clutch: $r = 0.80$ $p < 0.001$; egg volume: $r = 0.48$ $p = 0.07$; hatch rate: $r = 0.63$ $p = 0.02$). Visual examination of plotted data (not shown) revealed that these results were due to an increase in the number of small clutches and clutches with small eggs and/or low hatch rates produced by old females. Teneral weight of females (mean ± SE: 4.87 ± 0.174 mg) was not correlated with other variables, including total number of eggs laid and female lifespan (42.9 ± 4.37 days). Male lifespan (28.9 ± 4.72 days) was not correlated with other variables.

To examine for relationships between egg volume, hatch rate and clutch size, partial correlations were calculated with maternal age held constant. Angular transformed values of hatch rate were positively correlated with clutch size ($r = 0.44$, $p <$

0.0001, n = 103) but egg volume was not correlated with clutch size or hatch rate (clutch size: r = -0.18, p = 0.07, n = 103; hatch rate: r = -0.13, p = 0.19, n = 103).

Maternal age effects: population cages

Comparing 10 and 24-day-old females, younger flies produced larger eggs with higher hatch rate and lipid content; however, protein, carbohydrate and energy content were unaffected by maternal age (Table 2.1). Females in the N and R cages produced eggs that differed significantly in several parameters, even though females were from the same egg mass, and male replacement did not begin until after deposition of the first group of eggs examined. Females in the R cages produced larger eggs with significantly higher protein content but lower lipid content, before and after addition of young, replacement males. However, there were no significant interactions between treatment and maternal age, and thus no effects of male replacement, although the power of this test is relatively low. Additionally, all changes were in the same direction for both treatments; for protein (the only case in which the percent change differed between treatments by more than 5%) the increase in protein was less with male replacement than without (analyses not shown but based on Table 2.1). On days 10 and 23, mean female survivorship was 96 and 90% respectively and did not differ between treatments (not shown). Male survivorship in the N populations was 98 and 47% on days 10 and 23 respectively. Mating was observed in all cages throughout the experiment.

Maternal size effects: individual females

Maternal size was not significantly related to egg hatch rate, but was positively correlated with clutch size and egg volume, and negatively correlated with age at first oviposition (Figure 2.2). To examine the relationship between egg parameters, partial

correlations were calculated with maternal size held constant. Angular transformed values of hatch rate were negatively correlated with egg volume ($r = -0.447$, $p < 0.029$, $n = 25$) but no other significant correlations were found (analyses not presented). This unexpected relationship appeared to be due to a single, otherwise unremarkable, fly from the 6 eggs/g treatment that produced the largest eggs (59.7 ± 1.35 nanolitres) and those with the lowest hatch rate (0%). Dropping this fly from the analysis changed the partial correlation of hatch rate and egg volume to –0.354 ($n = 24$, $p = 0.0975$). Removing this fly did not significantly alter any other relationship.

Maternal size effects: population cages

Eggs from large and small females (144% difference in dry mass) did not differ in hatch rate or protein, carbohydrate, lipid, or energy content, but eggs from large females were larger than those from small females (Table 2.2). Eggs from small flies in this experiment had higher hatch rate and greater lipid, protein and energy content, but did not differ in volume or carbohydrate content from those of eggs produced by old flies (treatments pooled) in the maternal age effects population experiment. (Tables 2.1 and 2.2; volume: $t_{48} = 1.23$, $p = 0.22$; hatch rate: $\chi^2 = 5.58$, $p = 0.018$; protein: $t_{30} = 2.07$, $P = 0.047$; carbohydrate: $t_{28} = 0.38$, $p = 0.71$; lipid: $t_{30} = 2.93$, $p = 0.006$; energy content: $t_{28} = 3.19$, $p = 0.003$).

Discussion

It is often postulated that egg size accurately reflects maternal investment and egg quality. I found that egg size was either uncorrelated or negatively correlated with hatch rate and that egg size bore little relation to egg contents (Tables 2.1 and 2.2). The main

findings are summarized in Figure 2.3. Partial correlation analysis established that, although maternal age was negatively correlated with hatch rate and egg size, hatch rate was unrelated to egg size after removal of maternal age effects. Using similar logic, egg size and hatch rate were weakly negatively correlated after removal of maternal size effects, even though maternal size was positively related to egg size but was statistically unrelated to hatch rate. Egg size differences were not reflected in egg energy, protein, or carbohydrate content, however, lipid content was lower in eggs from old mothers but not in eggs from small mothers (Tables 2.1 and 2.2). Unfortunately I was unable to statistically separate maternal age and size effects from egg size effects in these experiments.

The results suggest that hatch rate is not tightly bound to egg size in house flies. As older flies lose the ability to produce numerous eggs they also lose the ability to produce viable eggs (Figure 2.1), but decreased viability is not directly associated with egg size. In the population maternal age experiment, for females aged 24 days, hatch rate and egg size had decreased but total egg energy content remained unchanged (Table 2.1). Therefore, an overall loss of egg provisioning did not cause the observed decrease in hatch rate. The reduced lipid content of small eggs from old mothers may be approaching the minimum lipid required for successful embryonic development; if this is true, young mothers may be over-provisioning their eggs with lipid. Interestingly, it took small females longer to produce eggs than it did large females (Figure 2.2). This increase could be explained if small flies must produce eggs with a minimum level of energy or lipid content, but are less efficient at nutrient acquisition or conversion. A similar relationship

between decreased yolk production rates and minimum egg energy levels might explain the increased inter-oviposition interval in aged females (unpublished observation).

Almost all models of egg size-number trade-offs and maternal investment treat egg size as the sole determinant of egg quality and offspring fitness (reviewed in Bernardo 1996). A notable exception is the work of McGinley and Charnov (1988) which considers the allocation of two fitness enhancing resources to propagules. They demonstrate that, with limited pools of two resources, optimal allocation to propagules depends on the relative size of the two pools. According to the McGinley and Charnov model, if the target of house fly egg production is a minimum energy content, and if egg size is a product of some other constraint, then the proportion of lipid in the egg should depend upon the relative availability of lipid and on egg size.

It could be argued that, in the benign environment experienced by our flies, egg size should not affect hatch rate since females have adequate resources to fully provision eggs of any size produced, within the constraints of age, ovariole number, and maternal size. This argument is unlikely to be correct since I found a weak negative partial correlation between egg size and hatch rate in the test of maternal size effects using individual females. The differences in egg lipid content also argue against this since, in the same environment, flies of different ages produced eggs with different compositions. Thus, 'fully provisioned' is different for eggs from different female phenotypes.

I did not directly assess the effects of male age on measured egg parameters. Comparing females with only same age males available with those provided with young replacement males on a weekly basis, I observed no effects attributable to the availability of young males (Table 2.1). For unknown reasons, females with access to young males

produced larger eggs with higher protein and lower lipid content before and after young males were added to the cages. However, there were no significant interactions of maternal age and male replacement, which is the true test of the effect of new males. Possibly my handling methods during experimental setup inadvertently sorted females by vigor, cold tolerance, or some other trait associated with egg production. Whatever the cause, the results suggest that differences between eggs of young and old females do not result from a lack of vigorous males or sperm, although the opposite result has been reported in other insects (e.g. Fox 1993).

It is easy to conflate the effects of egg size with the effects due to the underlying causes of egg size variation. These can not be adequately separated through simple phenotypic correlation of an egg or maternal trait with offspring fitness. Egg size of animals has been manipulated either directly (Sinervo and McEdward 1988; Sinervo and Huey 1990); or indirectly (Sinervo and Licht 1991a, 1991b; Azevedo et al. 1997; Fox 1997). Although these studies clarified the nature of egg size effects on offspring fitness and life history, independent of other maternal effects, none of the studies verified that egg composition was unaffected by the experimental manipulation.

I solved this problem by using two distinct measures to generate egg size variation (maternal age and maternal size effects) and comparing relationships among egg parameters between manipulations. Large and small house fly eggs differed primarily in size and lipid content in the maternal age effect experiment. However, eggs did not differ in energy, protein, or carbohydrate content, and egg size had either a weak negative correlation (maternal size experiment), or was not correlated (maternal age experiment) with hatch rate, after removal of maternal effects. I found also that small eggs produced

by large old females differed considerably from those produced by small young females. Eggs from small young flies had higher hatch rate, protein, lipid, and energy content than similarly sized eggs from large old flies. Egg size is therefore a poor estimator of egg quality, at least with regard to the parameters measured.

The assumptions that egg size can be used as a surrogate for egg quality and that large eggs are more expensive to produce than small eggs are not correct. This has been previously demonstrated in other taxa (reviews in Williams 1994; Jaeckle 1995; Bernardo 1996). The present study, and the reviews just cited lead me to conclude that future empirical studies and life history models of maternal investment and of egg size/egg number trade-offs should include measures of egg quality other than just size.

Table 2.1. Effects of maternal age and male replacement on hatch rate and mean (± SE) egg volume, and protein, carbohydrate, lipid and energy content. Results are provided for females caged with only the original same-aged males and for females provided on days 14 and 21 with teneral males (N.B. at 10 days both treatments are identical, see text). P-values are from a type III, two way ANOVA of maternal age and replacement except where noted.

	No Replacement				Male Replacement				P		
	n	10 day	n	24 day	n	10 day	n	24 day	maternal age	replacement	interaction
Egg Volume (nl)	25	56.6 ± 1.27	25	50.3 ± 1.41	25	61.7 ± 1.29	25	52.6 ± 1.12	0.0001	0.0046	0.26
Hatch Rate (%)[a]	50	94.0	50	80.0	50	96.0	50	78.0	0.001	1	1
Protein (µg/egg)	8	5.0 ± 0.10	8	5.4 ± 0.23	8	5.9 ± 0.28	8	6.0 ± 0.25	0.16	0.0024	0.51
CHO (µg/egg)	8	2.0 ± 0.10	8	2.0 ± 0.14	8	1.8 ± 0.07	7	1.8 ± 0.15	0.68	0.14	0.79
Lipid (µg/egg)	8	2.4 ± 0.10	8	2.0 ± 0.07	8	2.2 ± 0.04	8	1.9 ± 0.06	0.0001	0.0155	0.31
Energy Content (mJ/egg)	8	202.2 ± 4.38	8	194.5 ± 6.25	8	205.6 ± 4.93	7	198.4 ± 4.90	0.16	0.49	0.95

[a] Age Effect: Chi-Square=11.317, df=1, $p < 0.001$

Table 2.2. Effects of maternal size on hatch rate and mean (±SE) egg volume and egg protein, carbohydrate, lipid, and energy content. Mothers of two sizes were produced by rearing them at high and low density; mean (±SE) teneral dry mass of a sample from each group of females is also provided. Probabilities are from two-tailed t tests, except where noted.

	n	Small Mothers	Large Mothers	p
Teneral Dry Mass (mg)	16	0.9 ± 0.03	2.2 ± 0.09	0.0001
Egg Volume (nl)	50	49.8 ± 1.01	54.4 ± 0.77	0.0005
Hatch Rate (%)	50	94.0	84.0	0.11[a]
Protein (µg/egg)	16	6.2 ± 0.14	5.9 ± 0.15	0.16
CHO (µg/egg)	16	2.0 ± 0.08	2.1 ± 0.08	0.15
Lipid (µg/egg)	16/14	2.1 ± 0.04	2.1 ± 0.07	0.92
Energy Content (mJ/egg)	16/14	210.5 ± 2.26	209.9 ± 3.47	0.88

[a] X^2=2.55, df=1

Figure 2.1. Maternal age effects on daily means (± SE) of egg parameters for 15 female houseflies. A) clutch size (regression: (egg number) = -2.35*(maternal age) + 143.6, n = 13, R^2 = 0.85, p < 0.0001), B) egg volume (regression: ln(volume) = -0.09*ln(maternal age) - 2.7, n = 13, R^2 = 0.83, p < 0.0001), C) hatch rate (regression: arcsin√(proportion hatching) = -0.014*(maternal age) + 1.16, n = 13, R^2 = 0.75, p < 0.0001), D) batch number (regression: (batch) = -0.23*(maternal age) + 0.08, n = 13, R^2 = 0.99, p<0.0001).

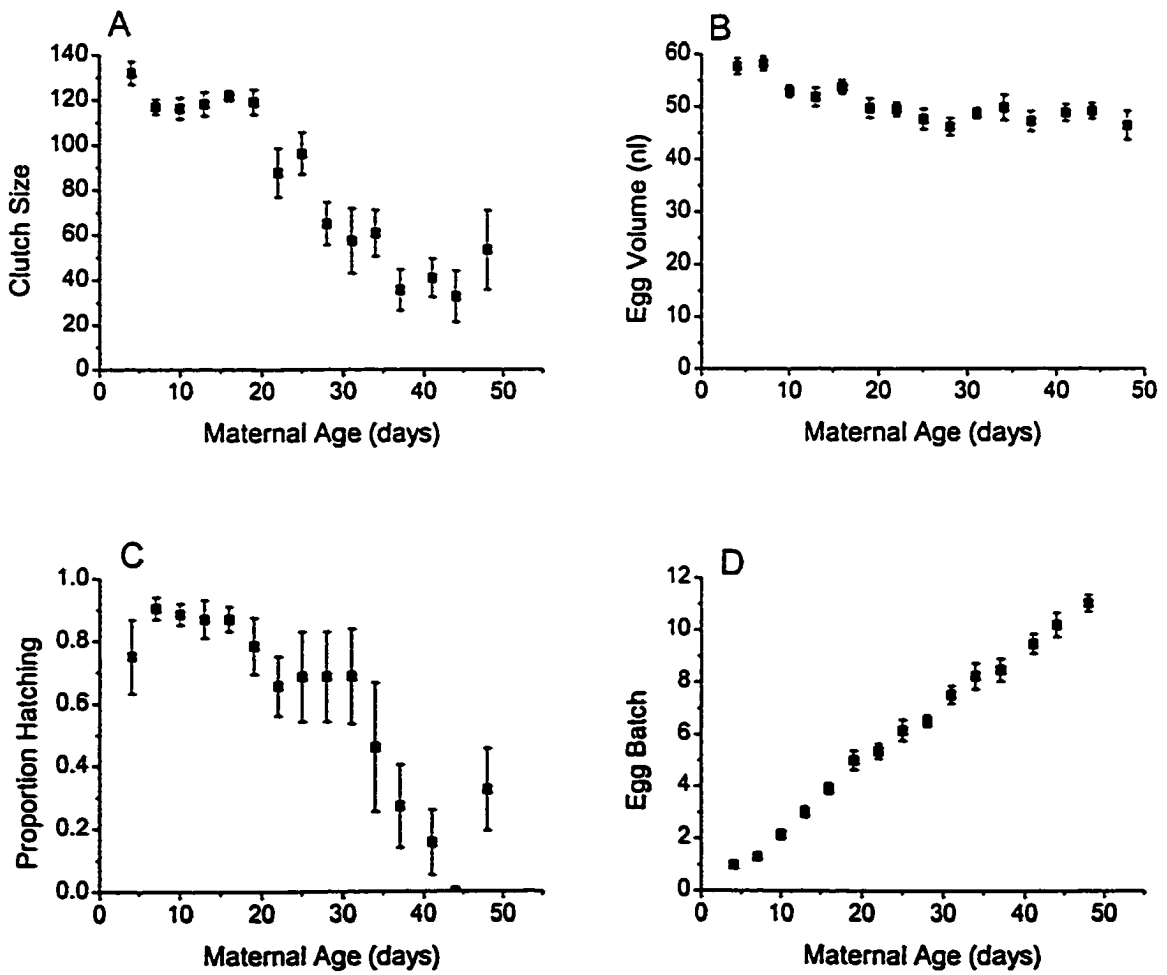


Figure 2.2. Maternal size effects on egg parameters for 24 female houseflies. A) clutch size (regression: ln(egg number) = 0.77*ln(mass(mg)) + 3.57, n = 24, R^2 = 0.84, p < 0.0001), B) egg volume (regression: (volume) = 0.0017*(mass(mg)) + 0.048, n = 24, R^2 = 0.38, p < 0.001), C) hatch rate (regression: arcsin√(proportion hatching) = 0.0076*(mass(mg)) + 0.98, n = 24, R^2 = 0.0, p = 0.88), D) age at first oviposition (regression: ln(age) = -0.38*ln(mass(mg)) + 2.07, n=24, R^2=0.45, p=0.0002).

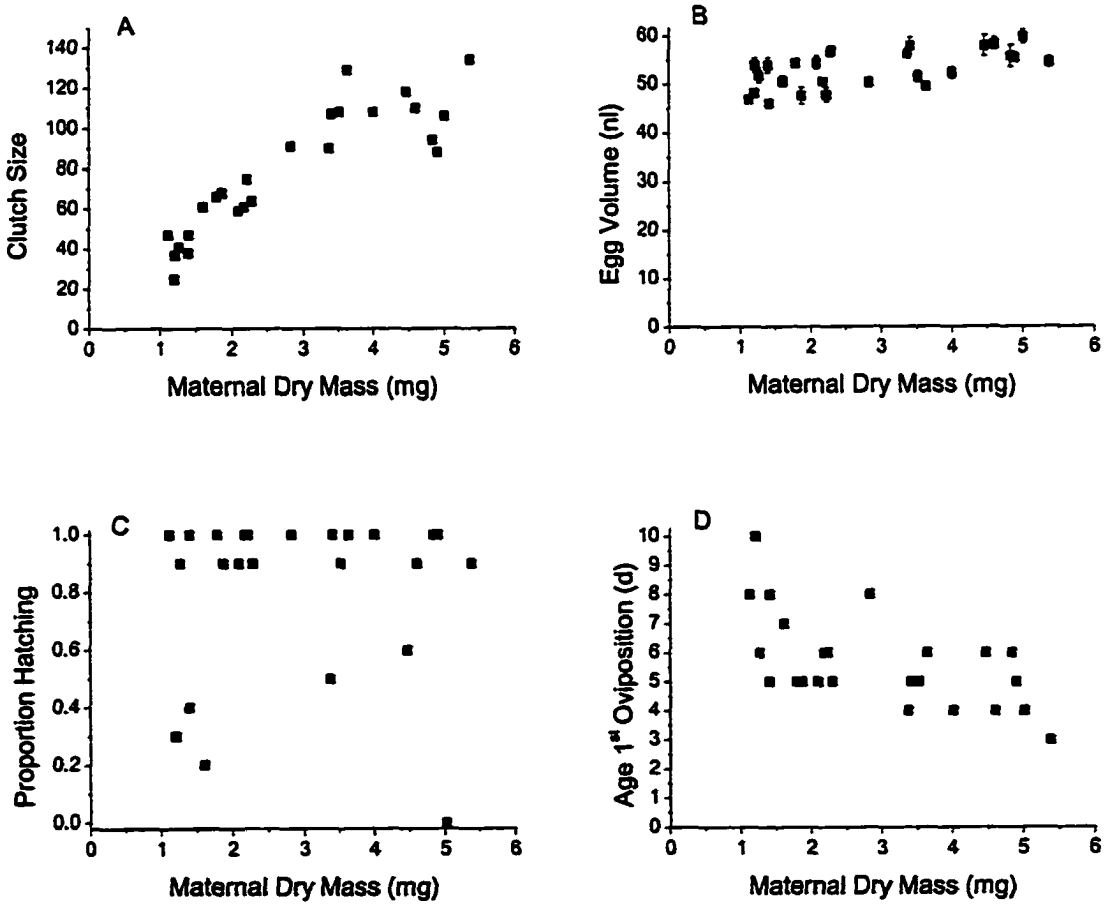
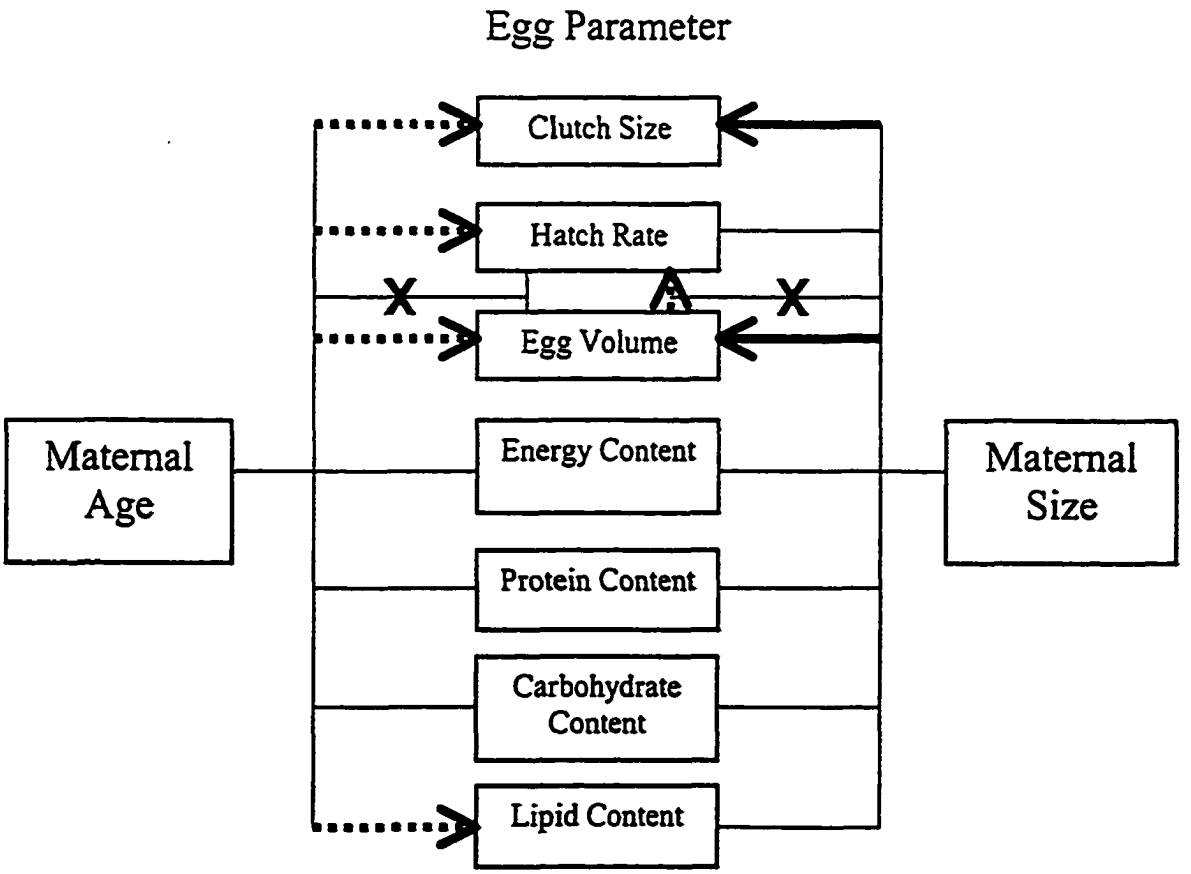


Figure 2.3. Maternal age and size effects upon egg parameters in house flies. For clutch size, hatch rate, and egg volume the results are from experiments monitoring both individual flies and groups of flies. For biochemical and energy content, the results are from single experiments with groups of flies, within these experiments there were no significant relationships of egg volume with biochemical contents. Solid and hatched arrows represent positive and negative correlations respectively, a plain line indicates lack of a significant relationship, and an X through a line indicates removal of maternal effects via partial correlation.



References

Adrian, J., Legrand, G., and Frangne, R. 1988. Dictionary of Food Nutrition and Biochemistry. Horwood. Chichester (England).

Azevedo, R. B. R., French, V., and Partridge, L. 1997. Life-history consequences of egg size in *Drosophila melanogaster*. Am. Nat. **150**: 250-282.

Baur, A., and Baur, B. 1997. Seasonal variation in size and nutrient content of eggs of the land snail *Arianta arbustorum*. Invertebrate Reproduction and Development **32**: 55-62.

Bernardo, J. 1996. The particular maternal effect of propagule size, expecially egg size: patterns, models, quality of evidence and interpretations. Amer. Zool. **36**: 216-236.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry **72**: 248-256.

Capinera, J. L., Barbosa, P., and Hagedorn, H. H. 1977. Yolk and yolk depletion of gyspy moth eggs: implications for population quality. Ann. Ent. Soc. Am. **70**: 40-42.

Diss, A. L., Kunkel, J. G., Montgomery, M. E., and Leonard, D. E. 1996. Effects of maternal nutrition and egg provisioning on parameters of larval hatch, survival and dispersal in the gypsy moth, *Lymantria dispar* L.. Oecologia **106**: 470-477.

Fox, C. W. 1993. The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). Oecologia **96**: 139-146.

Fox, C. W. 1997. Egg-size manipulations in the seed beetle *Stator limbatus*: consequences for progeny growth. Can. J. Zool. **75**: 1465-1473.

Jaeckle, W. B. 1995. Variation in the size, energy content, and biochemical composition of invertebrate eggs: correlates to the mode of larval development. In: McEdward. L. R. (ed). Ecology of Marine Invertebrate Larvae, pp. 49-77. Discorides Press, Portland.

Keiding, J., and Rosenkilde, I. 1990. A device for preventing houseflies from copulating. Ent. Exp. Appl. **55**:125-129.

McGinley, M. A., and Charnov, E. L. 1988. Multiple resources and the optimal balance between size and number of offspring. Evol. Ecol. **2**: 77-84.

McIntyre, G. S., and Gooding, R. H. 1995. Pteridine accumulation in *Musca domestica*. J. Insect Physiol **41**: 357-368.

Parsons, P. A. 1994. Developmental variability and the limits of adaptation: interactions with stress. In: Markow, T. A. (ed) Developmental Instability: Its Origins and Evolutionary Implications, pp. 247-255. Kluwer, The Netherlands.

Quickenden, K. L., and Roemhild, G. R. 1969. Maternal age and density effects on carbohydrate partitioned to eggs of the grasshopper, *Aulocara elliotti*. J. Insect Physiol. **15**: 1215-1223.

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

Roff, D. 1992. The Evolution of Life Histories: Theory and Analysis. Chapman and Hall, New York.

Sinervo, B., and Huey, R. B. 1990. Allometric engineering: An experimental test of the causes of interpopulational differences in locomotor performance. Science **248**: 1106-1109.

Sinervo, B., and Licht, P. 1991a. Proximate constraints on the evolution of egg size, egg number, and total clutch mass in lizards. Science **252**: 1300-1302.

Sinervo, B., and Licht, P. 1991b. The physiological and hormonal control of clutch size, egg size, and egg shape in *Uta stansburiana*: Constraints on the evolution of lizard life histories. J. Exp. Zool. **257**: 252-264.

Sinervo, B., and McEdward, L. R. 1988. Developmental consequences of an evolutionary change in egg size: an experimental test. Evolution **42**: 885-899.

Stearns S. C. 1992. The Evolution of Life Histories. Oxford University Press, New York.

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

Van Handel, E. 1985a. Rapid determination of glycogen and sugars in mosquitoes. J. Am. Mosq. Control Assoc. **1**: 299-301.

Van Handel, E. 1985b. Rapid determination of total lipids in mosquitoes. J. Am. Mosq. Control Assoc. **1**: 302-304.

Williams, T. D. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. Biol. Rev. **68**: 35-59.

Chapter 3: Effects of maternal age on larval competitiveness in house flies[2]

Introduction

Offspring of old female insects (hereafter referred to as late offspring) are usually thought of as inferior performers, relative to the offspring of young females (hereafter referred to as early offspring). Late offspring are often smaller as hatchlings, arising from smaller eggs with lower average viability, than their earlier siblings do, andthey often have decreased growth rates, and larval viability (for reviews see Mousseau and Dingle 1991a, b; Mousseau and Fox 1998; Chapter 1). Maternal age effects generally become attenuated as the offspring develop but in some cases they extend to later larval and even adult performance (e.g. adult size of offspring [Rossiter 1991; McIntyre and Gooding 1998]). But these later differences are usually measured as performance or fitness components of isolated groups or individuals. There have been few studies of maternal age effects in which offspring of young and old females interact. Therefore the intraspecific competitive fitness of offspring of different maternal ages is essentially unknown in insects, in spite of the considerable body of research seeming to address this.

Most maternal age effects are considered non-adaptive because they are thought to result from physiological constraints and deterioration (e.g., inability to adequately provision eggs at advanced ages [Murphy et al. 1983]). However, many maternal effects are adaptive, patterned responses of females to predictive cues resulting in improved offspring success (Mousseau and Fox 1998a, b). For example, the maternal age-related increase in the incidence of diapause in many seasonal populations corresponds well with

[2] A version of this chapter has been accepted for publication. McIntyre, G. S. and Gooding, R. H. *In press.* Effects of maternal age on larval competitiveness in house flies. Heredity.

the increasing need for offspring to enter diapause as the season advances (Mousseau and Dingle 1991a,b; Denlinger 1998). Furthermore, in species that experience consistent environmental changes during the life of individuals, maternal age effects that improve offspring performance may exist. If, due to seasonal changes or resource depletion, density consistently becomes challenging to their offspring as females age, maternal effects could evolve to avoid or otherwise cope with higher larval density. An initial step in demonstrating that a maternal age effect is an adaptive response to increasing density is to show that old mothers produce larvae that are more successful in competition in or avoidance of high density.

A common approach used to study maternal age effects is to raise isolated groups of offspring from mothers of various ages and compare performance among these groups. This approach can not reveal maternal age effects on larval competition, which is an important fitness component in species that have significant larval interactions. As pointed out by Lewontin (1955) "The supposition that one can predict the relative viabilities of genotypes from their absolute viabilities in pure culture is really a supposition that there is no interaction between these genotypes in exploiting the resources. . ." The critical experiment is to raise offspring from different aged females in competition with each other, as I report in this paper.

A common method for comparing competitive ability of two phenotypically indistinguishable strains is to compare both strains to a third, phenotypically distinct, "testor" strain (e.g., Santos et al. 1992). However, to extend the logic of Lewontin (1955), this assumes that the different genotypes interact with each other in a common fashion.

This assumption is unwarranted, unless there is a single major determinant of competitive success.

Studies on the bionomics of house fly larvae suggest that there are two particularly important phases in their development: initial medium conditioning by hatchlings and growth of the third (final) instar. Survival and adult size of house flies are determined by the performance of larvae during both of these phases. House fly larvae are bacterial feeders that actively condition their medium through the release of waste ammonium that stimulates yeast and bacterial growth (Bryant 1969; Schmidtmann and Martin 1992). Insufficient early larval density may result in poor medium conditioning and death of larvae through starvation or fungal takeover of the medium (Bryant and Sokal 1967). However, with very high initial density, high early mortality can lead to increased total survival and larger flies through reduced density during the growth phase of the final larval instar (Bryant and Sokal 1968; Kence and Jdeidi 1997). Flies developing at elevated larval density are smaller, take longer to develop, and have lower survival (Sullivan and Sokal 1963; and Bryant and Sokal 1967). Most of the negative effects of high density occur during the final larval instar, probably as a result of food depletion (Sullivan and Sokal 1963).

The outcome of larval competition in house flies can not be reliably predicted from performance in pure culture and it depends upon density, the identity of the competing strains, and the ratio of the strains (Sokal and Sullivan 1963; Bhalla and Sokal 1964; Sullivan and Sokal 1965). In the current study I confirm these results and extend them to the impact of maternal age on competitive ability. I tested genetically marked strains against each other to address a single main question: Are there maternal age

effects on competitive ability in house flies? This approach allowed me to address the following two corollaries: Do maternal age effects vary between strains? Are maternal age effects competitor dependent? The results are discussed with respect to the possible adaptive nature of maternal age effects in house flies and the use of testor strains to determine relative competitive ability.

Materials and methods

The Flies

The history and maintenance of my house fly colony was reported in McIntyre and Gooding (1995, Chapter 1). Three electrophoretically marked lines were selected from this colony. I used electrophoretic markers because they are more likely to be neutral than are morphological markers, some of which affect development and competition in house flies (Sokal and Sullivan 1963; Bhalla and Sokal 1964; Sullivan and Sokal 1965). The selected lines differed at either the glutathione reductase locus (*Gr*), the octanol dehydrogenase locus (*Odh*), or at both loci. The marked lines are C (homozygous Gr^s, Odh^f), D (homozygous Gr^s, Odh^s) and G (homozygous Gr^f, Odh^f). Polyacrylamide gel electrophoresis was used during selection and later to determine the origin of flies from the competition experiments. I used 7% gels at pH 8.9. Each gel, was stained in 25ml of 50 mM TRIS pH 7.2 containing 9mg NADP, 9mg nitro blue tetrazolium (NBT), 3mg phenazine methosulfate (PMS), plus substrate; for GSH this was 3mg reduced glutathione and for ODH 8 drops of octanol. Under these conditions the positions of the bands, relative to the bromophenol blue front (R_f) values were 0.70 for Gr^s, 0.74 for Gr^f, 0.35 for Odh^s and 0.39 for Odh^f. At *Odh* and *Gr* there were actually two very similar fast

alleles. Since I was interested only in using these as markers I did not distinguish between the fast alleles in these experiments.

Competition Experiments: Design and Setup

To compare the larval competitiveness of young and old house flies I used a modified replacement (substitution) series experiment (Mather and Caligari 1981; Novak et al. 1993) (Figure 3.1), i.e. the groups of interest were reared with each other at several ratios with the same overall density. To investigate maternal age effects, my design was more complex because 1) I can not simultaneously produce offspring from the same mother(s) at two different ages and 2) I can not distinguish offspring from the same kind of mother. For each pair-wise combination of marked lines I reared larvae from young and old females of each line in all possible combinations at five seeding ratios. Because of the fundamental difference between intra- and inter-group competition each experiment contains a logical subdivision into mixed cups (i.e., cups with larvae from two colonies; Exp. 1m, 2m, and 3m) and pure cups (i.e., cups with larvae from only one colony; Exp. 1p, 2p, and 3p).

In each experiment, 'young' and 'old' parent populations were established so the youngest and oldest flies within each age group differed in age by a maximum of 48 hours and the mean difference between flies from different age groups was 14 days. The left wings of a sample of 16 surplus females from each group were slide-mounted and measured as described below. Eggs were collected twice weekly, beginning at age seven days, from each group of mothers. The eggs used for the competition experiments were taken from 24 ± 1 day old 'old' mothers (the sixth egg collection) and 10 ± 1 day old 'young' mothers (the second egg collection). I chose these ages because 10 day old

females produce the largest eggs with the highest viabilities, and 24 day old females produce significantly smaller eggs with lower viabilities but females are not post-reproductive and have not begun to suffer significant mortality (Chapter 2).

Eggs were collected, on cotton soaked in evaporated milk, from 09:00 to 16:00 at which time they were separated from the adult flies. A sample of eggs was frozen at -20C in 0.85% saline for later measurement (eggs can be frozen for up to 3 months this way without significant alteration of size or shape [Appendix 1]). A second sample of 50 eggs was incubated for 24 hours at 25C. Hatch rate was recorded for these eggs. The remainder of the eggs were incubated, on the milk-soaked cotton, until 09:00 the following morning to allow hatching. Three replicate cups for each combination of line, age, and ratio were seeded with a total of 60 vigorous larvae in 3g of larval medium. This high larval density ensures that larvae experience stress and competition. In preliminary experiments these conditions resulted in ~70% larval mortality.

At emergence, each fly was frozen at -20C. Flies from the pure cups were dried for 72 hrs at 60C and weighed to the nearest 0.1mg. Both wings of all flies were mounted in Euparol, between two slides clamped with elastic bands to flatten the wings. Flies from mixed cups were electrophoresed to determine their parentage.

Wing measurement/dry mass regression

Four landmarks (the branching point of veins R_1 and R3, the intersection of veins C and R_1, the intersection of veins C and M_{1+2}, and the intersection of the posterior crossvein and vein M_{3+4} [landmarks, 1, 5, 9, and 14, Figure 4.1]) were digitized in x,y coordinates for the left wing of each fly using a dissecting microscope and camera lucida with a Summasketch III data tablet (Summagraphics, Seymour, Connecticut). The wing

centroid size (square root of the summed squared distance of all landmark coordinates from the mean x and y coordinates of the landmarks) was calculated and regressed against the teneral dry mass for flies from the pure cups. No significant effects of experiment, sex, line and maternal age on the slope of this regression were found. Accordingly a single regression, based on all available data, was used to estimate the teneral mass of the flies from the mixed cups.

Statistics

I chose to keep the analysis of pure and mixed cups entirely separate, since they are essentially different experiments. It is possible to partially combine the results into unbalanced analysis of variance (ANOVA) models (e.g., Novak et al. 1993), but it is not a completely satisfactory method and introduces several problems. Since the experimental unit is the replicate cup, and not each fly produced, I calculated a single value from the flies emerging from each cup for sex ratio, viability, teneral mass, and development time. To examine sex ratio I calculated the angular transformed proportion of male emergents for each replicate cup.

Viability in pure cultures was analyzed using angular transformed survival. Viability in mixed cups was analyzed using natural log transformed Haldane's $V = (a/(b + 1))/(A/B)$, where a and b are the number of adults emerging and A and B are the number of larvae seeded into the culture from each line (Haldane 1956; Santos et al. 1992). To reduce the number of cups for which I was unable to calculate V, because of missing values, I chose the line with the fewest number of cups with zero emergence for the numerators in the calculation of V.

For pure cups the use of mean mass was inappropriate since the same number of larvae were not competing throughout the experiment in each cup, given differences in larval mortality, and I was unable to use the number of emergents to correct for differences in effective larval density, since the assumptions of analysis of covariance were not met. I used natural log transformed total biomass in ANOVAs of line by age. Thus, I was assessing effects on productivity and not on fly size *per se*. For mixed cups I used the ratio of the mean mass of each type of fly. Ratios were calculated with the mean of the more successful line, in terms of viability, as the numerator. Mean development time was analyzed in pure cups since development time was not correlated with survival in these experiments. For mixed cups I used the ratio of the mean development time of each type of fly, calculated in the same fashion as the ratio of mean mass.

For each experiment I tested all variables in separate two-way (line by maternal age) and three-way (parental age line A by parental age line B by seeding ratio) ANOVAs for pure cups and mixed cups respectively. There were several mixed cups in the study that produced emergents from only one line. This forced an unbalanced analysis because of missing values. Therefore a portion of the total sums of squares could not be partitioned to any effect, interaction, or to the residual term. This precluded the estimation of two interaction terms in the analysis of Experiment 3m.

Results

Parental Differences

With the exception of a single contrast (Experiment 1, line D) there were no significant differences between the estimated masses of young and old females, but eggs from old females were significantly smaller than those from young females in four out of seven

cases after Bonferroni correction (Table 3.1). Eggs of old females had significantly lower viability than those of young females in two of seven comparisons, but the difference was in the same direction in all but one case (Table 3.1). After pooling data, egg hatch differed significantly between maternal ages (Young: 88.3%; Old: 76.6%; ($\chi^2 = 16.58$ $p < 0.001$, $df = 1$), lines (Line C: 93.0%; Line D: 76.7%; Line G: 80.5%; ($\chi^2 = 22.82$ $p < 0.001$, $df = 2$), and experiments (Exp 1: 88.0%; Exp 2: 80.5%; Exp 3: 80.0%; ($\chi^2 = 6.021$ $p = 0.049$, $df = 2$). In a randomized block ANOVA with line and maternal age as factors and with experiments as blocks, mothers for experiment 1 were slightly smaller than were mothers for the other experiments ($F_{2,216} = 4.17$, $p = 0.016$, $R^2 = 0.036$), but produced slightly larger eggs ($F_{2,342} = 8.82$, $p = 0.0002$, $R^2 = 0.047$). There were no line, age, or interaction effects for mass or egg volume.

Overall Survival and Sex Ratio

Total survival in experiment 3(m+p) was 12.7%, much lower than the survival rates of 26.0 and 25.3% from experiments 1(m+p) and 2(m+p) respectively. In experiment 3m there were 10 cups that produced no adult and seven cups that produced flies from only one of the parental lines. The net result was greater apparent variability within groups and uneven sample sizes within cells of the ANOVA.

Of the 1,910 flies that emerged in these experiments, 51.5% were male and the sex ratio did not depart significantly from 0.5 ($\chi^2 = 1.913$, $df = 1$, $P = 0.166$). Within experiments and in the pooled data there were no significant effects of experiment, line, or maternal age on sex ratio (analyses not shown). Accordingly, sex was excluded from the analyses of viability, mass, or development time.

Pure Cups

In pure cups, offspring of old mothers had higher viability and greater total biomass than did offspring of young mothers (Table 3.2, Figure 3.2). Line C had higher survival than line D in experiment 1p, and lower survival than line G in experiment 3p, but in experiment 2p survival of line G was only marginally higher than that of line D (Table 3.2, Figure 3.2). The maternal age effect differed significantly between lines in experiments 2p and 3p (Table 3.2, Figure 3.2). Line and line by maternal age interaction did not affect biomass except in experiment 1p, in which early line C larvae out-produced early line D larvae. There was no effect of maternal age on development time in experiments 1p and 2p, but in experiment 3p late offspring developed more rapidly than early offspring and this difference was much greater in line C than in line D (Table 3.2, Figure 3.2).

Mixed Cups

Maternal age affected viability in five out of six cases in the three mixed cup experiments, but the direction of the effect differed between lines and experiments (Table 3.3, Figure 3.3). Figure 3.3 contains plots of the performance of each maternal age for each line, relative to the two maternal ages from the competitor line. These plots are modifications of standard replacement plots (de Wit 1960) designed to facilitate comparison of competitive differences between maternal age classes.

Late C was better than early C when in competition with late D but there was no difference when competing with early D (Figure 3.3A). Against line G, late C did better than early C, especially against late G (Figure 3.3E). Early D was more competitive than late D against both ages of line C in experiment 1m (Figure 3.3B), but in experiment 2m

late D was better than early D against both age classes of G, with a non-significant trend for the difference to be greater at lower seeding ratios of G:D larvae (Figure 3.3D). Early G was slightly, but significantly, better than late G against both age classes of D (Figure 3.3C). In experiment 3m early G were marginally better than old G in competition with both age classes of C and there was a greater deficit of C emergents when the proportion of C larvae was high (Figure 3.3F).

There were few significant effects of maternal age on competitor mass and development time ratios. The pattern of effects is consistent with the observation that development time is negatively correlated with mass in these experiments ($r = -0.25$, $n = 1900$, $p < 0.0001$). Early line D offspring achieved larger sizes and completed development more rapidly than their line C competitors (especially against late line C), but late D offspring were smaller and developed more slowly than their line C competitors. In experiment 1m there was a small effect of seeding ratio on mass ratio, with line D 9% smaller than line C at the 1:1 seeding ratio, but > 6% larger at the other ratios (Table 3.3, Figure 3.4). In experiment 2m, even though there were no effects on mass ratios, young line G flies completed development more rapidly than late line G did, relative to their line D competitors. There was also a significant interaction between seeding ratio and maternal age of line G for development time. Early G flies developed 24% more slowly than their D counterparts at the high G:D seeding ratio while late G flies took 3% longer to develop than did their D counterparts at this ratio. At the other seeding ratios the development time ratios did not differ between maternal ages of line G (Table 3.3, Figure 3.4). In experiment 3m late line G flies were significantly larger relative to their line C competitors than were early G flies. There was also an interaction

between seeding ratio and maternal age of line G for mass ratio. Late G flies were almost 40% heavier than line C flies when the G:C seeding ratio was 1:3, but at other ratios and maternal ages G flies were approximately 16% lighter than their C competitors (Table 3.3, Figure 3.4).

Discussion

I have demonstrated that old female house flies can produce larvae that are more competitive, at high density, than larvae from early females. In most cases, maternal age was positively associated with larval viability, mass, and development rate. Competitive ability and the relationship of maternal age to competitive ability were strain specific. Strain and mixture specific nature of competitive ability has been previously demonstrated in house flies and is strong evidence that larval competitive ability is under genetic control (Sokal and Sullivan 1963; Bhalla and Sokal 1964; Sullivan and Sokal 1965). The results suggest that maternal age effects on larval competitive ability are also under genetic control.

Thus, maternal age effects in larval house flies are more than simple constraints of age-related physiological deterioration and they may be adaptive in nature. House fly populations increase throughout the summer in temperate areas (Black and Krafsur 1986). This implies that females will generally oviposit into more crowded situations as they age. Since larval density has strong life history consequences, females may experience evolutionary pressure to produce better competitors at advanced ages and the positive maternal age effects I observed on larval competitive ability may be a response to this pressure.

The basis of the competitive advantage of late larvae is unknown. Late larvae were less active than early larvae at the beginning of these experiments (qualitative observation) and they came from smaller eggs (Table 3.1). Egg size is not related to egg energy content in house flies (Chapter 1) so late hatchlings may have proportionately larger energy reserves. If initial nutrient levels are very low then more active larvae might do well at low density since they will condition the medium more rapidly (assuming that activity correlates with medium conditioning) and will search for and find sufficient food to keep them alive until bacterial growth accelerates. At high larval density, active larvae might exhaust their hatchling energy reserves and the initial food supply, and starve before medium conditioning takes effect. Less active larvae might be initially less prone to starvation in poor media and could outlast more active larvae. However, less active larvae might be at a disadvantage in nutrient rich media and also less able to condition nutrient poor media in the absence of more active larvae. This explanation fits the data for late D offspring which performed poorly in pure culture, but performed well when a few competitors, that performed well in pure culture, were present (Table 3.3, Figure 3.3). Similarly, *ge* house fly mutants are poor nutrient conditioners that perform well in the presence of larvae with normal nutrient conditioning activity (Bhalla and Sokal 1964; Bryant 1969)

If the Gr^f or Odh^5 allele (or closely linked genes) affect the rate of ageing in female house flies then this might explain line differences in maternal age effects. But line G (homozygous for Gr^f), which was least affected by maternal age in competition, exhibited the largest maternal age effects on egg size and viability (Table 3.1). Line D (homozygous for Odh^5), exhibited less difference in egg hatch than other lines, and egg

size differences were also small, while D was the only line with early larvae to dramatically outperform old larvae in any experiment (1m). In this line, less age related change in egg parameters may be related to less extreme maternal age effects upon larvae, but this does not explain the very weak performance of offspring of young D mothers in pure culture (experiments 1p and 2p).

One of the most salient features of this study is that the competitive effects of line, maternal age, and competition ratio could not be generalized. Rather competitive ability varied among combinations of lines and also between pure cups and mixed cups as has been previously demonstrated in house flies and *Drosophila* spp. (Lewontin 1955; Lewontin and Matsuo 1963; Sokal and Sullivan 1963; Bhalla and Sokal 1964; Sullivan and Sokal 1965). The difficulty in predicting performance in mixtures from performance in pure culture has led to the use of testor strains to indirectly determine relative competitive abilities of genotypes (e.g., Santos et al. 1992). But, as argued in the introduction, this requires that either there is a single major determinant of competitive success or that the different genotypes interact with each other in a common fashion. These assumptions are usually not demonstrated prior to use of the testor method. In *Drosophila*, for which the testor method is frequently used (e.g. Mueller 1988; Santos et al. 1992; James and Partridge 1998), feeding rate is often highly correlated with larval success and is sometimes taken as the primary determinant of larval success (Bakker 1961; Mueller 1988). However, *D. melanogaster* strains are differentially susceptible to metabolic waste accumulation (Weisbrot 1966; Botella et al. 1985), and sub-populations exhibit distinct, genetically based strategies for the utilization of the larval medium before and after larval density and waste concentrations increase (Borash et al. 1998). In

larval competition studies using *Drosophila*, yeast growth has been viewed as an undesirable complication and many larval culturing methods provide killed yeast as food in sterile media (Bakker 1961; Nunney 1983). This eliminates a facet of the hatchling/microbial interaction known to be important in species with similar larval ecology. Thus, in *Drosophila* there is more than a single factor important in determining larval competitive ability. It is therefore inadvisable to adopt the testor stock method unless the factors acting in a particular contrast are known to be identical to those in other comparisons of interest.

Would the testor strain approach have been useful here? In the competition experiments (mixed cups only) using larval survivorship as the measure of success and ignoring maternal age effects, there are three independent rankings of larval competitive ability: D>C, G>>D, and G>C. If line C had been the testor stock I would have predicted that G~D, which was not the result found in experiment 2m. If line D were the testor strain the resulting prediction is that G>>C, which is in the correct direction, but the observed magnitude of the difference is smaller than predicted. If line G were the testor strain, the prediction that C>D is in the wrong direction. Thus I have a correct qualitative prediction in only a single case, but the magnitude is less than expected. The use of testor strain competition experiments is clearly suspect in house flies and probably also in other species with complex larval competition dynamics. I invite detractors to develop analytical models demonstrating that the testor methodology is sound in cases with complex larval dynamics. Existing models of larval competition consider only the amount of food available and the feeding rate (de Jong 1976; Nunney 1983), which reduces to a single larval trait (feeding rate).

Table 3.1. Mean (±SE) maternal teneral mass, egg size, and hatch rate for young and old female house flies from lines used in three competition experiments. P values are from two-tailed t-tests for maternal and egg size, before Bonferroni correction. For hatch rate p values are from χ^2 tests, before Bonferroni correction. Sample sizes for each group are 16, 25, and 50 for estimates of female mass, egg volume, and hatch rate respectively.

		Female Mass (mg)			Egg Volume (mm^3 x 10^3)			Hatch Rate (%)		
Experiment	Line	Young	Old	P	Young	Old	P	Young	Old	P
1	C	4.4 ± 0.12	4.0 ± 0.12	0.031	56.2 ± 0.88	51.5 ± 1.12	0.002*	96	94	0.646
1	D	3.3 ± 0.12	4.0 ± 0.10	0.001*	55.9 ± 1.12	51.2 ± 1.34	0.001*	76	86	0.202
2	D	4.2 ± 0.11	4.0 ± 0.11	0.152	50.9 ± 1.28	53.0 ± 1.22	0.255	86	72	0.086
2	G	4.0 ± 0.12	4.2 ± 0.11	0.297	51.8 ± 1.14	45.2 ± 0.89	0.001*	98	66	0.001*
3	C	4.0 ± 0.11	3.9 ± 0.10	0.626	52.1 ± 1.13	48.9 ± 1.13	0.048	94	88	0.295
3	D	4.6 ± 0.17	4.2 ± 0.14	0.086	47.2 ± 1.07	49.0 ± 1.23	0.260	78	62	0.081
3	G	4.1 ± 0.09	4.2 ± 0.08	0.394	55.0 ± 0.90	49.6 ± 1.48	0.003*	90	68	0.007*

*P < 0.05 after sequential Bonferroni correction

Table 3.2. Analyses of Variance of angular transformed survival, natural log transformed biomass, and log transformed mean development time for flies from pure cups seeded with offspring of young and old females from each line in three pairwise competition experiments. Data is presented in Figures 3.3 and 3.4.

	Viability				Biomass				Development Time			
Source	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P
Experiment 1p (C & D)												
Line	1	0.104	24.36	0.0011	1	4.939	40.22	0.0002	1	0.000	0.00	0.9819
Maternal Age	1	0.230	53.98	0.0001	1	5.756	46.88	0.0001	1	0.148	4.63	0.0636
Line*Age	1	0.000	0.04	0.847	1	5.085	41.41	0.0002	1	0.027	0.84	0.3855
Error	8	0.004			8	0.123			8	0.032		
Experiment 2p (G & D)												
Line	1	0.024	1.63	0.237	1	0.177	0.47	0.5169	1	0.052	1.03	0.3503
Maternal Age	1	0.105	7.09	0.0287	1	3.729	9.97	0.0196	1	0.012	0.24	0.6406
Line*Age	1	0.206	13.92	0.0058	1	0.486	1.30	0.298	1	0.014	0.27	0.6199
Error	8	0.015			6	0.374			6	0.051		
Experiment 3p (C & G)												
Line	1	0.090	31.69	0.0005	1	0.792	3.51	0.098	1	0.001	0.31	0.5927
Maternal Age	1	0.141	49.85	0.0001	1	5.136	22.74	0.0014	1	0.149	65.37	0.0001
Line*Age	1	0.131	46.46	0.0001	1	0.013	0.06	0.815	1	0.088	38.54	0.0003
Error	8	0.003			8	0.226			8	0.002		

Table 3.3. Three-way factorial analyses of variance of Haldane's unbiased viability estimate (V, Haldane 1957), and ratios of mean masses and development times for competition experiments testing the effects of maternal age on larval competitive ability. For calculation of V lines D, G, and C are used in the numerator of the constituent ratios and mass and development time ratios were calculated as line D/C, G/D, and G/C in experiments 1m, 2m, and 3m respectively (see text). Maternal age of each line is entered into the ANOVA separately to allow comparison of maternal age effects between lines. Degrees of freedom differ between variables and experiments, depending upon the number of cups which produced no adults from one or both lines. Data are presented in Figures 3.3 and 3.4.

	Viability				Ratio of Masses				Ratio of Development Times			
Source	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P
Experiment 1m (C & D)												
Age(C)	1	4.65	15.66	0.0006	1	0.042	2.06	0.1653	1	0.001	0.06	0.8093
Age(D)	1	15.91	53.54	0.0001	1	0.501	24.6	0.0001	1	0.080	8.66	0.0075
Age(C)*Age(D)	1	1.97	6.62	0.017	1	0.051	2.53	0.126	1	0.047	5.11	0.034
Ratio	2	0.23	0.76	0.48	2	0.076	3.72	0.04	2	0.017	1.81	0.19
Age(C)*Ratio	2	0.72	2.43	0.11	2	0.017	0.82	0.45	2	0.001	0.13	0.88
Age(D)*Ratio	2	0.14	0.48	0.63	2	0.036	1.77	0.19	2	0.024	2.6	0.10
Age(C)*Age(D)*Ratio	2	0.10	0.35	0.71	1	0.001	0.04	0.83	1	0.001	0.14	0.72
Error	24	0.30			22	0.020			22	0.009		

Experiment 2m (G & D)												
Age(D)	1	10.04	42.39	0.0001	1	0.142	3.19	0.1046	1	0.001	0.63	0.4448
Age(G)	1	1.13	4.77	0.039	1	0.006	0.14	0.712	1	0.032	13.99	0.004
Age(G)*Age(D)	1	0.33	1.41	0.25	1	0.004	0.08	0.78	1	0.000	0.03	0.87
Ratio	2	0.11	0.47	0.63	2	0.064	1.43	0.28	2	0.004	1.64	0.24
Age(D)*Ratio	2	0.70	2.96	0.071	1	0.147	3.3	0.0992	1	0.001	0.25	0.627
Age(G)*Ratio	2	0.69	2.9	0.07	2	0.001	0.03	0.97	2	0.018	7.76	0.01
Age(G)*Age(D)*Ratio	2	0.61	2.56	0.10	1	0.030	0.67	0.43	1	0.000	0.05	0.83
Error	24	0.24			10	0.045			10	0.002		
Experiment 3m (C & G)												
Age(C)	1	22.08	15.81	0.0014	1	0.009	0.46	0.5148	1	0.014	2.01	0.1865
Age(G)	1	5.70	4.08	0.063	1	0.140	6.76	0.0265	1	0.055	8.01	0.0179
Age(C)*Age(G)	1	8.00	5.73	0.031	0	not estimable			0	not estimable		
Ratio	2	16.84	12.06	0.0009	2	0.062	2.99	0.0961	2	0.006	0.81	0.4719
Age(C)*Ratio	2	1.07	0.77	0.48	1	0.028	1.37	0.27	1	0.020	2.86	0.12
Age(G)*Ratio	2	0.44	0.32	0.73	1	0.136	6.58	0.03	1	0.027	3.97	0.07
Age(C)*Age(G)*Ratio	2	1.24	0.88	0.43	0	not estimable			0	not estimable		
Error	14	1.40			10	0.021			10	0.007		

Figure 3.1. Design of competition experiments: Separate experiments were conducted for each combination of the three marked lines. In each experiment I reared larvae from young and old females of each genotype separately with larvae from each maternal age class in the other line. Experiment 2 consisted of the following combinations: young D (Dy) with young G (Gy), Dy with old G (Go), old D (Do) with Gy, and Do with Go. Within each of these combinations I tested five larval seeding ratios (1:0, 3:1, 1:1, 1:3, and 0:1). There were three replicate cups within each treatment that were seeded with a total of 60 larvae in three grams of larval medium.

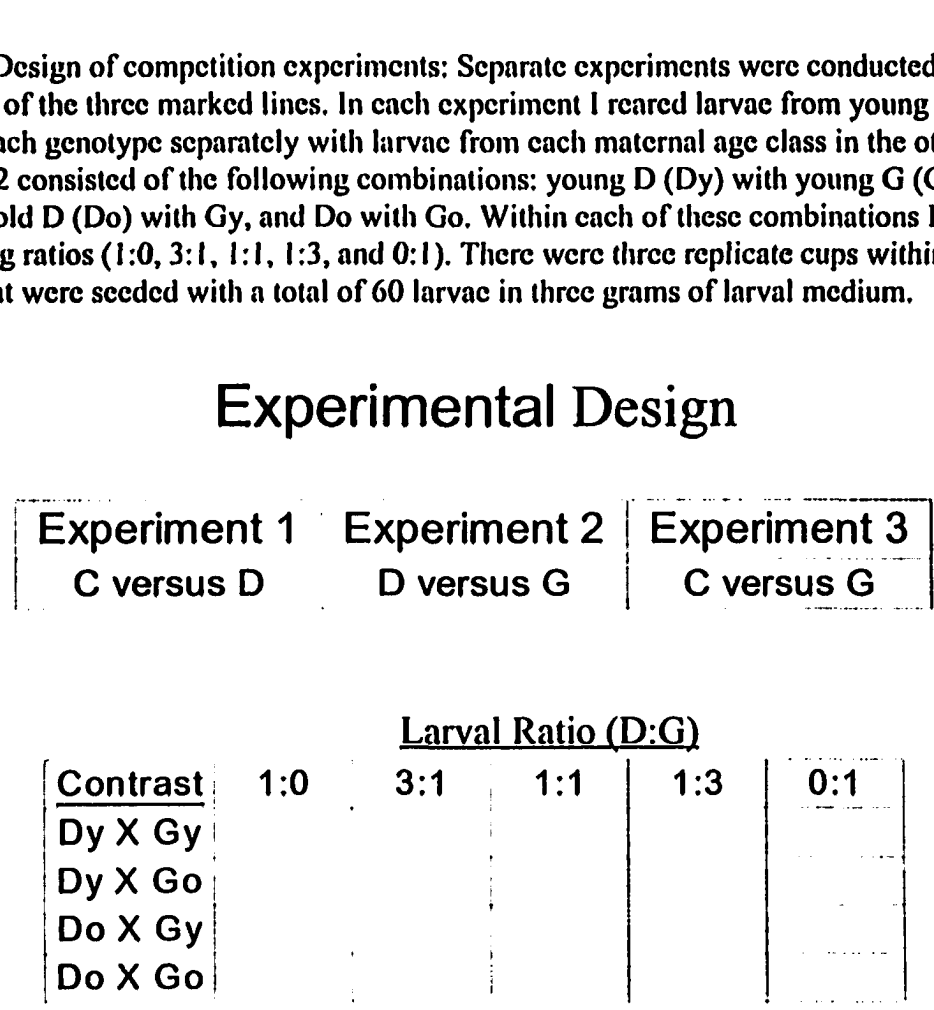


Figure 3.2. Mean (±SE) performance in pure culture of 60 larvae/3g of larval medium from young and old mothers. For experiment 1p: A) proportion surviving, B) total biomass, C) mean development time. For experiment 2p: D) survival, E) biomass, and F) development time. For experiment 3p: G) survival, H) biomass, and I) development time.

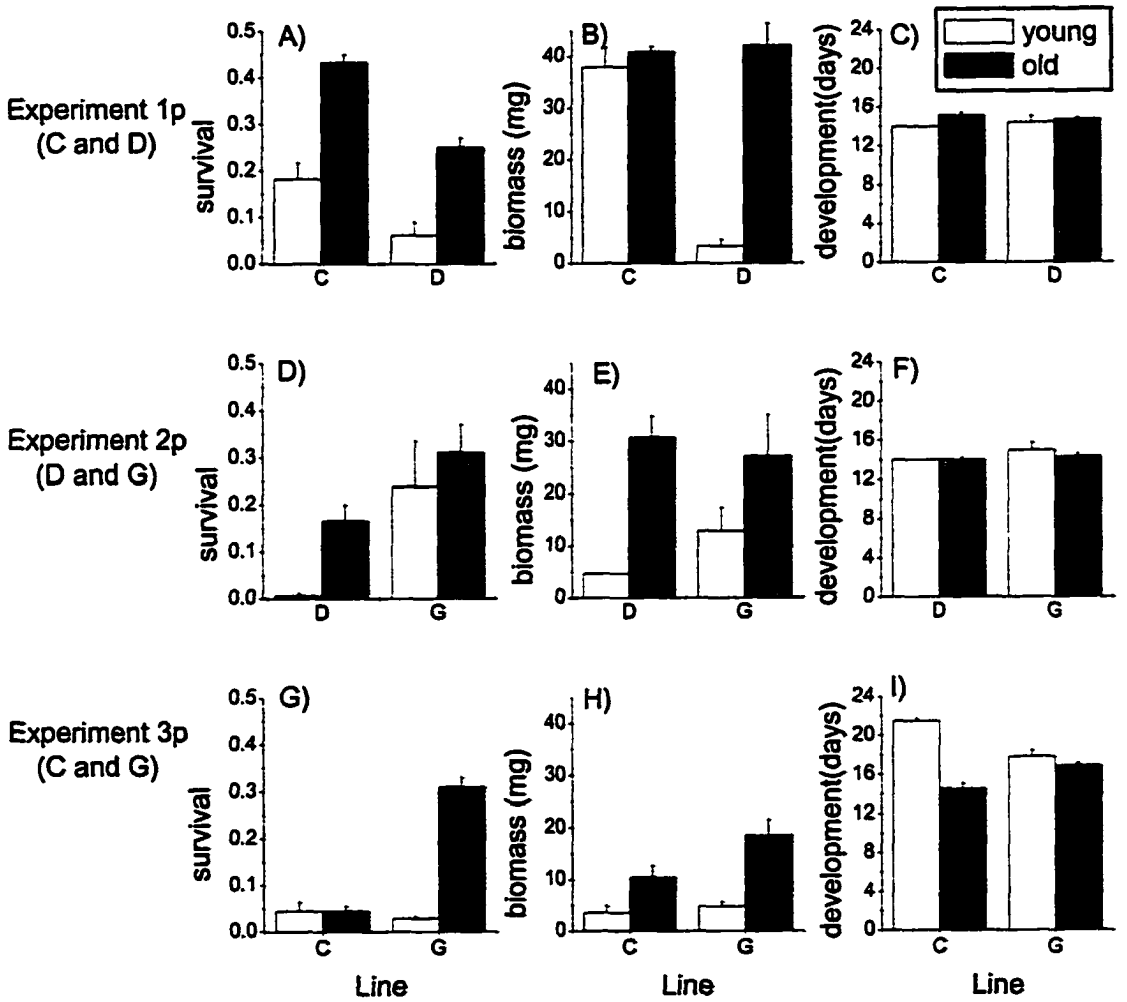


Figure 3.3. Modified replacement plots (see text) of competition between larvae produced by house flies from two maternal age classes from three genetically selected lines (C, D, and G). Each plot shows the performance (survivorship) of early and late offspring from one line in competition with early and late offspring from another line. A) performance of C offspring in experiment 1m. B) performance of D offspring in experiment 1m. C) performance of G offspring in experiment 2m. D) performance of D offspring in experiment 2m. E) performance of C offspring in experiment 3m. F) performance of G offspring in experiment 3m. Squares and circles are the performance of late and early offspring respectively. Solid and hollow symbols indicate competition against early and late competitor offspring respectively. In the absence of maternal age and line effects the expected proportion of emergents is equal to X in plots A, C, and E and (X - 1) in plots B, D, and F, where X is the x-axis value.

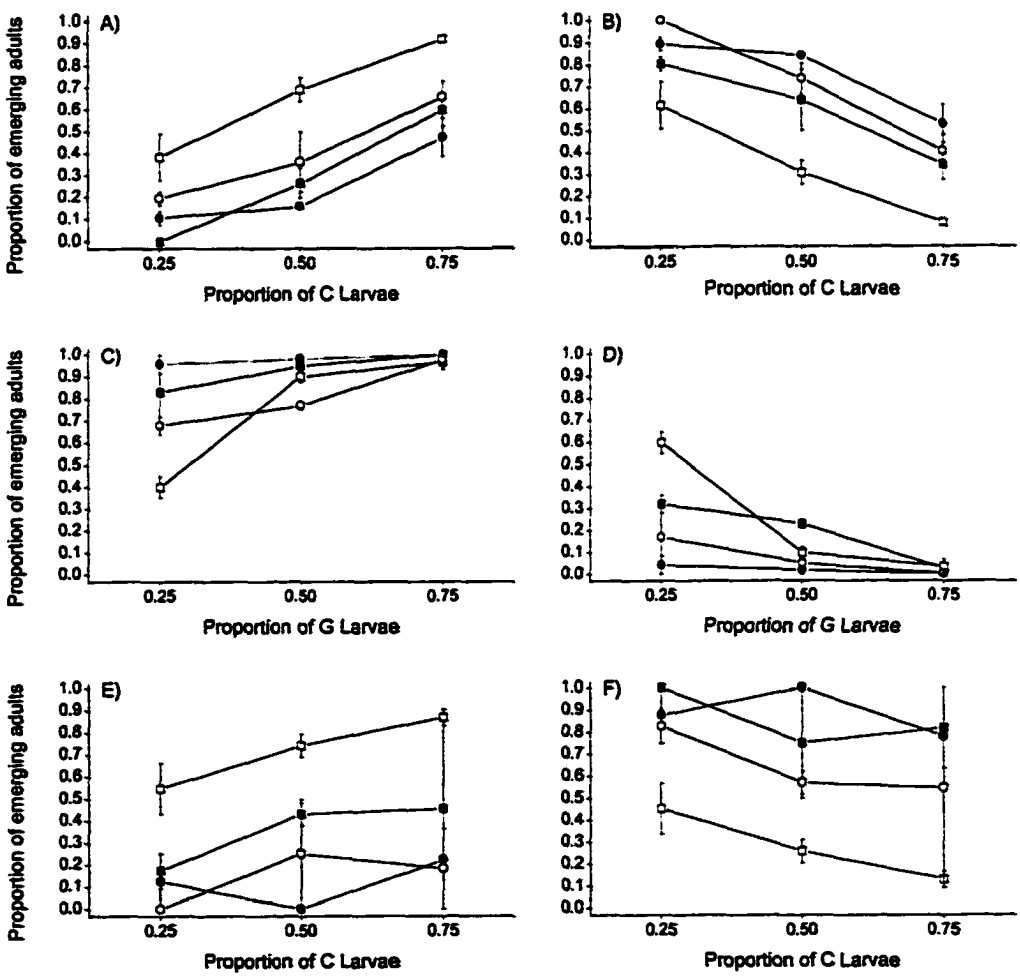
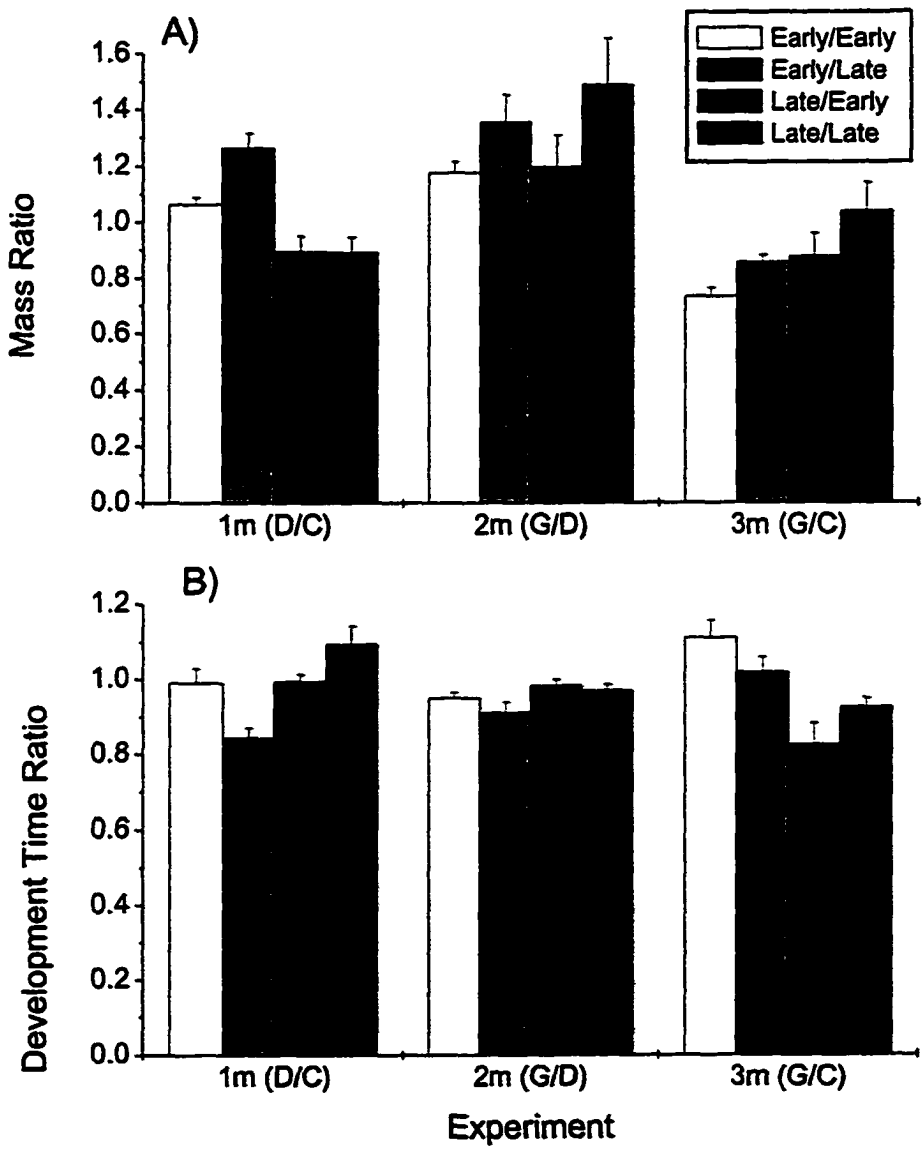


Figure 3.4. Mean (±SE) of ratios of mean fly mass (A) and mean development time (B) for flies from mixed cups in each of three competition experiments. In each experiment the ratio is the mean for the line indicated by the first letter in the X-axis label over the mean for the line indicated by the second letter. The legend indicates the maternal age class comparisons within each experiment (e.g. the leftmost bar in each plot is the mean ratio of values for larvae from young D mothers over the values for larvae from young C mothers).



References

Bakker, K. 1961. An analysis of factors which determine success in competition for food among larvae of *Drosophila melanogaster*. Archs Neerl. Zool., **14**: 200-281.

Bhalla, S. C. and Sokal, R. R. 1964. Competition among genotypes in the housefly at varying densities and proportions (the green strain). Evolution **18**:312-330.

Black IV, W. C. and Krafsur, E.S. 1986. Population biology and genetics of winter house fly (Diptera: Muscidae) populations. Ann. Ent. Soc. Am. **79**:636-644.

Borash, D. J., Gibbs, A. G., Joshi, A. and Mueller, L. D. 1998. A genetic polymorphism maintained by natural selection in a temporally varying environment. Am. Nat. **151**:148-156.

Botella, L. M., Moya, A., Gonzalez, M. C. and Mensua, J. L. 1985. Larval stop, delayed development and survival in overcrowded cultures of *Drosophila melanogaster*: effect of urea and uric acid. J. Insect Physiol. **31**: 179-185.

Bryant, E. H. 1969. The fates of immatures in mixtures of two housefly strains. Ecology **50**:1049-4069.

Bryant, E. H. and Sokal, R. R. 1967. The fate of immature housefly populations at low and high densities. Res. Popul. Ecol. **9**: 19-44.

Bryant, E. H. and Sokal, R. R. 1968. Genetic differences affecting the fates of immature housefly populations at two densities. Res. Popul. Ecol. **10**: 140-170.

De Jong, G. 1976. A model of competition for food. I. Frequency dependent viabilities. Am. Nat. **110**: 1013-1027.

De Wit, C. R. 1960. On competition. Versl. Landbouwkd. Onderz. **66**: 1-82.

Denlinger, D. L. 1998. Maternal control of fly diapause. In: Mousseau, T. A. and Fox, C. W. (eds) Maternal Effects as Adaptations, pp. 275-287. Oxford Univ. Press, New York.

Haldane, J. B. S. 1956. Estimation of viabilities. J. Genet. **54**: 294-296.

James, A. C. and Partridge, L. 1998. Geographic variation in competitive ability in *Drosophila melanogaster*. Am. Nat. **151**: 530-537.

Kence, M. and Jdeidi, T. 1997. Effect of malathion on larval competition in house fly (Diptera: Muscidae) populations. J. Econ. Entomol. **90**: 59-65.

Lewontin, R. C. 1955. The effects of population density and composition on viability in *Drosophila melanogaster*. *Evolution* **9**, 27-41.

Lewontin, R. C. and Matsuo Y. 1963. Interaction of genotypes detemining viability in *Drosophila busckii*. Proc. Natl. Acad. Sci. USA **49**: 270-278.

Mather , K. and Caligari, P. D. S. 1981. Competitive interactions in *Drosophila melanogaster*. II Measurement of competition. Heredity **46**: 239-254.

McIntyre, G. S. and Gooding, R. H. 1995. Pteridine accumulation in *Musca domestica*. J. Insect Physiol **41**: 357-368.

McIntyre, G. S. and Gooding, R. H. 1998. Effect of maternal age on offspring quality in tsetse (Diptera: Glossinidae). J. Med. Ent. **35**: 210-215.

Mousseau, T. A. and Dingle, H. 1991a. Maternal effects in insect life histories. Annu. Rev. Entomol. **36**: 511-534.

Mousseau, T. A. and Dingle, H. 1991b. Maternal effects in insects: examples, constraints, and geographic variation. In: Dudley, E. C. (ed) The Unity of Evolutionary Biology, vol. II, pp. 745-761. Dioscoridies Press, Portland.

Mousseau, T. A. and Fox, C. W. 1998. Maternal Effects as Adaptations. Oxford Univ. Press, New York.

Mueller, L. D. 1988. Evolution of competitive ability in *Drosophila* by density-dependent natural selection. Proc. Natl. Acad. Sci. USA **85**: 4383-4386.

Murphy, D. D., Launer, A. E. and Ehrlich, P. R. 1983. The role of adult feeding in egg production and population dynamics of the checkerspot butterfly *Euphydryas editha*. Oecologia **56**: 257-263.

Novak, M. G., Higley, L. G., Christianssen, C. A. and Rowley, W. A. 1993. Evaluating larval competition between *Aedes albopictus* and *A. triseriatus* (Diptera: culicidae) through replacement series experiments. Environ. Entomol. **22**: 311-318.

Nunney, L. 1983. Sex differences in larval competition in *Drosophila melanogaster*: The testing of a competition model and its relevance to frequency-dependent selection. Am. Nat. **121**: 67-93.

Rossiter, M. C. 1991. Maternal effects generate variation in life history: consequences of egg weight plasticity in the gypsy moth. Funct. Ecol. **5**:386-393.

Santos, M., Fowler, K. and Partridge, L. 1992. On the use of tester stocks to predict the competitive ability of genotypes. Heredity **69**:489-495.

Schmidtmann, E. T. and Martin, P. A. W. 1992. Relationship between selected bacteria and the growth of immature house flies, *Musca domestica*, in an axenic test system. J. Med. Ent. **29**: 232-235.

Sokal, R. R. and Sullivan, R. L. 1963. Competition between mutant and wild-type house-fly strains at varying densities. Ecology **44**: 314-322.

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

Sullivan, R. L. and Sokal, R. R. 1965. Further experiments on competition between strains of house flies. Ecology, **46**, 172-182.

Weisbrot, D. R. 1966. Genotypic interactions among competing strains and species of *Drosophila*. Genetics **53**: 427-435.

Chapter 4: Independent response of size FA and shape FA to two developmental stresses, larval density and maternal age

Introduction

Fluctuating asymmetry (FA, small random departures from bilateral symmetry) has been proposed as a sensitive indicator of organismal quality that may reveal quality differences between groups of organisms, before such differences are detectable using conventional fitness measures (Leary and Allendorf 1989; Graham et al. 1993). Use of FA as an indicator of quality is based on the putative relationship of FA and developmental stability (DS, the ability of a bilaterally symmetrical organism to develop identical mirror image forms on both sides). FA is thought to result from a lack, or impairment, of DS, which is jointly determined by the degree of genetic control (not to be confused with canalization [see Zhakarov 1992]) of the developing organism, or trait, and the level of developmental stress experienced (reviewed in Palmer 1996).

For FA of individual traits to be a useful indicator of organism-wide traits such as fitness, FA must accurately reflect DS, but how DS can be estimated from FA is currently under debate (Whitlock 1996, 1998; Van dongen 1998). Much of this debate stems from empirical observations that FA is often uncorrelated among traits within individuals. When FA is uncorrelated among traits within individuals its use to estimate DS may still be possible by calculating repeatabilities of FA of different traits (Whitlock 1996, 1998; Van dongen 1998). However, these methods can lead to widely divergent estimates of DS based on different trait sets, and they do not apply to traits with correlated development, thus eliminating a large subset of possible traits, especially since appendages developing

from distinct imaginal discs have correlated development and FA (Klingenberg and Nijhout 1998; Van Dongen et al. 1999).

A logical step towards understanding organism-wide DS, is to examine structure-wide DS by quantifying within structure correlations of trait FA. Within structures we expect complex patterns of trait FA correlations, regardless of the organism-wide asymmetry or DS. Since trait, or landmark, locations are related on complex structures we expect patterns of FA covariation among traits to depend, in part, upon the tightness of the developmental relationship of traits (Leamy 1993; Leamy et al. 1997; Klingenberg and McIntyre 1998). In tsetse wings, closely related structures (e.g., location of opposite ends of crossveins) share common patterns of both inter-individual shape variation and shape FA variation (Klingenberg and McIntyre 1998; but see Debat et al. 2000). However, final form is a combination of size and shape and it is differences in this combination that result in FA and in covariation of FA of traits in bilaterally paired structures. Thus the relationship of overall shape FA and size FA is more relevant to our understanding of the FA-DS relationship than is the relationship of the FA of specific linear traits within a structure. Recently Procrustes superimposition techniques that allow separate analysis of size and shape variation have been modified to study FA (Bookstein 1991; Auffray et al. 1996; Smith et al. 1997; Klingenberg and McIntyre 1998). If size FA and shape FA are loosely or uncorrelated within structures, then our ability to estimate structure-wide, much less organism-wide, DS is called into question.

Even if FA of two traits is correlated, their use to estimate organism-wide DS and quality will be confounded if their FA levels respond predictably, but independently or in different directions, to developmental stress. Stress during development will lead to an

increase in FA if it causes a decrease in DS (i.e., the stress induced developmental noise exceeds the buffering capacity of the developing organism). While closely related traits can respond to stresses very differently (e.g., Held 1990) many studies report a relationship between FA and stress, but which traits will exhibit elevated FA in response to specific stresses is generally difficult to predict (Leung and Forbes 1996; Bjorksten et al. 2000). In this context the stress response of shape and size asymmetry of a single structure is particularly interesting. If different aspects of the same structure respond independently to particular stresses it bodes poorly for the use of FA as an indicator of overall DS.

I used Procrustes techniques to determine the effects of maternal age and larval density upon house fly wing FA. Offspring of old female insects, i.e., late offspring, are usually inferior performers, relative to the offspring of young female insects, i.e., early offspring. Late offspring are often smaller as hatchlings, arising from smaller eggs with lower average viability, than early offspring do. They can also have decreased growth rates and larval viability (for reviews see Mousseau and Dingle 1991a, b; Roff 1992; Mousseau and Fox 1998; Chapter 1). However, maternal age effects, and other maternal effects, generally become attenuated as the offspring age and environmental effects reduce differences due to maternal effects (Mousseau and Dingle 1991a, b). From the attenuation of maternal age effects it is unclear whether or not the surviving adult offspring of old females are really any different from the surviving offspring of young females.

In my study of maternal age effects on house flies, *Musca domestica* L., I found that eggs of old females do not differ in total energy content, even though they are

smaller and have lower hatch rate (Chapter 2). In addition, in direct competition at high larval density, which decreases house fly survival, size, and development rate (Sullivan and Sokal 1963), late offspring often outperform early offspring (Chapter 3). In house flies, surviving offspring of old females therefore do not appear to have lower quality than those of young females, even though they arise from smaller eggs with lower hatch rates. Altered FA levels may thus provide indications of maternal age effects on adult offspring quality, although empirical studies provide equivocal evidence for maternal age effects on FA in insects (Parsons 1962; Wakefield et al. 1994; McIntyre and Gooding 1998; Klingenberg and McIntyre 1998).

I used Procrustes techniques (Dryden and Mardia 1998; Klingenberg and McIntyre 1998) to determine the effects of maternal age and larval density upon house fly wing FA and then compared the responses of shape FA and size FA to these stresses.

Materials and Methods

Flies and Wing Measurements

The history and maintenance of the house fly colony is described in McIntyre and Gooding (1995; Chapter 2). Eggs were collected twice per week from a large group of females, reared from 10 day old females and maintained under benign conditions. Eggs were used to seed two replicate cups at two larval densities (0.03 and 0.09ml eggs/30g medium, i.e., approximately 6 and 18 eggs/g medium) on days 7, 14, 21, 28, and 35 of the experiment. On day 35 there were only enough eggs to seed a single high density cup. After emergence, 15 males and 15 females were chosen haphazardly from all high-density cups and from both low-density cups from days 14 and 28. In cups seeded on days 28 and 35 there were fewer emergents, so I used all available flies. Wings from the

selected flies were mounted in Euparol and clamped, with elastic bands, between two slides to flatten them.

Two replicate measurements (taken blind and on different days) were made of 14 landmarks on each wing (Figure 4.1). Landmark locations were digitized using a dissecting microscope and camera lucida with a Summasketch FX data tablet (Summagraphics, Seymour, Connecticut). To estimate the precision of this apparatus, 10 measurements were made of a 1.0 mm stage micrometer. The mean (SE) of these measurements was 1.000 ± 0.0027 mm. I had previously measured a set of wings normally, with reversed right-left orientation, and upside down on the microscope stage to verify that orientation did not affect any of the parameters analyzed below (unpublished data).

Fluctuating asymmetry analyses

As a measure of overall wing size I used centroid size (the square root of the summed squared distances of all landmarks from the centroid [mean landmark position]). Analysis of ideal behavior for FA of centroid size followed Palmer (1994) and used standard statistical tests since centroid size is a well-behaved univariate quantity. However, when testing for differences in size FA between samples I used permutation tests as described below. Significance of FA above measurement error was tested with a mixed model ANOVA of individual (random) by side (fixed), with the significance of side tested against the interaction indicating presence or absence of directional asymmetry, DA. Skewness and kurtosis were calculated to assess departures from normality. Size dependence of FA was tested using regressions of unsigned centroid size FA, |R-L|, on mean centroid size. All assessments of the presence of ideal FA were

conducted separately on males and females for each replicate within each treatment and were subjected to the sequential Bonferroni correction. I also tested for differences in size FA between replicate cups before pooling cups for comparison of treatment effects on FA (Appendix 3).

Procrustes analysis of asymmetry using landmark data (described in detail by Klingenberg and McIntyre [1998]) consists of 4 basic steps: (1) reflection of the left (or right) side to align corresponding landmarks between sides; (2) scaling each configuration to unit centroid size; (3) superimposition of centroids of all configurations; and (4) rotation of configurations around the centroid to achieve an optimal fit of landmarks (I used generalized orthogonal least squares fit [Bookstein 1991]). Using the aligned coordinates, shape asymmetry corresponds to the deviations of right and left landmarks from each other across individual flies. Similarly, other shape differences can be analyzed as deviations between appropriate groupings of landmark configurations. These can be compared using a variety of multivariate methods including Procrustes ANOVA (Dryden and Mardia 1998, Klingenberg and McIntyre 1998). Procrustes ANOVA consists of computing sums of squares for each level of the ANOVA for each landmark coordinate. Sums of squares are then added across all landmark coordinates and divided by the appropriate degrees of freedom to arrive at mean squares (Klingenberg and McIntyre 1998). (Procrustes degrees of freedom are normal degrees of freedom multiplied by the number of landmark coordinates less four: one, two and one degrees of freedom lost to scaling, superimposition and rotation respectively.) Significance of effects within the ANOVA can then be tested using standard methods or permutation tests.

For shape, I used the mixed model Procrustes ANOVA of Klingenberg and McIntyre (1998) to test for significance of variation among individuals, between sides, and of the interaction term (FA) for each sex from each replicate cup. I tested for significance of these effects using separate permutation tests (10,000 repetitions) for each effect (permuting wings across individuals within sides, wings across sides within individuals, or wings across sides and individuals after removing main effects by subtracting fly and side means and adding the grand mean [Klingenberg and McIntyre 1998]). To assess antisymmetry of shape using aligned configurations I examined scatter plots of left minus right differences for each landmark. Bivariate bimodal distributions indicate antisymmetry. To assess the size dependence of shape asymmetry, I conducted multivariate regressions of signed and unsigned asymmetry vectors on centroid size following Klingenberg and McIntyre (1998). Individual signed asymmetry vectors consist of the mean (R-L) values at each coordinate for each fly, after removal of the mean directional shape asymmetry within the data set. Unsigned vectors were the individual asymmetry vectors with sign of all coordinates reversed if the deviation of the X coordinate for landmark 1 was negative. The null hypothesis was that the slope of the asymmetry was indistinguishable from zero for each coordinate.

To test for differences between replicates in shape, shape DA and shape FA, I conducted the mixed model Procrustes ANOVA of individuals by sides for each group and used permutation tests to determine if the between-group difference in mean squares for fly, side, and FA was greater than expected. For example, the test for DA differences between sexes compared the difference between the side mean squares of males and females with the mean square difference between equivalently sized groups generated

from randomly resampling individuals without replacement. For each test I ran 10000 permutations exchanging individuals between test groups. Because there was significant shape DA throughout the study, I subtracted the mean shape asymmetry, of all flies, from individuals before calculating FA mean squares. With a single exception (see below), males and females did not differ between replicate cups in shape DA or shape FA, and cups were pooled in the subsequent analyses (Appendix 3).

Because of the unbalanced sampling design, I conducted two separate balanced factorial ANOVAs: a two-way ANOVA of maternal age by sex for all high density flies and a three-way ANOVA of maternal age by sex by larval density for the flies from two and four week old mothers. I conducted ANOVAs for centroid size and |R-L| of centroid size. To test for effects on shape asymmetry, I calculated Procrustes mean squares of the individual signed asymmetry vectors after removal of DA, for each effect in the ANOVAs. Since unsigned asymmetry of centroid size, and asymmetry vectors do not conform well to the assumptions of ANOVA, I derived significance values using permutation tests, with 10,000 iterations, that the observed mean squares were greater than expected based on resampling individuals without replacement.

As a direct test of the relationship between size FA and shape FA I regressed an unsigned univariate estimator of individual shape FA, Procrustes distance between wings (the square of the summed distances between mean right and left configurations [Dryden and Mardia 1998]), on unsigned centroid size FA.

Results

Centroid size was positively correlated with maternal age, negatively correlated with larval density and higher in females than in males (Tables 4.1 and 4.2). Main effects

did not interact except that centroid size in offspring of two and four week old mothers differed little at low density, but at high density the offspring of two week old mothers were much smaller than the offspring of four week old mothers (Tables 4.1 and 4.2).

Centroid size exhibited ideal FA in most samples after pooling replicates (Table 4.3, Appendix 3). DA was absent except in females from a single replicate of offspring of 2 week old mothers raised at low density. Rather than complicate interpretations of results these flies were excluded from analyses of size and size FA. In the remaining data set, FA was significantly higher than measurement error and DA was non-significant (Table 4.3). There was significant leptokurtosis and right skew in low-density male offspring and significant left skew in low density female offspring of two week old mothers (Table 4.1). However since I used permutation tests to assess significance of FA differences between replicates and groups I included these samples in the ensuing analyses. There were no significant relationships of |R-L| with mean centroid size for any group or subgroup of flies after Type I error correction, nor was there any difference in centroid size FA between replicates within any treatment after sequential Bonferonni correction (Appendix 3). Accordingly, I pooled cups for analysis of treatment effects on size FA.

At high larval density, unsigned centroid size FA was elevated in flies from older mothers, but there was no significant effect of sex (Tables 4.1 and 4.2). This maternal age effect was primarily due to an increase in centroid size FA among female offspring of mothers greater than three weeks of age but the interaction was not significant (Tables 4.1 and 4.2).

Among flies from mothers at 2 and 4 weeks of age, size FA was greater in females (0.029 ± 0.0026 mm) than in males (0.022 ± 0.0019 mm) and the effect of maternal age approached significance (Tables 4.1 and 4.2). The three-way interaction of maternal age, sex, and density was also significant: there was a reversal of density effects between maternal ages in females but not in males. (Tables 4.1 and 4.2).

For shape, the only difference between replicates at any level of variation was a difference in DA between males with 2-week-old mothers from low-density cups (Replicate 1: MS = 6.69×10^{-5}; Replicate 2: MS = 2.78×10^{-5}; $P_{permutation} < 0.0001$). These groups of males did not differ in other respects nor did the difference appear to be affected by the presence of outliers (Appendix 3). Based on these tests I pooled all replicate cups for further analyses. After pooling replicates, FA variation was significant in all groups (Tables 4.1 and 4.2, Appendix 4). After removal of DA, multivariate regression of signed asymmetry vectors revealed that signed shape asymmetry was dependent on centroid size (n = 331, Wilks' Lambda = 0.78, F = 3.64, df = 24,306, $p<0.0001$). (Recall that shape asymmetry is the deviation between aligned configurations of right and left wings and the signed asymmetry vector describes the magnitude and direction of asymmetry, but in the unsigned asymmetry vector directional information is removed.) Unsigned asymmetry vectors were not related to centroid size when separate samples (analyses not shown) or the entire data set were considered (n = 331, Wilks' Lambda = 0.94, F = 0.82, df = 24,306, p = 0.71). This suggests that the direction of shape FA is related to size, but the magnitude of shape FA is not. Since the method for comparing shape FA differences is based on magnitude and not direction I was justified in treating shape FA as size independent.

Procrustes ANOVA, of maternal age and sex for flies raised at high density, revealed no significant effects on shape asymmetry (Table 4.2). However, in the comparison of shape FA between maternal ages two and four weeks, all main effects were significant (Table 4.2). Early offspring (MS = 4.047×10^{-6}) were slightly less symmetrical than late offspring (MS = 3.972×10^{-6}). High-density flies (MS = 4.650×10^{-6}) were less symmetrical than low-density flies (MS = 3.967×10^{-6}) and males (MS = 3.967×10^{-6}) were more symmetrical than females (MS = 4.047×10^{-6}). Since shape FA is positively related to density, the observed maternal age effect on shape FA may result from decreasing larval density late in the experiment. (Hatch rate, which decreases with maternal age [Chapter 2], was not controlled for in this experiment, and larval density is negatively correlated with size [Sullivan and Sokal 1963] which increased with age in the high density treatment in this experiment [Table 4.1].) After removing density and sex effects, maternal age did not significantly influence shape FA (2 week old mothers MS = 3.886×10^{-6}; 4 week old mothers MS = 3.950; $F_{1344,2880} = 1.02$, $P_{permutation} = 0.36$). Since there is no *a priori* reason to expect density or maternal age to differentially affect shape FA of males and females, I did not conduct similar analyses for these factors.

Shape FA was weakly positively related to centroid size FA in the entire data set (Figure 4.2). The slope of the relationship did not vary between treatment groups in a test of homogeneity of slopes ($F_{13,288} = 0.81$, $P = 0.65$) nor was the relationship significant within any single treatment group after sequential Bonferroni correction (analyses not shown).

Discussion

Maternal age, larval density, and sex significantly affected house fly wing FA but, even though they were positively correlated (Figure 4.2), size FA and shape FA responded differently to the experimental treatments (summarized in Figure 4.3). Increased larval density elevated shape FA but did not affect size FA, and increased maternal age elevated size FA but did not affect shape FA after removal of the confounding density effect (Tables 4.1 and 4.2). Although there appears to be a small underlying whole-wing DS that affects both size FA and shape FA, reflected by their positive correlation (Figure 4.2), this is outweighed by trait specific stress responses of FA. Most of the observed effects on size FA and shape FA are independent.

Close correlation of independent trait FA within individuals is not expected (Whitlock 1996). However, this does not hold for developmentally interdependent traits (Whitlock 1996; Van Dongen et al. 1999) and the closer the developmental relationship between traits, the closer their FA, and the response of their FA to stress (McIntyre and Gooding 1998). The primarily independent response of size FA and shape FA to maternal age and density raises several questions: How independent is the development of wing shape and wing size? Can the differences in stress response be related to the kind or timing of stress induced by maternal age and larval density? Is size FA or shape FA a better indicator of developmental stability?

Our understanding of wing development in higher flies rests primarily on studies of *Drosophila*, in which wing size and shape are clearly independent at some level. In spite of the large amount of size variation naturally present and inducible by environmental (e.g., James et al. 1997) and genetic (e.g., Bohni et al. 1999) factors, wing

shape differs only slightly between large and small flies (Azevedo et al. 1998; Haas and Tolley 1998). A comparison of the genetics of wing size and wing vein location reveals further disjunction of control of these traits. Wing vein locations are determined during the late third instar and early puparial stage at morphogen boundaries on the developing wing blade. These boundaries signal a cascade of other regulatory genes leading to specification of vein and intervein areas (Lunde et al. 1998). Misplacement of the morphogen boundaries or disruption of this cascade affects vein properties and location, and because individual veins develop at specific morphogen boundaries, individual veins can respond independently if stress has morphogen specific effects (Biehs et al. 1998; Mohler et al. 2000). In contrast to vein location, wing size is determined by cell proliferation and growth in the wing imaginal disc, which begins at the end of the first instar and continues until the second day after pupariation in *Drosophila* (Bryant and Schmidt 1990). However, final size and shape are independent of specific local patterns of cell growth and division, and final size may be specified by total volume or by absolute distance between structures (Milan et al. 1996, Weigmann et al. 1997, Neufeld et al. 1998, Bohni et al. 1999). Wing size and shape are clearly related characters, in spite of partially independent developmental control. In the data, a multiple regression of wing shape vectors on centroid size is highly significant ($n = 331$, Wilks' Lambda = 0.26, $F_{24,306} = 37.20$, $P < 0.0001$). Such shape allometry is probably only possible if control of wing size and control of wing shape share common elements or are jointly regulated.

The partially independent determination of wing size and wing vein location makes the primarily independent response of size FA and shape FA less surprising. The independent response may be due to the differential timing of developmental

disturbances likely to result from maternal age effects and from high larval density. Most maternal age effects are confined to the embryo and early immature stages (Mousseau and Dingle 1991a, b; Mousseau and Fox 1998), although some do persist (e.g. adult size of offspring [e.g., Phelan and Frumhoff 1991; McIntyre and Gooding 1998]). However, the effects of high larval density act primarily during the third larval instar (Sullivan and Sokal 1963). Wing imaginal discs are set aside half way through embryogenesis as a group of approximately 30 cells on each side of the body and they begin to proliferate at the end of the first larval instar (Bryant and Schmidt 1990). In contrast, wing vein locations are determined during the third instar and early pupa (e.g., Mohler et al. 2000). It is possible that maternal age could affect size asymmetry through between side differences in the initial size or early proliferation of wing discs before maternal age effects become attenuated. A very small early difference could be magnified to measurable amounts in adults due to the ~1000 fold size increase that occurs during development. In contrast, maternal age effects are likely to have disappeared before wing veins are determined. However, density stress during the third instar might affect local cell proliferation patterns, or morphogen concentrations or boundaries, that affect vein location but are compensated for by the rest of the wing to maintain size symmetry.

How should wing size FA and wing shape FA be used to estimate DS or individual quality? The positive correlation of wing size FA and wing shape FA (Figure 4.2) seems to support the notion of an underlying wing DS that jointly affects FA of these traits. However, the difference in response of two aspects of the same structure to specific stresses suggests that FA largely reflects trait specific, not organism-wide or even structure-wide, DS. Combining size FA and shape FA into a single estimate of quality

may thus confound stress- and trait-specific DS with organism-wide DS. There have been numerous attempts to combine traits into composite FA indices but the common underlying logic is that traits exhibit different degrees of a common stress response (reviewed in Leung et al. 2000). In a modelling exercise, composite FA indices were more likely to reveal a stress response than were individual traits (Leung et al. 2000). This result supports the presence of an organism-wide DS only if, as the model assumes, there is an equal degree of stress response across all traits, but this is an empirically unfounded assumption (e.g. Bjorksten et al. 2000; this study).

My results suggest that maternal age does decrease DS sufficiently to affect FA of at least one trait in adult offspring of old female house flies, even though this species does not otherwise exhibit detrimental, post-hatching, maternal age effects (Chapters 2 and 3). FA also reveals negative effects of increased larval density on DS of wing shape corresponding with those revealed through survival, size and density (Sullivan and Sokal 1963). However these effects are quite small, as is the effect of sex.

There is variable evidence for maternal age effects on FA in insects. For example, FA of sternopleural bristle number increased with maternal age in one *Drosophila melanogaster* population (Parsons 1962). However, in another population, maternal age did not affect FA of sternopleural bristle number, wing length or wing area (Wakefield et al. 1994). In tsetse (*Glossina palpalis gambiensis*) wing FA decreased slightly with maternal age (McIntyre and Gooding 1998; Klingenberg and McIntyre 1998). These divergent results suggest that maternal age effects on FA vary among species and populations. This variation may be related to the adaptive nature of some maternal age effects since it seems to depend upon the life history, particularly the relative contribution

of late-born offspring to female fitness (McIntyre and Gooding 1998). The result suggests that this variability might also be due to the trait, species (and population) specific nature of the FA response to maternal age.

It is unclear why FA was higher, and more stress sensitive in female than in male house flies. Females are larger than males and take longer to develop, increasing the temporal windows during which maternal age and larval density can act to disrupt symmetry of wing development. Alternatively, flight may be more important to males than to females, since they actively pursue females on the wing for mating, and there is no evidence that females use elaborate flight maneuvers to avoid contact with males. This may lead to tighter control of wing symmetry in males.

The biological significance of small differences in FA is often questioned since it is difficult to demonstrate that they directly affect performance. Rather the biological significance of small FA differences is based on the assumption that they reflect organism-wide DS and hence organismal quality. If, as the results suggest, a large component of DS is trait specific, then I must look elsewhere for the biological significance of small FA differences. A better use of FA might be to use developmental stress and trait specific developmental stability to investigate specific hypothesis about species biology rather than trying to assess organism wide quality.

Table 4.1. Wing size and asymmetry statistics for male and female house flies reared at high and low larval density, from eggs produced by the same females at 5 different ages. For centroid size means (±SE) of centroid size, signed symmetry (R-L) and unsigned symmetry (|R-L|) are provided as are skew and kurtosis values for unsigned asymmetry. Shape mean squares (multiplied by 10^6) are from separate Procrustes ANOVAs of the multivariate data set including replicate measurements of each wing, for each sample. For signed size asymmetry and shape FA the bracketed values are the percent variation in the total data set explained by FA. Degrees of freedom for Procrustes mean squares are normal degrees of freedom times 24 (e.g., in samples of 30 flies, with two replicate measurements per wing, there are 696 and 1440 degrees of freedom for the FA and residual terms respectively).

				Centroid Size					Procrustes Mean Squar	
Sex	Maternal Age (weeks)	Density	n	Size (mm)	(R-L) (μm)	Kurtosis (R-L)	Skew (R-L)	|R-L| (μm)	Fluctuating Asymmetry	Residual (error)
F	1	H	30	4.89 ± 0.048	1.0 ± 5.28 (0.30)	1.83	-0.40	22.0 ± 3.42	3.90 (5.7)	1.10
F	2	H	30	4.79 ± 0.037	2.3 ± 5.32 (0.52)	0.07	-0.20	24.0 ± 3.03	5.10 (7.0)	1.06
F	2	L	15/30#	6.07 ± 0.055	22.2 ± 9.02 (0.67)	2.80	1.75	26.7 ± 8.16	3.19 (5.2)	0.75
F	3	H	30	5.29 ± 0.042	-5.1 ± 6.17 (0.52)	3.12	-1.51 ***	24.8 ± 4.24	3.79 (4.8)	0.96
F	4	H	9	5.66 ± 0.079	1.6 ± 19.00 (1.41)	-1.67	0.01	48.8 ± 8.97	5.29 (4.9)	1.06
F	4	L	19	5.98 ± 0.098	12.4 ± 7.05 (0.13)	0.11	-0.63	28.5 ± 3.65	3.15 (3.8)	0.87
F	5	H	19	5.76 ± 0.087	2.0 ± 11.52 (0.44)	-0.19	-0.34	38.3 ± 7.71	6.25 (11.0)	0.89
M	1	H	30	4.55 ± 0.048	3.0 ± 4.53 (0.22)	-0.99	0.17	21.7 ± 2.26	4.12 (5.7)	1.01
M	2	H	30	4.53 ± 0.050	4.8 ± 5.01 (0.25)	0.19	0.19	22.2 ± 3.04	4.28 (5.6)	1.19
M	2	L	30	5.78 ± 0.033	12.2 ± 4.89 (0.56)	3.22 *	1.34 ***	21.9 ± 3.73	3.25 (6.2)	0.90
M	3	H	30	5.04 ± 0.037	3.2 ± 5.37 (0.52)	1.29	-0.43	23.0 ± 3.36	3.46 (4.3)	1.04
M	4	H	5	5.31 ± 0.054	3.2 ± 15.27 (1.92)	3.90	1.96	23.2 ± 10.15	4.35 (6.4)	0.88
M	4	L	23	5.78 ± 0.065	6.1 ± 5.75 (0.20)	-0.65	0.53	23.3 ± 3.36	4.26 (5.6)	0.83
M	5	H	16	5.22 ± 0.103	5.7 ± 8.68 (0.18)	-0.82	0.34	29.0 ± 4.79	4.78 (8.5)	0.91

#15 individuals in size analyses, 30 individuals in shape analyses

* Bonferroni corrected $P < 0.05$, *** corrected $P < 0.001$

Table 4.2. Effects of maternal age, sex and larval density on wing centroid size, unsigned asymmetry of centroid size, and shape asymmetry vectors (corrected for DA) for house flies raised from eggs produced by the same mothers at different ages. Analyses are separated into two balanced factorial ANOVAs. Centroid size ANOVAs employ type III SS. For size and shape FA, sums of squares were calculated independently and interactions were adjusted for main effects. Mean squares for size FA and shape FA are multiplied by 10^3 and *P*-values are from permutation tests that the observed mean square for each term is greater than expected based on 10^4 resamplings without replacement.

	Centroid Size				Centroid Size FA |R-L|				Shape FA			
Source	DF	MS	*F*	*P*	DF	MS	*F*	*P*	DF	MS	*F*	*P*
High Density Flies												
Maternal Age	4	6.170	85.55	0.0001	4	1.662	4.05	0.0046	96	0.005	1.14	0.2471
Sex	1	4.816	66.78	0.0001	1	1.196	2.92	0.0959	24	0.003	0.73	0.7063
Maternal Age * Sex	4	0.136	1.88	0.1140	4	0.741	1.81	0.2317	96	0.005	1.24	0.4837
Residual	219	0.072			219	0.410			5256	0.004		
Maternal Ages 2 and 4 Weeks												
Maternal Age	1	4.312	60.77	0.0001	1	1.255	3.19	0.0847	24	0.008	1.97	0.0427
Sex	1	2.149	30.28	0.0001	1	1.598	4.06	0.0459	24	0.011	2.71	0.0067
Maternal Age * Sex	1	0.000	0.00	0.9953	1	0.729	1.85	0.1866	24	0.006	1.56	0.1201
Density	1	19.133	269.61	0.0001	1	0.111	0.28	0.6075	24	0.011	2.78	0.0044
Maternal Age * Density	1	5.199	73.26	0.0001	1	0.628	1.59	0.7381	24	0.016	4.11	0.3843
Sex * Density	1	0.026	0.37	0.5423	1	0.078	0.20	0.7052	24	0.002	0.51	0.9207
Age * Sex * Density	1	0.051	0.72	0.3964	1	1.576	4.00	0.0488	24	0.007	1.84	0.5068
Residual	153	0.071			153	0.394			4032	0.004		

Table 4.3. Mixed model ANOVAs of individual by side for centroid size and Procrustes aligned shape configurations. Main effects were tested over the interaction term (FA) which was tested against the residual. Mean squares for size and shape are multiplied by 10^3 and 10^6 respectively. Bracketed values are the percentage of the total variation accounted for by each term. Procrustes degrees of freedom are normal degrees of freedom multiplied by 24 (i.e., the total number of coordinates in the alignment less two lost to alignment, one to rotation and one to scaling). Procrustes P values are from permutation tests that the MS of each term is not larger than expected.

Source	DF	MS	*F*	*P* <
Centroid Size Asymmetry				
Individuals	330	1,346.2 (0.9990)	1281.5	0.0001
Sides	1	4.0 (0.0000)	3.8	0.0520
Fluctuating Asymmetry	330	1.1 (0.0008)	10.7	0.0001
Residual	662	0.1 (0.0002)		
Procrustes Shape Asymmetry				
Individuals	7920	89.6 (0.92)	21.3	0.0001
Sides	24	435.3 (0.01)	103.4	0.0001
Fluctuating Asymmetry	7920	4.2 (0.04)	4.3	0.0001
Residual	15888	1.0 (0.02)		

Figure 4.1. Landmark locations measured on house fly wings. All landmarks were measured as the interior junction of wing veins except landmark 7 which is a campaniform sensillum on the anterior crossvein and landmark 10 which was measured as the invagination of the posterior wing margin where it is met by vein 5 (M_{3+4}).

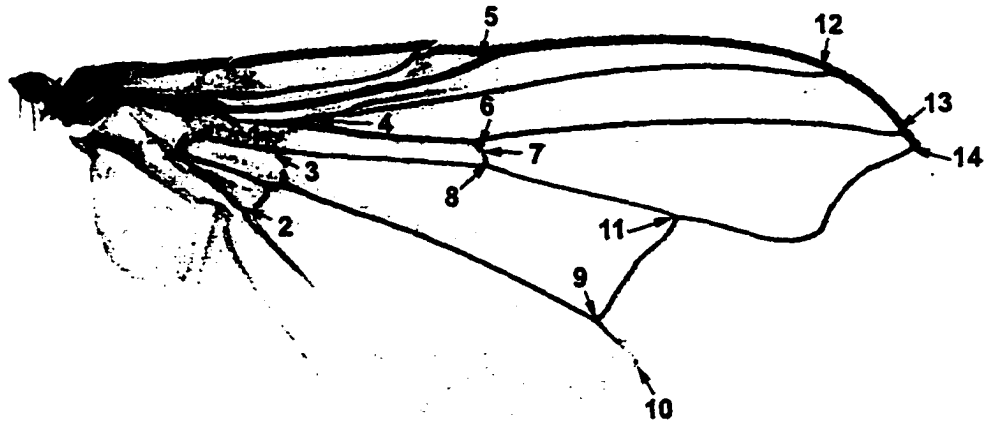


Figure 4.2. Relationship between wing shape FA (Procrustes distance) and unsigned centroid size FA for individual houseflies. Regression: Shape FA = 0.034 x (Size FA) + 0.010, n = 316, P < 0.0001, R^2 = 0.07.

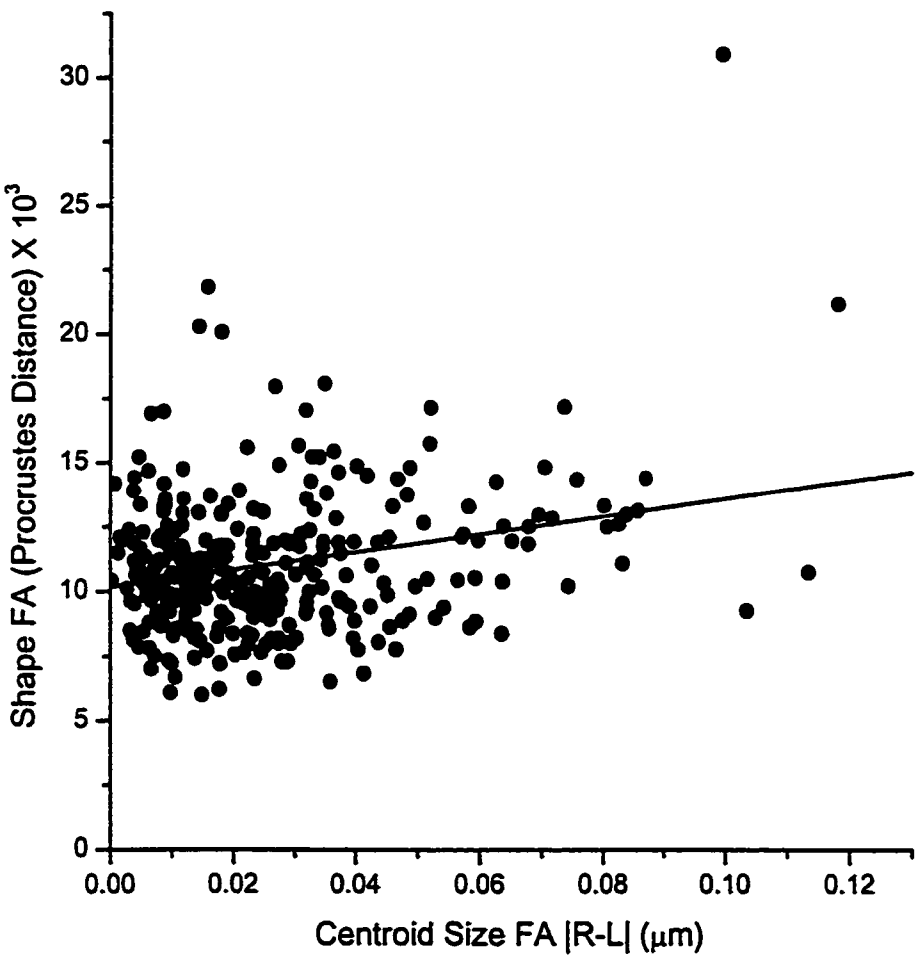
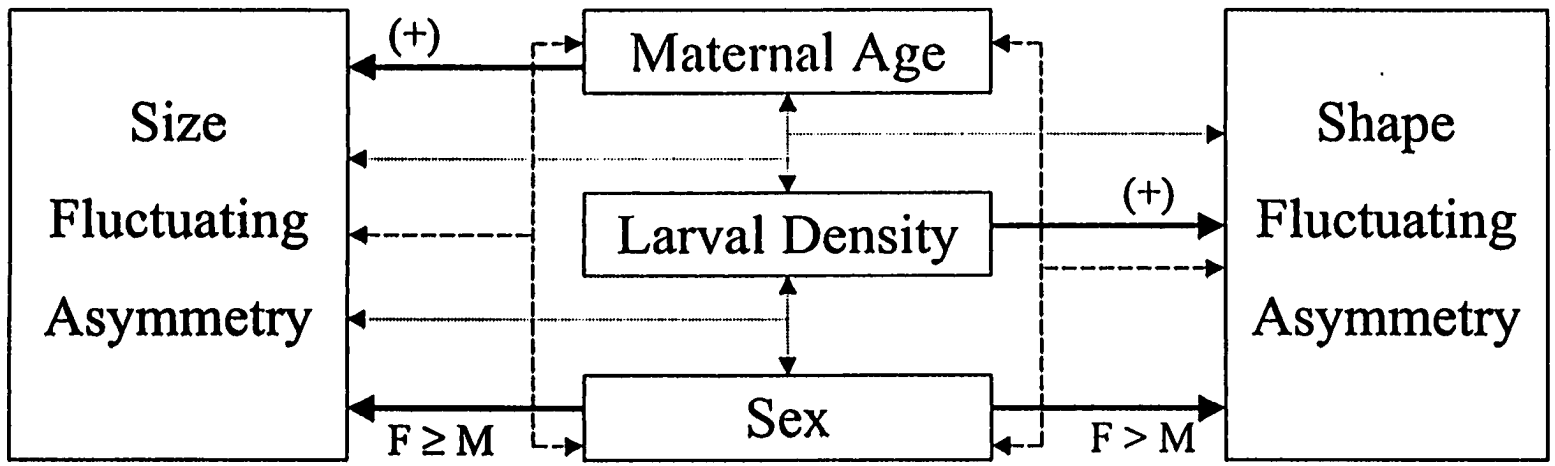


Figure 4.3. Summary of the relationships of maternal age, larval density, and sex with size FA and shape FA of house fly wings. Main effects are solid arrows and interactive effects are dotted or dashed arrows. Interaction effects are described in the text. (+) indicates a positive correlation, otherwise the correlations are negative and lack of an arrow indicates a non-significant effect.



References

Auffray, J. C., Alibert, P, Renaud, S., Orth, A. and Bonhomme, F. 1996. Fluctuating asymmetry in *Mus musculus* sub-specific hybridization: traditional and Procrustes comparative approach. In Marcus, L.F., Corti, M., Loy, A., Naylor, G. J. P. and Slice, D. E. (eds), Advances in Morphometrics, pp 275-283. Plenum Press, New York..

Azevedo, R. B. R., James, A. C., McCabe, J. and Partridge, L. 1998. Latitudinal variation of wing:thorax size ratio and wing-aspect ratio in *Drosophila melanogaster*. Evolution **52**: 1353-1362.

Biehs, B., Sturtevant, M. A. and Bier, E. 1998. Boundaries in the *Drosophila* wing imaginal disc organize vein-specific genetic programs. Development **125**: 4245-4257.

Bjorksten, T. A., Fowler, K. and Pomiankowski, A. 2000. What does sexual trait FA tell us about stress? Trends Ecol. Evol. **15**: 163-166.

Bohni, R., Riesgo-Escovar, J, Oldham, S., Brogiolo. W., Stocker, H., Andruss, B. F., Beckingham, K. and Hafen, E. 1999. Autonomous control of cell and organ size by CHICO, a *Drosophila* homolog of vertebrate IRS1-4. Cell **97**: 865-875.

Bookstein, F. L. 1991. Mophometric Tools for Landmark Data: Geometry and Biology. Cambridge University Press, Cambridge, MA.

Bryant, P. J. and Schmidt, O. 1990. The genetic control of cell proliferation in *Drosophila* imaginal discs. J. Cell Sci. Suppl.**13**: 169-189.

Debat, V., Alibert, P., David, P. and Paradis, E.. 2000. Independence between developmental stability and canalization in the skull of the house mouse. Proc R. Soc. London B **267**: 423-430.

Dryden, I. L. and Mardia, K. V. 1998. Statistical Shape Analysis. Wiley, New York.

Graham, J. H., Freeman, D. C. and Emlen, J. M. 1993. Developmental stability: a sensitive indicator of populations under stress. In Landis, W.G., Hughes, J.S., and Lewis, M.A. (eds). Environmental Toxicology and Risk Assessment, pp. 136-158. American Society for Testing and Materials, Philadelphia (PA).

Haas, H. L. and Tolley, K. A. 1998. Geographic variation of wing morphology in three Eurasian populations of the fruit fly, *Drosophila lummei*. J. Zool. Lond. **245**: 197-203.

Held, L. I. Jr. 1990. Sensitive periods for abnormal patterning on a leg segment in *Drosophila melanogaster*. Roux's Archiv. Dev. Biol. **199**: 31-47.

James, A. C., Azevedo, R. B. R. and Partridge, L. 1997. Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. Genetics **146**: 881-890.

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

Klingenberg, C. P. and Nijhout, H. F. 1998. Competition among growing organs and developmental control of morphological asymmetry. Proc. R. Soc. London B **265**: 1135-1139.

Leamy, L. 1993. Morphological integration of fluctuating asymmetry in the mouse mandible. Genetica **89**: 139-153.

Leamy, L. J., Routman, E. J. and Cheverud, J. M. 1997. A search for quantitative trait loci affecting asymmetry of mandibular characters in mice. Evolution **51**: 957-969.

Leary, R. F. and Allendorf, F. W. 1989. Fluctuating asymmetry as an indicator of stress: Implications for conservation biology. Trends Ecol. Evol. **4**: 214-217.

Leung, B. and Forbes, M. R. 1996. Fluctuating asymmetry in relation to stress and fitness: Effects of trait type as revealed by meta-analysis. Ecoscience **3**: 400-413.

Leung, B., Forbes, M. R. and Houle, D. 2000. Fluctuating asymmetry as a bioindicator of stress: Comparing efficacy of analyses involving multiple traits. Am. Nat. **155**: 101-115.

Lunde, K., Biehs, B., Nauber, U. and Bier, E. 1998. The *knirps* and *knirps*-related genes organize development of the second wing vein in *Drosophila*. Development **125**: 4145-4154.

McIntyre, G. S. and Gooding, R. H. 1995. Pteridine accumulation in *Musca domestica*. J. Insect Physiol. **41**: 357-368.

McIntyre, G. S. and Gooding, R. H. 1998. Effect of maternal age on offspring quality in tsetse (Diptera: Glossinidae). J. Med. Ent. **35**: 210-215.

Milan, M., Campuzano, S. and Garcia-Bellido, A. 1996. Cell cycling and patterned cell proliferation in the *Drosophila* wing during metamorphosis. Proc Natl. Acad. Sci. USA **93**: 11687-11692.

Mohler, J., Seecoomar, M., Agarwal, S., Bier, E. and Hsai, J. 2000. Activation of *knot* (KN) specifies the 3-4 intervein region in the *Drosophila* wing. Development **127**: 55-63.

Mousseau, T. A. and Dingle, H. 1991a. Maternal Effects in Insect Life Histories. Annu.

Rev. Entomol. **36**: 511-534.

Mousseau, T. A. and Dingle, H. 1991b. Maternal effects in insects: examples, constraints, and geographic variation. In: Dudley, E. C. (ed) The Unity of Evolutionary Biology, vol. II, pp. 745-761. Dioscoridies Press, Portland.

Mousseau, T. A. and Fox, C. W. 1998. Maternal effects as adaptations. Oxford University Press, New York.

Neufeld, T. P., de la Cruz, A. F. A., Johnston, L. A. and Edgar, B. A. 1998. Coordination of growth and cell division in the *Drosophila* wing. Cell **93**: 1183-1193.

Palmer, A. R. 1994. Fluctuating asymmetry analyses: A primer. In: Markow, T. A. (ed) Developmental Instability: Its Origins and Evolutionary Implications, pp. 335-364. Kluwer, The Netherlands

Palmer, A. R. 1996. Waltzing with asymmetry. Bioscience **46**:518-532.

Parsons, P. A. 1962. Maternal age and developmental variability. J. Exp. Biol. **39**:251-260.

Phelan, J. P. and Frumhoff, P. C. 1991. Differences in the effects of parental age on offspring life history between tropical and temperate populations of milkweed bugs (*Oncopeltus* spp.). Evol. Ecol. **5**: 160-172.

Roff, D. 1992. The Evolution of Life Histories: Theory and Analysis. Chapman & Hall, New York.

Smith, D. R., Crespi, B. J. and Bookstein, F. L. 1997. Fluctuating asymmetry in the honey bee, *Apis mellifera*: effects of ploidy and hybridization. J. Evol. Biol. **10**: 551-574.

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

Van Dongen, S. 1998. How repeatable is the estimation of developmental stability by fluctuating asymmetry? Proc. R. Soc. Lond. B **265**: 1423-1427.

Van Dongen, S., Sprengers, E. and Lofstedt, C. 1999. Correlated development, organism-wide asymmetry and patterns of asymmetry in two moth species. Genetica **105**: 81-91.

Wakefield, J., Harris, K. and Markow, T. A. 1994. Parental age and developmental stability in *Drosophila melanogaster*. In: Markow, T. A. (ed) Developmental Instability: Its Origins and Evolutionary Implications, pp. 237-246. Kluwer, The Netherlands.

Weigmann, K., Cohen, S. M. and Lehner, C. F. 1997. Cell cycle progression, growth and

patterning in imaginal discs despite inhibition of cell division after inactivation of *Drosophila* Cdc2 kinase. Development **124**: 3555-3563.

Whitlock, M. 1996. The heritability of fluctuating asymmetry and the genetic control of developmental stability. Proc. R. Soc. Lond. B **263**: 849-854.

Whitlock, M. 1998. The repeatability of fluctuating asymmetry: a revision and extension. Proc. R. Soc. Lond. B **265**: 1429-1431.

Zakharov, V. M. 1992. Population phenogenetics: Analysis of developmental stability in natural populations. Acta Zoologica Fennica **191**: 7-30.

Chapter 5: Wing fluctuating asymmetry extends the insights into developmental stresses caused by larval competition and maternal age

Introduction

It has often been suggested that fluctuating asymmetry (FA, i.e., small random deviations from perfect bilateral symmetry) may be a sensitive measure of developmental stress (Leary and Allendorf 1989; Graham et al. 1993). Extension of this idea to the use of FA as a general indicator of developmental or environmental stress has met with only limited success. Stress can elevate FA, but often it does not, even in cases where stress is evident through decreased survival or other effects (reviewed in Leung and Forbes 1996). FA seems to respond to stress in a trait specific fashion even for closely related traits. For instance, house fly wing shape FA is elevated by increasing larval density but not by maternal age, and vice versa for size FA (Chapter 4). Although, FA may not be a generally useful indicator of an organism-wide response to developmental stress, FA differences may nonetheless provide insight into stress responses not revealed by other stress indicators.

Although wing FA does not appear to indicate organismal quality in house flies, it may be useful in understanding the stresses and dynamics of larval development. Larval survival and adult size of house flies are determined primarily by performance during two larval stages: initial medium conditioning by hatchlings and growth of the third (final) instar (Sullivan and Sokal 1963; Bhalla and Sokal 1964; Bryant and Sokal 1967, 1968; Kence and Jdeidi 1997). The activities of house fly larvae modify or "condition" their medium, enhancing microbial growth, and low density of young larvae results in poor medium conditioning and in death of larvae through starvation or fungal takeover of the

medium (Bryant and Sokal 1967;Schmidtmann and Martin 1992). Flies developing at elevated larval density during the final larval instar, are smaller, take longer to develop, and have lower survival, probably as a result of food depletion (Sullivan and Sokal 1963; and Bryant and Sokal 1967). In competition experiments between genetically marked house fly (*Musca domestica* L.) lines, I measured the effects of maternal age on hatchling to adult survival, development time, and adult size (Chapter 3). In general, older females produced larvae that had higher viability and attained larger sizes, but developed more slowly. Maternal age effects were line specific, suggesting they are genetically determined, and there were significant interactions of maternal age effects between pair-wise line comparisons. Maternal age effects on performance in pure culture were not predictive of performance in mixed cultures. Competitor identity significantly affected the success of each line and maternal age class (Chapter 3).

These experiments did not directly reveal dynamics of larval competition although they allowed me to develop hypotheses concerning these dynamics. In the present study I evaluate FA of a subset of flies from the earlier competition experiments to determine whether: 1) the competition response of FA was similar to that of survival; 2) the FA response provided additional insight into larval competition dynamics; 3) the response of wing shape FA was similar to that of wing size FA; and 4) the previously observed maternal age effect on FA could be confirmed in lines that differed in their pattern of maternal age effects on survival and competition.

Materials and Methods

Competition experiments

The history and maintenance of the house fly colony and the details of the competition experiments appear elsewhere (McIntyre and Gooding 1995; Chapters 1 and 3). In brief: I compared the larval competitiveness of offspring of ~10-day-old females (hereafter referred to as early offspring) and offspring of ~24-day-old females (hereafter referred to as late offspring) from three electrophoretically marked lines in a modified replacement (substitution) series experiment (Chapter 3). The flies measured for FA were those emerging from pure culture controls and equal mixtures of lines C (homozygous Odh^f) and D (homozygous Odh^s). There were four pure cultures and four 1:1 mixtures of line and maternal age, each with three replicates of 60 vigorous larvae in 3g of medium. These conditions are stressful, resulting in ~70% larval mortality. At emergence, each fly was frozen at -20C. Both wings from each fly were mounted in Euparol, between two slides clamped with elastic bands to flatten the wings. Flies from mixed cups were electrophoresed to determine their parentage.

Since the experimental unit is the replicate cup, and not each fly, I calculated a single value from the flies emerging from each cup for viability, teneral mass, and development time. Sex ratio was not affected by any treatment in the original study, so I did not consider it here. For pure cups, viability was analyzed in a line by maternal age analysis of variance (ANOVA) of angular transformed survival. For mixed cups, viability was analyzed using natural log transformed Haldane's relative viability, $V = (a/(b + 1)) / (A/B)$, where a and b are the number of adults emerging and A and B are the number of larvae seeded into the culture from each line (Haldane 1956; Santos et al. 1992). Ratios

were calculated with line D values in the numerator. V was analyzed in a two-way ANOVA of parental age line C by parental age line D. Survival between mixed and pure cultures were compared using chi-squared tests.

Wing measurements

Two sets of replicate measurements were made of 14 landmarks on each wing (Figure 4.1). Locations of landmarks were digitized using a dissecting microscope and camera lucida with a Summasketch III data tablet (Summagraphics, Seymour, Connecticut). To estimate the accuracy of this apparatus, 10 measurements were made of a 1.0 mm stage micrometer. The mean (± sd) of these measurements (1.000 ± 0.0024 mm) did not differ significantly from one. Some flies had damaged wings so fewer flies were measured than emerged in each treatment.

Analysis of FA

As a univariate measure of wing size, I used centroid size (the square root of the summed squared distances of all landmarks from the centroid [mean landmark position]). Analysis of ideal behavior for FA of centroid size followed Palmer (1994) and used standard statistical tests, since centroid size is a well-behaved univariate quantity. However when testing for differences in size FA between samples, I used permutation tests as described below. Significance of FA above measurement error was tested with a mixed model ANOVA of individual (random) by side (fixed), with the significance of side tested against the interaction indicating presence or absence of directional asymmetry, DA. Skewness and kurtosis were calculated to assess departures from normality. Size dependence of FA was tested using regressions of unsigned centroid size FA, |R-L|, on mean centroid size of individuals. All assessments of the presence of ideal

FA were conducted separately on males and females for each replicate within each treatment and were subjected to the sequential Bonferroni correction. I used F-tests of the variance of |R-L| to detect differences in size FA between replicate cups before pooling cups.

Procrustes analysis of asymmetry using landmark data (described in detail by Klingenberg and McIntyre [1998]) consists of 4 basic steps: 1) reflection of the left (or right) side to align corresponding landmarks between sides; 2) scaling each configuration to unit centroid size; 3) superimposition of centroids of all configurations; and 4) rotation of configurations around the centroid to achieve an optimal fit of landmarks (I used generalized orthogonal least squares fit [Bookstein 1991]). Using the aligned coordinates, shape asymmetry corresponds to the deviations of right and left landmarks across individual flies. Similarly, other shape differences can be analyzed as deviations between appropriate groupings of landmark configurations. These can be compared using a variety of multivariate methods including Procrustes ANOVA (Dryden and Mardia 1998, Klingenberg and McIntyre 1998). Procrustes ANOVA consists of computing sums of squares for each level of the ANOVA for each landmark coordinate. Sums of squares are then added across all landmark coordinates and divided by the appropriate degrees of freedom to arrive at effect mean squares (Klingenberg and McIntyre 1998). (Procrustes degrees of freedom are normal degrees of freedom multiplied by the number of landmark coordinates less four degrees of freedom, one of which is lost to scaling, two to superimposition, and one to rotation.) Significance of effects within the ANOVA are tested using standard methods or permutation tests.

I used the mixed model Procrustes ANOVA of Klingenberg and McIntyre (1998) to test for significance of shape variation among individuals, between sides, and of the interaction term (FA) for each sex from each replicate cup. I tested for significance of these effects using separate permutation tests (10,000 repetitions) for each effect (permuting wings across individuals within sides, wings across sides within individuals, or wings across sides and individuals after removing main effects by subtracting fly and side means and adding the grand mean [Klingenberg and McIntyre 1998]). To assess antisymmetry of shape using aligned configurations I examined scatter plots of left minus right differences for each landmark. Bivariate bimodal distributions indicate antisymmetry. To assess the size dependence of shape asymmetry, I conducted multivariate regressions of signed and unsigned asymmetry vectors on centroid size. Individual signed asymmetry vectors consist of the mean (R-L) values at each coordinate for each fly, after removal of the mean directional shape asymmetry of the entire data set. Unsigned vectors were the individual asymmetry vectors with sign of all coordinates reversed, if the deviation of the X coordinate for landmark 1 was negative. The null hypothesis was that the slope of the asymmetry was indistinguishable from zero for each coordinate.

To test for differences between replicates in shape, shape DA and shape FA, I used permutation tests with 10,000 iterations to determine if the between group difference in mean squares for fly, side, and FA was greater than expected. For example, the test for DA differences between sexes compared the difference between the side mean squares of males and females with the mean square difference between equivalently sized groups generated from randomly resampling individuals without replacement. Because

there was significant shape DA throughout the study, I subtracted the mean shape FA, of all flies, from individuals before calculating FA mean squares. Sexes did not differ between replicate cups in shape DA or FA, and cups were pooled in the subsequent analyses.

Analysis of Competition Effects on FA

FA is a population trait, and requires large sample sizes for even modest hopes of detecting differences between groups (Palmer 1994). To achieve sufficient sample sizes I treated individuals as the experimental unit. This was reasonable since there were no effects of replicates on shape FA or size FA of wings in this study or in a previous study (Chapter 4, Appendices 3 and 5). I tested for effects of culture (mixed or pure), line and maternal age on FA with a three way factorial ANOVA. Significance was assessed with permutation tests, of 10,000 iterations, that the observed mean squares were greater than expected, based on resampling without replacement from the original data. To examine effects of competitive differences on FA between lines and maternal ages in mixed cups, I employed a two-way ANOVA of maternal age line C by maternal age line D similar to that used for survival. However, since lines C and D emerge from the same mixed cups it was necessary to enter lines into the ANOVA separately, otherwise the difference between early and late offspring within lines would be confounded with the effect of maternal age class on competitors. For unsigned centroid size asymmetry and for DA corrected shape asymmetry vectors, significance was assessed using permutation tests as above. Significance values derived from permutation tests are designated as 'P_{perm}' to avoid confusion with significance values derived from standard statistical tests.

Results

The competition analyses presented here are based on a subset of the data used in the original study and closely follow the results using the entire data set (Chapter 3). Sex ratio was not affected by any treatment in the original study, suggesting that males and females are equally competitive under the experimental conditions and was excluded from other analyses.

When all data were pooled there were no survival differences between lines or mixed and pure cultures (analysis not shown). However, line C had higher survival in pure culture than in mixed culture, and line D had higher survival in mixed than in pure culture (line C: mixed 78/360, pure 111/360, $\chi^2 = 7.8$, d.f. = 1, P = 0.0052; Line D: mixed 120/360, pure 56/360, $\chi^2 = 30.8$, d.f. = 1, P < 0.0001). In pure culture line C had higher survival than line D (Figure 5.1, $F_{1,8} = 24.36$, P = 0.0011) and late offspring had higher survival than did early offspring (Figure 5.1, $F_{1,8} = 53.98$, P < 0.0001), but line and maternal age did not interact.

In mixed cups line D had higher overall survival than did line C (Figure 5.1; $\chi^2 = 12.3$, d.f. = 1, P = 0.0005), early D larvae had greater relative viability than late D larvae (Figure 5.1, $F_{1,8} = 23.62$, P = 0.0013), but late C had greater relative viability than early C larvae (Figure 5.1, $F_{1,8} = 8.98$, P = 0.017). Thus line C outperformed line D and late offspring outperformed early offspring in pure culture, but in mixed culture the relationship of lines was reversed, and the relationship of maternal ages was reversed in line D but not in line C.

Centroid size exhibited ideal FA in most samples. However, in samples with less than 12 flies FA could not be distinguished from measurement error, but in larger

samples and in the entire data set measurement error was much smaller than FA (Tables 5.1 and 5.2, Appendix 5). As in a previous study (using replicates with up to 30 flies per replicate) I found no evidence for cup effects on FA, DA, or measurement error (Chapter 4, Appendices 3 and 5) so pooling flies among replicates was justified. There were no significant departures of (R-L) from normality (Table 5.2, i.e., no signifcant skew or kurtosis) and there was no evidence of dependence of unsigned asymmetry on individual centroid size (not shown). Females had higher size FA than males (females: 0.023 ± 0.0014 mm; males: 0.019 ± 0.0013; $F_{1,251} = 4.57$, $P_{perm} = 0.0311$). This sex effect did not interact with any other treatment level for flies (pooled or separated by culture method or line) so data for males and females were pooled for further analyses.

There were no significant effects of culture method, line or maternal age on centroid size FA in the overall ANOVA, nor in the test for competition effects on line C. However line D flies raised in competition with late C larvae were more symmetrical for centroid size than were those raised with early C larvae (with early C larvae: 0.023 ± 0.0022 mm; with late C larvae: 0.018 ± 0.0014; $F_{1,94} = 4.25$, $P_{perm} = 0.0400$, $R^2 = 0.043$).

For Procrustes aligned configurations, permutation tests of significance of individual, side, and FA variation were highly significant although separation of FA from measurement error decreased when sample size was less than 6 individuals. In larger samples, and in the entire data set, measurement error was much smaller than FA (Table 5.1). The few significant differences in DA or FA between replicates, were based on very small samples, so I pooled flies within replicates (Appendix 5). There was no evidence of antisymmetry at any level of analysis (plots not shown). After removal of DA, multivariate regression of signed asymmetry vectors revealed that shape asymmetry was

dependent on centroid size (n = 190, Wilks' Lambda = 0.714, F = 2.75, df = 24,165 , p < 0.0001). Unsigned asymmetry vectors were not related to centroid size when separate samples or the entire data set were tested (n = 190, Wilks' Lambda = 0.910, F = 0.68, df = 24,165, p = 0.86). This suggests that the magnitude, but not the direction, of shape asymmetry variation (FA *sensu latu*) around mean shape asymmetry (DA), is unrelated to size. Since the method for comparing shape FA differences is based on magnitude and not direction, I was justified in treating shape FA as size independent. Females had marginally higher shape FA than males (females: MS = 4.01×10^6, n = 128; males: MS = 3.79×10^6, n = 124; $F_{24,6000} = 1.73$, $P_{perm} = 0.031$). This sex effect did not interact with any other treatment level for flies (pooled or separated by culture method or line) so data for both sexes were pooled for further analyses.

Flies from mixed culture had significantly higher FA than those raised in pure culture (Pure: MS = 3.65×10^6, n = 92; Mixed: MS = 3.88×10^6, n = 160, $F_{24,5856} = 8.95$, $P_{perm} < 0.0001$, $R^2 = 0.034$). Culture conditions had a greater effect on line C than on line D and the rank of the lines reversed between culture conditions (C Pure: MS = 3.50×10^6, n = 48; C Mixed: MS = 3.99×10^6, n = 62; D Pure: MS = 3.68×10^6, n = 44; D Mixed: MS = 3.78×10^6, n = 98, $F_{24,5856} = 2.93$, $P_{perm} = 0.0039$, $R^2 = 0.011$). When lines were considered separately in mixed culture, line C flies were more symmetrical when produced by old mothers (young: MS = 4.47×10^6, n = 18; Old: MS = 3.72×10^6, n = 44; $F_{24,1392} = 1.91$, $P_{perm} = 0.040$, $R^2 = 0.031$).

Discussion

FA will increase and survival will decrease if both are adversely affected by the same developmental stress, but not otherwise. This was evident in the reversal of line

survival and shape FA between mixed and pure cups: line C had lower survival and higher FA than line D in mixed cups, but the opposite held in pure cups. Also, in mixed cups survival of late C was greater and shape FA was lower than that of early C. In contrast, line D had lower viability and lower size FA when reared with late C than with early C. Thus, the response of FA only partially corresponds with that of viability, and FA was not a more sensitive measure of stress since viability differences revealed maternal age effects in more cases than did FA differences. The differential responses suggest that wing FA tracks a slightly different component of the larval environment than does viability. This would explain the frequently reported lack of correspondence between survival and FA (Leung and Forbes 1996).

Because of the indirect link between FA and survival, FA differences provided insight into larval competition dynamics, not revealed by viability differences. The mere presence of competitors from different lines caused elevated shape FA in both lines, although overall survival did not differ between pure and mixed cups. Even though survival of line D was facilitated by the presence of line C larvae, there was still a negative effect of mixing, manifested as increased wing shape FA. Thus there is something detrimental to these house fly larvae about developing with larvae of another genotype. This implies that either larvae recognized the presence of 'foreign' larvae and reacted negatively or that the lines utilized/modified their environment differently and these differences adversely affected larvae from the other line. Distinct, genetically based, larval competition strategies have been demonstrated in house flies and in *Drosophila* (Sullivan and Sokal 1965; Bhalla and Sokal 1964; Bryant and Sokal 1968; Bryant 1969; Ruiz-debruil et al. 1996; Sokolowski et al. 1997; Borash et al. 1998), but

the ability of dipteran larvae to respond differentially to kin and non-kin has not been demonstrated, although this ability does exist in other non-social insects (reviewed in Fellowes 1998).

I hypothesized previously (Chapter 3) that the competitive advantage of late larvae involved a lower activity rate (unpublished observations) and possibly higher energy concentration or reserves (late eggs are smaller but have similar energy content [Chapter 2]). This could allow late larvae to survive in nutrient poor conditions until the medium conditioning activities of all larvae present had taken effect, especially if late larvae are better medium conditioners. More active larvae from young mothers would exhaust their reserves more rapidly and die sooner. In the current study, late line C had lower shape FA than did early line C, and they reduced the size FA of D of both maternal ages, relative to D raised with early line C. This suggests that late C larvae may rely on a strategy of conditioning the larval medium and waiting for microbes to grow and for over-active scramble competitors to die. This strategy would benefit all larvae that survive until the conditioning takes effect, regardless of line. If line D larvae are primarily scramble competitors, with poor medium conditioning ability, then they should perform better in the presence of conditioning larvae, possibly out-competing them later during development. In this case, line D would not confer an advantage on other larvae present and should not affect competitor FA except via density effects and these were controlled in this study. A similar pattern exists in *ge* mutants in house flies. They are poor nutrient conditioners that perform well in the presence of larvae with normal nutrient conditioning activity (Bhalla and Sokal 1964; Bryant 1969).

A third difference between responses of FA and survival to competition is that while there were no sex effects on viability, females have higher shape and size FA than males. This is consistent with a previous report and is further evidence that FA is not directly related to survival since FA differs between sexes but survival does not (Chapter 3). Two studies of FA and mating success in house flies and survival did not show a difference between sexes (Moller 1996; Goulson et al. 1999), but this may have been due to decreased resolution from the use of only two simple metric characters (Leung et al. 2000), whereas I used multivariate characters.

The response of FA to competitors was exhibited primarily by shape FA and not by size FA (except for the difference between sexes and the effect of line C age on line D). Size FA, but not shape FA, responded to stresses of maternal age, but shape FA was influenced more by density in house flies than was size FA. This independence of size FA and shape FA may arise because maternal age effects are strongest during embryogenesis and early larval life, which is well before vein locations are determined, but density effects are strongest during late larval life when vein locations are being determined (Sullivan and Sokal 1963; Mohler et al. 2000). The above notwithstanding, the effect of the maternal age of line C on line D size FA is consistent with the idea that early larval environment and medium conditioning affect size FA.

It is interesting that FA accounts for a larger portion of shape variation than it does of size variation (Tables 5.1 and 5.2). This suggests that shape FA may be more revealing than size FA in other instances. Simultaneous analysis of shape FA and size FA in other systems is needed to address this.

I showed previously, in a longitudinal study of line C, that increasing maternal age elevated size FA (Chapter 4). This maternal age effect was not evident in the present study, possibly because the maternal age differences and/or sample sizes were smaller. Or maternal age effects on adult FA could vary among lines, as do maternal age effects on larval performance (Chapter 3). Maternal age effects on FA vary between taxa (Parsons 1962; Wakefield et al. 1994; McIntyre and Gooding 1998, Klingenberg and McIntyre 1998), and it is likely that these differences have evolved. In light of this, maternal age effects on FA could differ between strains, especially if FA is mediated by subtle patterns of line by environment interactions as I have demonstrated in house flies.

Table 5.1. Mixed model ANOVAs of individual by side for centroid size and Procrustes aligned shape configurations. Main effects were tested over the interaction term (FA) which was tested against the residual. Mean squares for size and shape are multiplied by 10^3 and 10^6 respectively. Bracketed values are the percentage of the total variation accounted for by each term. Procrustes *P* values are from permutation tests that the MS of each term is not larger than expected based on random resampling without replacement.

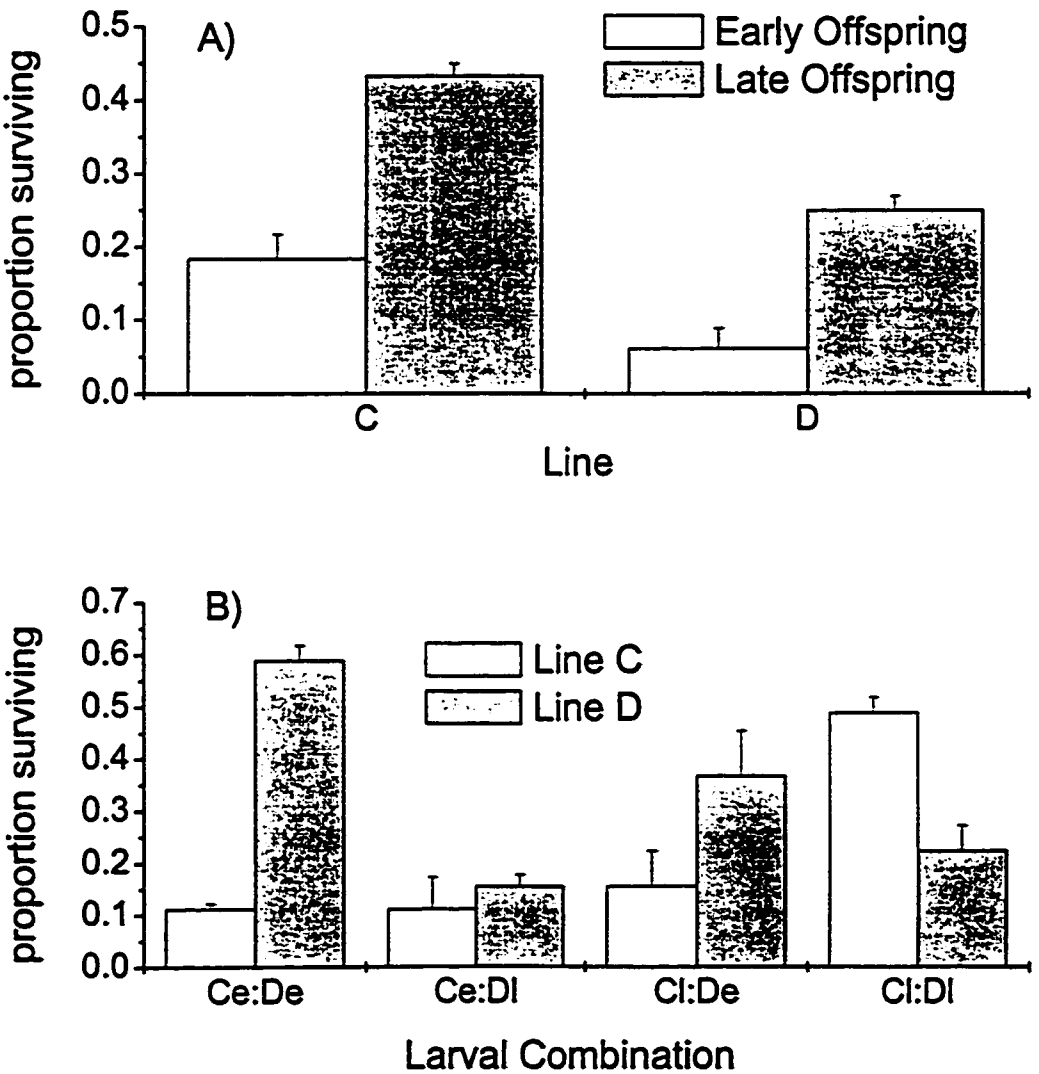
Source	DF	MS	*F*	*P* <
Centroid Size				
Individuals	253	1,235.4 (99.89)	4699.0	0.0001
Sides	1	2.2 (0.00)	2.6	0.1086
Fluctuating Asymmetry	253	0.8 (0.07)	3.2	0.0001
Residual	508	0.3 (0.04)		
Procrustes Shape				
Individuals	6024	94.8 (91.44)	24.2	0.0001
Sides	24	781.0 (3.00)	199.6	0.0001
Fluctuating Asymmetry	6024	3.9 (3.77)	4.2	0.0001
Residual	12096	0.9 (1.79)		

Table 5.2. Wing size and asymmetry statistics for offspring of young and old female house flies of two genetically marked lines. Flies were raised in mono- (pure) or duo-cultures (mixed). Mixed culture flies are grouped with their competitors. Means (±SE) of centroid size, signed asymmetry (R-L) and unsigned asymmetry (|R-L|) are provided, as are skew and kurtosis values for signed asymmetry. Shape mean squares (multiplied by 10^6) are from Procrustes ANOVAs of the full multivariate data set for each group. For signed size asymmetry and shape FA the percent of total variation explained by FA is presented in brackets. Degrees of freedom for Procrustes mean squares are normal degrees of freedom times 24 (e.g., in samples of 10 flies there are 216 and 480 degrees of freedom for the FA and residual terms respectively).

				Centroid Size					Shape Mean Squares	
Culture	Line	Age*	n	Size (mm)	(R-L) (mm)	Kurtosis	Skew	|R-L| (mm)	FA	Residual
Pure	C	Y	18	6.05 ± 0.092	-6.8 ± 6.19 (0.21)	2.64	-1.47	18.8 ± 4.63	3.19 (3.2)	0.82
Pure	C	O	30	5.34 ± 0.065	3.9 ± 4.80 (0.13)	0.35	-0.58	22.1 ± 2.69	3.70 (4.1)	0.78
Pure	D	Y	9	4.72 ± 0.071	8.6 ± 4.83 (0.11)	0.03	0.31	17.1 ± 3.00	6.32 (6.1)	1.11
Pure	D	O	35	5.91 ± 0.078	4.9 ± 4.69 (0.24)	0.82	0.55	22.6 ± 2.85	3.03 (3.5)	0.95
Mixed	C	Y	10	5.11 ± 0.077	-9.1 ± 8.19 (0.28)	1.93	1.17	22.2 ± 4.62	5.24 (5.1)	0.88
Mixed	D	Y	43	5.20 ± 0.040	-1.1 ± 4.20 (0.27)	0.44	-0.35	23.2 ± 2.34	4.16 (4.7)	1.04
Mixed	C	O	10	4.64 ± 0.075	11.0 ± 7.93 (0.28)	-1.71	0.13	22.1 ± 4.69	5.00 (6.0)	0.91
Mixed	D	Y	24	4.91 ± 0.050	-5.3 ± 3.06 (0.09)	0.97	0.94	15.8 ± 1.61	3.75 (3.6)	1.06
Mixed	C	Y	8	6.24 ± 0.098	2.2 ± 8.24 (0.18)	0.04	0.09	20.5 ± 4.28	3.47 (5.4)	0.76
Mixed	D	O	13	6.34 ± 0.078	5.3 ± 8.76 (0.31)	2.10	0.63	24.0 ± 5.70	3.05 (3.2)	0.68
Mixed	C	O	34	5.56 ± 0.061	-11.0 ± 3.92 (0.10)	1.19	-0.77	19.5 ± 2.80	3.43 (3.9)	0.94
Mixed	D	O	18	5.49 ± 0.053	-12.7 ± 4.25 (0.16)	-0.16	0.61	19.9 ± 2.28	3.33 (4.2)	0.91

* O and Y refer to maternal age of females from which eggs were collected (ca 10 and 24 days old respectively)

Figure 5.1. The mean (± SE) proportion of house fly larvae completing development in competition experiments between offspring of young (10-day-old) and old (24-day-old) mothers from two genetically selected house fly lines. A) Survival in mono-culture. B) Survival in 1:1 mixtures. "e" and "l" indicate that the flies are offspring of young and old females respectively.



References

Bhalla, S. C. and Sokal, R. R. 1964. Competition among genotypes in the housefly at varying densities and proportions (the green strain). Evolution **18**:312-330.

Bookstein, F.L. 1991. Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge Univ. Press, Cambridge, MA.

Borash, D. J., Gibbs, A. G., Joshi, A. and Mueller, L. D. 1998. A genetic polymorphism maintained by natural selection in a temporally varying environment. Am. Nat. **151**:148-156.

Bryant, E. H. , 1969. The fates of immatures in mixtures of two housefly strains. Ecology **50**:1049-4069.

Bryant, E. H. and Sokal, R. R. 1967. The fate of immature housefly populations at low and high densities. Res. Popul. Ecol. 9:19-44.

Bryant, E. H. and Sokal, R. R. 1968. Genetic differences affecting the fates of immature housefly populations at two densities. Res. Popul. Ecol. **10**:140-170.

Dryden, I. L. and Mardia, K. V. 1998. Statistical Shape Analysis. Wiley, New York..

Fellowes, M. D. E. 1998. Do non-social insects get the (kin) recognition they deserve?. Ecol. entomol. **23**: 223-227.

Graham, J. H., Freeman, D. C. and Emlen, J. M. 1993. Developmental stability: a sensitive indicator of populations under stress. In Landis, W.G., Hughes, J.S., and Lewis, M.A. (eds). Environmental Toxicology and Risk Assessment, pp. 136-158. American Society for Testing and Materials, Philadelphia (PA).

Goulson, D., Bristow, L., Elderfield, E., Brinklow, K., Parry-Jones, B. and Chapman, J.W. 1999. Size, symmetry, and sexual selection in the housefly, *Musca domestica*. Evolution **53**: 527-534.

Haldane, J. B. S. 1956. Estimation of viabilities. J. Genet. **54**: 294-296.

Kence, M. and Jdeidi, T. 1997. Effect of malathion on larval competition in house fly (Diptera: Muscidae) populations. J. Econ. Entomol. **90**: 59-65.

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

Leary, R. F. and Allendorf, F. W. 1989. Fluctuating asymmetry as an indicator of stress: Implications for conservation biology. Trends Ecol. Evol. **4**: 214-217.

Leung, B. and Forbes, M. R. 1996. Fluctuating asymmetry in relation to stress and fitness: Effects of trait type as revealed by meta-analysis. Ecoscience **3**: 400-413.

Leung, B., Forbes, M. R. and Houle, D. 2000. Fluctuating asymmetry as a bioindicator of stress: Comparing efficacy of analyses involving multiple traits. Am. Nat. **155**: 101-115.

McIntyre, G. S. and Gooding, R. H. 1995. Pteridine accumulation in *Musca domestica*. J. Insect Physiol. **41**: 357-368.

McIntyre, G. S. and Gooding, R. H. 1998. Effect of maternal age on offspring quality in tsetse (Diptera: Glossinidae). J. Med. Ent. **35**: 210-215.

Mohler, J., Seecoomar, M., Agarwal, S., Bier, E. and J. Hsai. 2000. Activation of *knot* (KN) specifies the 3-4 intervein region in the *Drosophila* wing. Development **127**: 55-63.

Moller, A. P. 1996. Sexual selection, viability selection, and developmental stability in the domestic fly *Musca domestica*. Evolution **50**: 746-752.

Palmer, A. R. 1994. Fluctuating asymmetry analyses: A primer. In: Markow, T. A. (ed) Developmental Instability: Its Origins and Evolutionary Implications, pp. 335-364. Kluwer, The Netherlands

Parsons, P. A. 1962. Maternal age and developmental variability. J. Exp. Biol. **39**:251-260.

Ruiz-Dubreuil, G., Burnet, B., Connolly, K. and Furness, P. 1996. Larval foraging behaviour and competition in *Drosophila melanogaster*. Heredity **76**: 55-64.

Santos, M., Fowler, K. and Partridge, L. 1992. On the use of tester stocks to predict the competitive ability of genotypes. Heredity **69**:489-495.

Schmidtmann, E. T. and Martin, P. A. W. 1992. Relationship between selected bacteria and the growth of immature house flies, *Musca domestica*, in an axenic test system. J. Med. Ent. **29**: 232-235.

Sokal, R. R. and Sullivan, R. L. 1963. Competition between mutant and wild-type house-fly strains at varying densities. Ecology **44**: 314-322.

Sokolowski, M. B., Pereira, H. S. and Hughes, K. 1997. Evolution of foraging behavior in *Drosophila* by density-dependent selection. Proc. Natl. Acad. Sci. USA **94**: 7373-7377.

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

Sullivan, R. L. and Sokal, R. R. 1965. Further experiments on competition between

strains of house flies. Ecology, **46**, 172-182.

Wakefield, J., Harris, K. and Markow, T. A. 1994. Parental age and developmental stability in *Drosophila melanogaster*. In: Markow, T. A. (ed) Developmental Instability: Its Origins and Evolutionary Implications, pp. 237-246. Kluwer, The Netherlands.

Chapter 6: The adaptive nature of house fly maternal age effects

Introduction

Maternal age effects are usually considered to be non-adaptive declines in maternal reproductive performance (Mousseau and Dingle 1991a, b; Roff 1992; Mousseau and Fox 1998; Chapter 1). However, a review of the literature reveals that, except for a widespread decline in clutch size, maternal age effects are quite variable around the generally accepted trends of declining egg size, embryonic development rates, hatch rate, and offspring fecundity (Tables 1.1 and 1.2). This variability, coupled with cases of variable maternal age effects that can be explained through morph, strain, host or other differences (Chapter 1), suggests that maternal age effects are likely to be adaptive more often than is generally thought.

One way to gain further insight into maternal age effects is to make a detailed comparison of offspring of young and old females from a single species at several stages of development and to examine appropriate fitness related traits at each stage. I have conducted such a study of house flies (*Musca domestica* L.) testing for maternal age effects on egg number, size, hatch rate, and contents and also on larval competitiveness and adult fluctuating asymmetry. In this chapter I summarize those aspects of this study most directly relating to maternal age effects. I use these results to develop an adaptive hypothesis for maternal age effects in house flies. A program for testing this hypothesis is presented, as are implications of this study for further investigation of maternal age effects.

Maternal age effects and egg contents

The main assumption underlying the non-adaptive view of insect maternal age effects is that many insects (especially endopterygotes) produce fewer smaller eggs at advanced ages (Table 1.2). Egg size is thought to reflect female investment per egg and therefore egg quality as well. Thus late eggs are thought to be of lower quality than early eggs because they are smaller (e.g., Azevedo et al. 1997). However, egg size has been shown to vary independently of egg content (an alternate estimator of maternal investment) in some insects and in other taxa (Quickenden and Roemhild 1969; Capinera et al. 1977; Williams 1994; Jaeckle 1995; Bernardo 1996; Diss et al. 1996). Accordingly, I began my investigation of maternal age effects in house flies by comparing eggs of young and old flies.

To understand the true nature of maternal age effects on eggs it is necessary to untangle direct effects of maternal age and effects due to maternal age related egg size decline. I solved this problem by using two distinct measures to generate egg size variation (maternal age and maternal size: these are negatively and positively correlated with egg size respectively) and comparing relationships among egg parameters between manipulations. This allowed me to separate the effects of egg size that are common to these two phenotypic manipulations from the egg size independent maternal age effects on eggs.

Egg size was either uncorrelated or negatively correlated with hatch rate, and egg size bore little relation to egg contents (Tables 2.1 and 2.2, Figure 2.3). Partial correlation analysis revealed that, although maternal age was negatively correlated with hatch rate and egg size, hatch rate was unrelated to egg size after removal of maternal age effects. Using similar logic, egg size and hatch rate were weakly negatively correlated after removal of

maternal size effects, even though maternal size was positively related to egg size but was statistically unrelated to hatch rate. Egg size differences were not reflected in egg energy, protein, or carbohydrate content. However, lipid content was lower in small eggs from old mothers but not in small eggs from small mothers (Tables 2.1 and 2.2). Unfortunately I was unable to separate statistically maternal age and size effects from egg size effects in the egg content experiments.

The assumptions that egg size can be used as a surrogate for egg quality and that large eggs are more expensive to produce than small eggs may hold for many species (Azevedo et al. 1997), but they do not hold for house flies (Chapter 2), or for some members of other taxa (Williams 1994; Jaeckle 1995; Bernardo 1996). Thus, late eggs are not simply smaller versions of early eggs, since they differ in content as well as in size. Although late eggs have reduced hatch rates (a negative maternal age effect that is independent of egg size), hatchlings from small late eggs may nonetheless be better suited to certain conditions than are hatchlings from large early eggs as discussed below.

Maternal age and larval competition

One arena in which late hatchlings might outperform early hatchlings is in competition for resources, since larval competition is important to success for house flies (Sullivan and Sokal 1963; Bryant and Sokal 1967, 1968; Kence and Jdeidi 1997). By conducting experiments in which early and late offspring, from genetically marked strains, competed against each other I demonstrated that late larvae are more competitive, at high density, than are early larvae. In most cases, maternal age was positively associated with larval viability, mass, and development rate (Tables 3.2 and 3.3; Figures 3.2-3.4). Both competitive ability and the effect of maternal age on competitive ability

were strain specific. Strain and mixture specific nature of competitive ability were previously demonstrated in house flies and is strong evidence that larval competitive ability is under genetic control (Sokal and Sullivan 1963; Bhalla and Sokal 1964; Sullivan and Sokal 1965). My results suggest that maternal age effects on larval competitive ability are also under genetic control.

One of the most salient features of my competition study is that the competitive effects of line, maternal age, and competition ratio could not be generalized. Rather competitive ability varied among combinations of lines and also between pure cups and mixed cups as has been previously demonstrated in house flies and *Drosophila* spp. (Lewontin 1955; Lewontin and Matsuo 1963; Sokal and Sullivan 1963; Bhalla and Sokal 1964; Sullivan and Sokal 1965). The difficulty in predicting performance in mixtures from performance in pure culture has led to the use of testor strains to indirectly determine relative competitive abilities of genotypes (e.g., Santos et al. 1992). But, as I have argued (Chapter 3), this requires that either there is a single major determinant of competitive success or that the different genotypes interact with each other in a common fashion. My results demonstrate that the use of testor strain competition experiments is inappropriate in house flies and probably also in other species with complex larval competition dynamics.

The basis of the competitive advantage of late house fly larvae is unknown. Late larvae were less active than early larvae at the beginning of these experiments (qualitative observation), they came from smaller eggs (Table 3.1), and they may have increased medium conditioning ability (Chapter 3). Egg size is not related to egg energy content in house flies (Chapter 2) so late hatchlings may have proportionately larger energy reserves

also. Different levels of larval activity and medium conditioning may be favored depending upon environmental conditions. More active larvae would be at an advantage at low-moderate larval density since they will search for and find sufficient food to keep themselves alive until bacterial growth accelerates due to their medium conditioning activity. However, at high larval density, active larvae would be at a disadvantage since they may rapidly exhaust their hatchling energy reserves and the initial food supply, thus starving before their weak medium conditioning activities takes effect. Less active larvae would be at an advantage at high density because they are less prone to starvation in poor media and could outlast more active larvae while their strong medium conditioning activity takes effect. However, less active larvae may be at a disadvantage in nutrient rich media since they feed less rapidly than active larvae. This explanation fits the data for late D offspring which performed poorly in pure culture, but performed well when a few competitors, that performed well in pure culture, were present (Table 3.3, Figure 3.3). Similarly, *ge* house fly mutants are poor nutrient conditioners that perform well in the presence of larvae with normal nutrient conditioning activity (Bhalla and Sokal 1964; Bryant 1969).

Thus, as is the case with eggs, larvae from old females are not simply smaller versions of larvae from young females, rather they perform better in larval competition. It is likely that these differences are due to the employment of distinct genetically based larval strategies of resource utilization. Such strategies have been previously demonstrated in house flies and in *Drosophila*, but they have not been related to maternal age (Sullivan and Sokal 1965; Bhalla and Sokal 1964; Bryant and Sokal 1968; Bryant 1969; Ruiz-Debruil et al. 1996; Sokolowski et al. 1997; Borash et al. 1998). This

difference in competitive ability is further evidence that maternal age effects in house flies may be adaptive in nature.

Maternal age and adult fluctuating asymmetry

In an attempt to assess maternal age effects on adult house fly offspring, I compared offspring fluctuating asymmetry (FA, a potential indicator of developmental stress and organismal/population quality [reviewed in Palmer 1996]) across maternal ages. In a longitudinal study of the effects of maternal age on shape FA and size FA of wings of offspring raised at high and low densities, maternal age, larval density, and sex had effects on house fly wing FA but the response of size FA and shape FA differed. Centroid size FA was positively correlated with maternal age, higher in females, and unaffected by larval density (Tables 4.2 and 4.3). Shape FA was unaffected by maternal age, higher in females than in males, and positively correlated with larval density, especially in females (Tables 4.2 and 4.3). These results suggest that increasing maternal age decreases developmental stability, at least in some traits, and that the use of FA as an indicator of overall developmental stability, developmental stress, or individual quality is a very uncertain endeavor, as has been argued elsewhere (e.g. Leung and Forbes 1996; Bjorksten et al. 2000).

To make use of this information it is necessary to relate FA levels to the biology of the house flies, since FA does not unambiguously indicate organismal quality. The partially independent and temporally distinct determination of wing size and wing vein location (reviewed in Chapter 4) makes the primarily independent response of size FA and shape FA less surprising. Wing imaginal discs are set aside half way through embryogenesis and begin to proliferate at the end of the first larval instar (Bryant and

Schmidt 1990). This coincides with the period when maternal effects are usually strongest (Mousseau and Dingle 1991a,b; Mousseau and Fox 1998; Chapter 1 this volume). Maternal age could affect size asymmetry through between side differences in the initial size or early proliferation of wing discs. In contrast, maternal age effects are likely to have disappeared before wing vein determination, which occurs in late the third instar larvae and early pupae (Mohler et al. 2000 and references therein). Since the effects of high larval density act primarily during the third larval instar (Sullivan and Sokal 1963), density stress might affect vein location but not wing size, if local alterations in vein pattern are compensated for by the rest of the wing to maintain appropriate size.

The longitudinal study of maternal age and wing FA (Chapter 4) suggests that maternal age negatively affects the developmental stability of specific traits of adult offspring. However these traits are determined during larval development and thus, the maternal age effects are probably trait specific responses to particular stresses. To conclude that the FA differences are negative maternal age effects on adult quality requires the demonstration that either the small degree of wing asymmetry observed is related to adult performance, or that wing FA is truly representative of organism-wide developmental stability and quality.

Relating FA to larval competition strategies

To elucidate further the relationships of maternal age, wing FA, and larval development, I evaluated the FA of a subset of flies from the competition experiments (Chapter 3). This was done by determining whether the response of FA to competition was similar to that of survival and by determining if the FA response could provide additional insight into larval dynamics.

When comparing lines, maternal ages and culture method (pure or mixed), flies raised in competition with larvae from another line had higher wing shape FA than those raised in pure culture, even in line D which had higher survival in mixed than in pure culture (Chapter 5). Wing centroid size FA was unaffected by maternal age, line, or culture method (Chapter 5).

When comparing only flies from mixed cultures, late C offspring had lower wing shape FA than did early C offspring, and D flies raised with late C offspring had lower size FA than D flies raised with early C offspring. The results are consistent with the hypothesis that late C larvae alter their medium in a manner that benefits development (as measured by survival and FA) of all larvae present, but that this is not the case with D larvae of either maternal age class (Chapter 5).

FA will increase and survival will decrease if both are adversely affected by the same developmental stress(es). This was evident in the reversal of line survival and shape FA between mixed and pure cups: line C had lower survival and higher FA than line D in mixed cups, but the opposite held in pure cups. Also, in mixed cups survival of late C was greater and shape FA was lower than that of early C. In contrast, line D had lower viability and lower size FA when reared with late C than with early C. Thus, the response of FA only partially corresponds with that of viability. The differential responses suggest that wing FA tracks a slightly different component of the larval environment than does viability.

I hypothesized previously (above, Chapter 3) that the competitive advantage of late larvae involves a lower activity rate (unpublished observations) and possibly higher energy concentration or reserves (late eggs are smaller but have similar energy content

[Chapter 2]). This could allow late larvae to survive in nutrient poor conditions until the medium conditioning activities of all larvae present take effect, especially if late larvae are better medium conditioners. More active larvae from young mothers would exhaust their reserves more rapidly and die sooner. In the study of FA and larval competition, late line C had lower shape FA than did early line C, and they reduced the size FA of D of both maternal ages, relative to D raised with early line C (Chapter 5). This suggests that late C larvae may rely on a strategy of conditioning the larval medium and waiting for microbes to grow and for over-active scramble competitors to die. This strategy would benefit all larvae that survive until the conditioning takes effect, regardless of line. If line D larvae are primarily scramble competitors, with poor medium conditioning ability, then they should perform better in the presence of conditioning larvae, possibly out-competing them later during development. In this case, line D would not confer an advantage on other larvae present and should not affect competitor FA, except via density effects and these were controlled in this study.

In the longitudinal study of line C, maternal age elevated size FA (Chapter 4). This maternal age effect was not evident in the study of larval competition and FA (Chapter 5), possibly because the maternal age differences and/or sample sizes were smaller. It is also possible that maternal age effects on adult FA vary between lines, as do maternal age effects on larval performance (Chapter 3). Maternal age effects on FA vary between taxa (Parsons 1962; Wakefield et al. 1994; McIntyre and Gooding 1998, Klingenberg and McIntyre 1998), and it is likely that these differences have evolved. In light of this, maternal age effects on FA could differ between strains, especially since FA appears to be mediated by subtle patterns of line by environment interactions (chapter 5).

An adaptive hypothesis of house fly maternal age effects

I argued in the introduction (Chapter 1) that adaptive maternal age effects could evolve in species that experience one or more consistent environmental changes during the life of individuals. In temperate areas house fly populations are seasonal and increase during the spring to a peak during late summer (Black and Krafsur 1986, 1987). This implies that females will generally oviposit into more crowded situations as they age. Since larval density has strong life history consequences, females may experience evolutionary pressure to produce larvae better suited to high larval density at advanced ages.

There are several steps involved in demonstrating that a maternal effect is adaptive. First, the environmental cue must be a reliable predictor of future conditions. Second, it must consistently elicit a specific change in the kind of offspring produced. And finally, the 'changed' offspring must outperform 'normal' offspring in the predicted environment. In this study, of maternal age effects in house flies, I have argued that age could be a reliable indicator of increased density experienced by later larvae. I have demonstrated changes in the kind of eggs, larvae, and adult offspring produced by older females. I have shown that under high-density conditions late larvae outperform early larvae in direct competition. Together this is evidence that maternal age effects may be adaptive in house flies, i.e., that late offspring are better suited to high larval density than are early offspring.

The simplest hypothesis consistent with the data on maternal age effects is that there is a continuum between two alternative larval competition strategies. At one end of the continuum are highly competent medium conditioners, with low activity and poor

scramble competition ability that are superior competitors at high larval densities. At the opposite end of the continuum are highly competent scramble competitors, with high activity but poor medium conditioning ability that are superior competitors at low larval densities. If the above is correct, the production of late offspring that are closer to the medium-conditioning end of the continuum, than predicted by their genetically determined position on the continuum, would be the result of an adaptive maternal age effect. Making predictions from this model will become more complicated if, as seems likely, the relative competitive ability of a specific strategy depends upon the nature of the competitors.

Future Work

Testing the adaptive hypothesis of house fly maternal age effects

Further work is required to conclusively demonstrate that the observed maternal age effects are adaptive. It will be crucial to demonstrate that age is a reliable indicator of increased density within the life span of individuals and that the conditions of competition experiment are sufficiently realistic.

The specifics of my hypothesis concerning maternal age effects on larval competition strategies also require verification. This could be accomplished by comparing starvation resistance, ability to condition the medium, and larval feeding rates of offspring of young and old females in isolation and relating this to performance in mixed culture.

Ideally this work should be verified under field conditions. This would be quite challenging given the size and habits of flies and their offspring. However if wild caught

females are found to exhibit similar maternal age effects, and variability in their maternal age effects, this would support the general validity of my results.

General extensions to maternal age studies

The results presented here suggest that the assumption that late, small eggs/offspring are simply smaller, less competitive versions of early, large eggs/offspring must be reconsidered. Close consideration should be given to differences between the environments usually experienced by early and late offspring, this of course will depend upon the biology of the species in question. Future studies should include an examination of egg parameters other than size, preferably including egg content. If it is biologically relevant, then early and late offspring should be raised together to see how they compete with each other. This should be done in the environment normally experienced by both kinds of offspring. If these suggestions are followed it is likely that researchers will find many more examples of adaptive maternal age effects than previously thought.

References:

Azevedo, R.B.R., French, V., and Partridge, L. 1997. Life-history consequences of egg size in *Drosophila melanogaster*. Am. Nat. **150**: 250-282.

Bernardo, J. 1996. The particular maternal effect of propagule size, expecially egg size: patterns, models, quality of evidence and interpretations. Amer. Zool. **36**: 216-236.

Bhalla, S. C. and Sokal, R. R. 1964. Competition among genotypes in the housefly at varying densities and proportions (the green strain). Evolution **18**: 312-330.

Bjorksten, T. A., Fowler, K., and Pomiankowski, A., 2000. What does sexual trait FA tell us about stress? Trends Ecol. Evol. 15: 163-166.

Black IV, W. C. and Krafsur, E.S. 1986. Population biology and genetics of winter house fly (Diptera: Muscidae) populations. Ann. Ent. Soc. Am. **79**: 636-644.

Black IV, W. C. and Krafsur, E. S., 1987. Fecundity and size in the housefly:

investigations of some environmental sources and genetic correlates of variation. Med. Vet. Entomol. **1**: 369-382.

Borash, D. J., Gibbs, A. G., Joshi, A. and Mueller, L. D. 1998. A genetic polymorphism maintained by natural selection in a temporally varying environment. Am. Nat. **151**:1 48-156.

Bryant, E. H. 1969. The fates of immatures in mixtures of two housefly strains. Ecology **50**: 1049-4069.

Bryant, E. H. and Sokal, R. R. 1967. The fate of immature housefly populations at low and high densities. Res. Popul. Ecol. **9**: 19-44.

Bryant, E. H. and Sokal, R. R. 1968. Genetic differences affecting the fates of immature housefly populations at two densities. Res. Popul. Ecol. **10**; 140-170.

Bryant, P. J. and Schmidt, O. 1990. The genetic control of cell proliferation in *Drosophila* imaginal discs. J. Cell Sci. Suppl.**13**: 169-189.

Capinera, J. L., Barbosa, P. and Hagedorn, H. H. 1977. Yolk and yolk depletion of gyspy moth eggs: implications for population quality. Ann. Ent. Soc. Am. **70**: 40-42.

Diss, A. L., Kunkel, J. G., Montgomery, M. E., and Leonard, D. E. 1996. Effects of maternal nutrition and egg provisioning on parameters of larval hatch, survival and dispersal in the gypsy moth, *Lymantria dispar* L.. Oecologia **106**: 470-477.

Jaeckle, W. B. 1995. Variation in the size, energy content, and biochemical composition of invertebrate eggs: correlates to the mode of larval development. In: McEdward. L. R. (ed). Ecology of Marine Invertebrate Larvae, pp. 49-77. Discorides Press, Portland.

Kence, M., and Jdeidi, T. 1997. Effect of malathion on larval competition in house fly (Diptera: Muscidae) populations. J. Econ. Entomol. **90**: 59-65.

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

Leung, B. and Forbes, M. R. 1996. Fluctuating asymmetry in relation to stress and fitness: Effects of trait type as revealed by meta-analysis. Ecoscience **3**: 400-413.

Lewontin, R. C. 1955. The effects of population density and composition on viability in *Drosophila melanogaster*. *Evolution*, **9**, 27-41.

Lewontin, R. C. and Matsuo Y. 1963. Interaction of genotypes detemining viability in *Drosophila buscktii*. Proc. Natl. Acad. Sci. USA **49**: 270-278.

McIntyre, G. S. and Gooding, R. H. 1998. Effect of maternal age on offspring quality in

tsetse (Diptera: Glossinidae). J. Med. Ent. **35**: 210-215.

Mohler, J., Seecoomar, M., Agarwal, S., Bier, E. and J. Hsai. 2000. Activation of *knot* (KN) specifies the 3-4 intervein region in the *Drosophila* wing. Development **127**: 55-63.

Mousseau, T. A. and Dingle, H. 1991a. Maternal effects in insect life histories. Annu. Rev. Entomol. **36**: 511-534.

Mousseau, T. A. and Dingle, H. 1991b. Maternal effects in insects: examples, constraints, and geographic variation. In: Dudley, E. C. (ed) The unity of evolutionary biology, vol. II, pp. 745-761. Dioscoridies Press, Portland.

Mousseau, T. A. and Fox, C. W. 1998. Maternal Effects as Adaptations. Oxford University Press, New York.

Palmer, A. R. 1996. Waltzing with asymmetry. Bioscience **46**:518-532.

Parsons, P.A., 1962. Maternal age and developmental variability. J. Exp..Biol. **39**: 251-260.

Quickenden, K. L. and Roemhild, G. R. 1969. Maternal age and density effects on carbohydrate partitioned to eggs of the grasshopper, *Aulocara elliotti*. J. Insect Physiol. **15**: 1215-1223.

Roff, D. 1992. The evolution of life histories: theory and analysis. Chapman & Hall, New York.

Ruiz-Dubreuil, G., Burnet, B., Connolly, K. and Furness, P. 1996. Larval foraging behaviour and competition in *Drosophila melanogaster*. Heredity **76**: 55-64.

Santos, M., Fowler, K. and Partridge, L. 1992. On the use of tester stocks to predict the competitive ability of genotypes. Heredity **69**: 489-495.

Sokal, R. R. and Sullivan, R. L. 1963. Competition between mutant and wild-type house-fly strains at varying densities. Ecology **44** ; 314-322.

Sokolowski, M. B., Pereira, H. S. and Hughes, K. 1997. Evolution of foraging behavior in *Drosophila* by density-dependent selection. Proc. Natl. Acad. Sci. USA **94**: 7373-7377.

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

Sullivan, R. L. and Sokal, R. R. 1965. Further experiments on competition between strains of house flies. Ecology **46** ; 172-182.

Wakefield, J., Harris, K. and Markow, T. A. 1994. Parental age and developmental

stability in *Drosophila melanogaster*. In: Markow, T. A. (ed) Developmental Instability: Its Origins and Evolutionary Implications, pp. 237-246. Kluwer, The Netherlands.

Williams, T. D. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. Biol. Rev. **68**: 35-59.

Appendix 1: Effect of storage on house fly egg size and content.

To test the effects of storage in 0.85% saline at –20C I compared egg dimensions, volume, and content of fresh eggs and eggs from the same sample that had been stored in 0.85% saline at –20C for three months using the methods described in Chapter 2. There were no significant differences in any of the measured parameters (Table A1.1).

Table A1.1. Mean (± SE) of length, width, volume, protein content, lipid content, and carbohydrate content for eggs from 10 day old female house flies measured fresh and after storage in 0.85% saline at -20C for 3 months. Sample sizes for dimensions are actual numbers of eggs measured and for egg content are number of determinations made of separate 5 egg samples (see Chapter 2: Materials and Methods).

Egg Trait	n	Fresh	n	Frozen	*P*
Length (mm)	50	1.269 ± 0.0065	50	1.271 ± 0.0056	0.76
Width (mm)	50	0.290 ± 0.0016	50	0.292 ± 0.0016	0.36
Volume microl	50	55.8 ± 0.72	50	56.7 ± 0.64	0.38
Protein (μg/egg)	8	5.38 ± 0.154	8	5.20 ± 0.162	0.43
CHO (μg/egg)	8	2.58 ± 0.118	8	2.58 ± 0.259	0.99
Lipid (μg/egg)	8	1.70 ± 0.047	8	1.66 ± 0.050	0.56

Appendix 2: House fly eggs do not change size during the first 9 hours post laying.

To ensure that house fly egg measurements were not affected by changes in size during, development I measured a group of eggs at 1, 3, 5, 7, and 9 hours post laying. Between measurement times eggs were incubated at 25C. All eggs included in the analysis successfully completed development. There were no differences in egg length, width, or volume during this time period (Table A2 .1). This range covers the possible age of eggs used in the experiments described in this thesis, since eggs were collected over a maximum seven hour period and then measured immediately or frozen in 0.85% saline at -20C for later measurement.

Table A2.1. Mean (± SE) of length, width, and volume of 20 eggs measured at five times after laying. Between measurements eggs were incubated at 25C. All eggs hatched.

Hours Post-Laying	Length	Width	Volume
1	1.264 ± 0.0122	0.290 ± 0.0027	55.8 ± 1.30
3	1.265 ± 0.0124	0.291 ± 0.0032	56.3 ± 1.47
5	1.264 ± 0.0119	0.292 ± 0.0032	56.8 ± 1.49
7	1.264 ± 0.0121	0.293 ± 0.0040	57.3 ± 1.80
9	1.263 ± 0.0124	0.288 ± 0.0029	55.1 ± 1.27

Appendix 3: Replicates differ little in FA or DA in the longitudinal study of maternal age effects on FA.

The only difference between replicates in the longitudinal study of maternal age effects on FA was that DA differed between replicates of low density male offspring of 2-week-old mothers (Tables A3.1 and A3.2) To determine if this might be due to the presence of outliers I plotted individual deviations from the mean asymmetry (R-L) of each landmark (Figure A3.1). One fly from replicate A had greater asymmetry than any other fly, with the average deviation across all landmarks was 2.4 times that of the average fly in these replicates. However after removal of this fly the replicates still differed significantly with respect to DA and other effects were unchanged (Table A5). Although this fly had extremely asymmetrical wings, it did not explain the difference between replicates, so I included this fly in the analysis of the longitudinal experiment (Chapter 4).

Table A3.1. Comparison of DA and FA wing centroid size variation between replicates within treatments. Mean squares are multiplied by 10^3, 10^4 for DA and FA respectively.

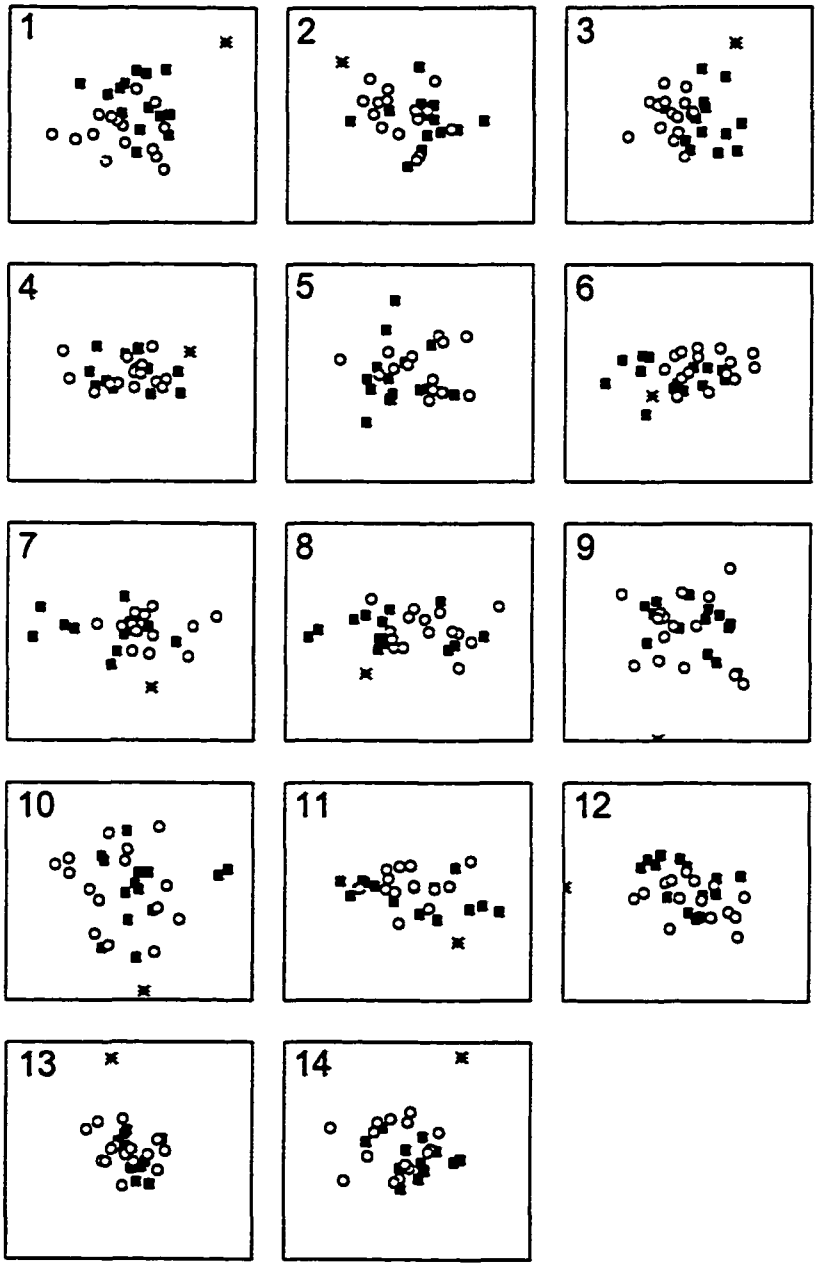
			n		DA			FA		
Density	Age	Sex	Rep A	Rep B	Rep A	Rep B	*P*	Rep A	Rep B	*P*
H	1	F	15	15	1.20	0.74	0.13	5.21	10.75	0.44
H	1	M	15	15	1.82	0.37	0.07	4.05	7.34	0.17
H	2	F	15	15	0.55	0.03	0.48	6.73	10.54	0.42
H	2	M	15	15	1.76	0.02	0.23	7.09	7.72	0.87
H	3	F	15	15	3.30	0.33	0.11	16.41	5.24	0.24
H	3	M	15	15	0.30	0.05	0.84	11.58	6.30	0.39
L	2	F	15	15	7.38	19.60	0.26	12.20	7.84	0.51
L	2	M	15	15	3.66	1.16	0.50	11.13	3.49	0.17
L	4	F	14	5	5.30	0.28	0.09	7.50	11.39	0.67
L	4	M	17	6	1.12	0.00	0.49	7.38	9.27	0.72

Table A3.2. Comparison of DA and FA shape variation between replicates within treatments. Mean squares are multiplied by 10^6.

			n		DA			FA		
Density	Age	Sex	Rep A	Rep B	Rep A	Rep B	P	Rep A	Rep B	P
H	1	F	15	15	36.1	22.1	0.07	3.00	4.50	0.01
H	1	M	15	15	20.0	25.2	0.54	3.44	4.79	0.08
H	2	F	15	15	19.4	20.0	0.95	4.68	5.50	0.56
H	2	M	15	15	26.3	28.3	0.79	4.32	4.20	0.84
H	3	F	15	15	15.5	24.6	0.16	4.75	2.87	0.10
H	3	M	15	15	16.5	19.1	0.68	3.65	3.35	0.59
L	2	F	15	15	25.1	25.5	0.95	3.02	3.34	0.67
L	2	M	15	15	66.9	27.8	0.0*	3.61	2.51	0.21
L	4	F	14	5	24.4	12.7	0.71	3.37	2.18	0.18
L	4	M	17	6	28.9	17.7	0.84	4.38	3.36	0.12

*significant after sequential Bonferroni correction for Type 1 error

Figure A3.1. Individual deviations around the mean asymmetry at each landmark for low-density male offspring from 2-week-old females. Each panel displays deviations for the landmark indicated by the number at the upper left. Vertical and horizontal axes run from -0.0063 mm to 0.0063 mm. Filled squares are for replicate A flies, hollow circles are for replicate B flies, and the asterisk is for the extremely asymmetrical fly from replicate A.



Appendix 4: After pooling replicates, FA is significantly greater than measurement error in all treatments in the longitudinal study.

Wing size FA and shape FA were significantly greater than measurement error in all treatments after pooling replicate cultures, but not always before pooling. Size DA was significant in a single replicate of low density male offspring from 2 week old mothers and remained significant after pooling replicates within this treatment (Tables A4.1 and A4.2). This replicate was excluded from further analysis of size FA to avoid complicating the interpretation of FA patterns. Interestingly, detection of shape DA required relatively large sample sizes and in most cases was significant only after pooling replicates (Tables A4.1 and A4.2).

Table A4.1. Mean squares and significance of DA, and FA of centroid size and shape variation within treatment replicates. The expectation is for non-significant DA but significant FA. *P*-values are from permutation tests of the Procrustes ANOVA for each trait for each group.

					Size DA		Size FA		Shape DA		Shape FA	
Density	Age	Sex	rep	n	MS.x 10^4	*P*	MS x 10^4	*P*	MS x 10^5	*P*	MS x 10^6	*P*
H	1	F	A	15	12.00	0.1619	5.21	0.0000	3.61	0.0038	3.00	0.0104
H	1	F	B	15	7.37	0.4293	10.75	0.0000	2.21	0.0438	4.50	0.0005
H	1	M	A	15	18.20	0.0510	4.05	0.0000	2.00	0.0302	3.44	0.0014
H	1	M	B	15	3.72	0.4773	7.34	0.0000	2.52	0.0378	4.79	0.0003
H	2	F	A	15	5.47	0.3800	6.73	0.0000	1.94	0.0613	4.68	0.0004
H	2	F	B	15	0.30	0.8705	10.54	0.0000	2.00	0.0773	5.50	0.0001
H	2	M	A	15	17.59	0.1421	7.09	0.0000	2.63	0.0272	4.32	0.0008
H	2	M	B	15	0.23	0.8658	7.72	0.0000	2.83	0.0211	4.20	0.0030
H	3	F	A	15	32.95	0.1768	16.41	0.0000	1.55	0.0921	4.75	0.0001
H	3	F	B	15	3.28	0.4323	5.24	0.0050	2.46	0.0111	2.87	0.0071
H	3	M	A	15	2.96	0.6254	11.58	0.0000	1.65	0.0517	3.65	0.0016
H	3	M	B	15	0.54	0.8051	6.30	0.0000	1.91	0.0318	3.35	0.0039
H	4	F	B	9	12.03	0.5477	32.50	0.0000	0.85	0.2412	5.29	0.0022
H	4	M	B	5	0.53	0.8789	11.67	0.0192	0.89	0.2269	4.35	0.0187[b]
H	5	F	A	19	27.50	0.3023	25.21	0.0000	3.11	0.0387	6.25	0.0000
H	5	M	A	16	5.20	0.5253	12.07	0.0000	2.90	0.0263	4.78	0.0000
L	2	F	A	15	73.77	0.0079	12.20	0.0000	2.51	0.0121	3.02	0.0003
L	2	F	B	15	196.02	0.0002[a]	7.84	0.0000	2.55	0.0153	3.34	0.0006
L	2	M	A	15	36.60	0.0818	11.13	0.0715[b]	6.69	0.0007[a]	3.61	0.0014
L	2	M	B	15	11.59	0.0894	3.49	0.0003	2.78	0.0050	2.51	0.0044
L	4	F	A	14	53.02	0.0218	7.50	0.0000	2.44	0.0186	3.37	0.0010
L	4	F	B	5	2.77	0.6966	11.39	0.0002	1.27	0.0729	2.18	0.1318[b]
L	4	M	A	17	11.21	0.2244	7.38	0.0000	2.89	0.0206	4.38	0.0000
L	4	M	B	6	0.00	0.9678	9.27	0.0021	1.77	0.0701	3.36	0.0324[b]

[a]significant after sequential Bonferroni correction

[b]non-significant after sequential Bonferroni correction

Table A4.2. Mean squares and significance of DA, and FA of centroid size and shape variation within treatments (replicates pooled). The expectation is for non-significant DA but significant FA. *P*-values are from permutation tests of the Procrustes ANOVA for each trait for each group.

				Size DA		Size FA		Shape DA		Shape FA	
Density	Age	Sex	n	MS x 10^4	*P*	MS x 10^4	*P*	MS x 10^5	*P*	MS x 10^6	*P*
H	1	F	30	0.28	0.8557	8.36	0.0000	5.02	0.0000[a]	3.90	0.0000
H	1	M	30	2.73	0.5175	6.16	0.0000	4.10	0.0000[a]	4.12	0.0000
H	2	F	30	1.60	0.6637	8.48	0.0000	3.40	0.0000[a]	5.10	0.0000
H	2	M	30	6.90	0.3436	7.52	0.0000	4.96	0.0000[a]	4.28	0.0001
H	3	F	30	7.72	0.4354	11.44	0.0000	3.69	0.0000[a]	3.79	0.0000
H	3	M	30	3.01	0.5664	8.65	0.0000	3.33	0.0000[a]	3.46	0.0002
H	4	F	9	12.03	0.5578	32.50	0.0000	0.85	0.1325	5.29	0.0024
H	4	M	5	0.53	0.8752	11.67	0.0186	0.89	0.0618	4.35	0.0242
H	5	F	19	27.50	0.3133	25.21	0.0000	3.11	0.0000[a]	6.25	0.0000
H	5	M	16	5.20	0.5352	12.07	0.0000	2.90	0.0000[a]	4.78	0.0000
L	2	F	30	255.14	0.0000[a]	10.18	0.0000	4.71	0.0000[a]	3.19	0.0000
L	2	M	30	44.69	0.0136	7.18	0.0049	8.62	0.0000[a]	3.25	0.0001
L	4	F	19	29.12	0.0981	9.43	0.0000	3.30	0.0000[a]	3.15	0.0006
L	4	M	23	8.48	0.3021	7.60	0.0000	3.97	0.0000[a]	4.26	0.0000

[a]significant after sequential Bonferroni correction

Appendix 5: Replicates differ little in FA or DA in the study of larval competition effects on FA.

Directional and fluctuating asymmetry of replicate cultures from the competition trial involving lines C and D (Chapter 3, Experiment 1) were compared using permutation tests, with 1000 iterations, that replicate mean squares did not differ more than expected based on random resampling without replacement. With 3 replicates, there were 3 comparisons in each treatment of 8 larval combinations. Accordingly, results were corrected simultaneously for type I error for all 24 tests for each trait using the sequential Bonferroni correction.

Although there were some significant differences between replicates, these always involved very small samples (Tables A5.1 and A5.2). Since very low survival indicates a highly stressful environment, and higher stress is expected to lead to greater variance in morphological traits (Parsons 1994) it is not surprising that DA and FA differences might exist between these replicates. This is because with increased morphological variance and decreased sample sizes, the chance of sampling two very different individuals (or wings) is increased also.

Reference:

Parsons, P. A., 1994. Developmental variability and the limits of adaptation: interactions with stress. *In* Developmental Instability: Its Origins and Evolutionary implications. *Edited by* T.A. Markow. Kluwer, The Netherlands. pp. 247-255

Table A5.1. Comparison of DA and FA wing centroid size variation between replicates within treatments. *P*-values are the minimum of the three comparisons between replicates. Bonferroni correction was made considering all 24 tests for DA and separately for the 24 tests for replicate effects on FA.

	n			DA (MS x 10^4)				FA (MS x 10^4)			
Larvae	Rep A	Rep B	Rep C	Rep A	Rep B	Rep C	min *P*	Rep A	Rep B	Rep C	min *P*
D early	2	5	2	4.12	1.43	2.32	0.644	0.06	3.24	2.46	0.000*
D early: C early	17	18	18	8.73	1.01	0.40	0.196	7.18	6.93	8.50	0.397
C early	9	1	8	0.04	0.27	18.47	0.000*	3.14	---	11.67	0.276
D early: C late	7	11	16	3.49	2.94	0.36	0.574	3.45	5.53	2.94	0.311
C late	11	9	10	4.07	1.59	0.12	0.689	6.60	4.12	11.12	0.009
D late	12	14	9	1.02	12.07	6.69	0.365	11.26	7.85	3.05	0.285
D late: C early	9	5	7	0.11	5.32	0.90	0.081	6.61	20.29	3.54	0.000*
D late: C late	18	19	15	26.66	36.20	9.55	0.440	4.13	3.17	6.99	0.247

*significant after sequential Bonferroni correction

Table A5.2. Comparison of DA and FA wing shape variation between replicates within treatments. *P*-values are the minimum of the three comparisons between replicates. Bonferroni correction was made considering all 24 tests for DA and separately for the 24 tests for replicate effects on FA.

	n			DA (MS x 10^4)				FA (MS x 10^4)			
Larvae	Rep A	Rep B	Rep C	Rep A	Rep B	Rep C	min *P*	Rep A	Rep B	Rep C	min *P*
D early	2	5	2	0.69	1.01	0.39	0.000*	4.09	7.89	6.85	0.000*
D early: C early	17	18	18	9.05	8.25	6.28	0.059	5.03	4.36	3.75	0.209
C early	9	1	8	2.13	0.90	1.75	0.694	3.39	---	2.53	0.389
D early: C late	7	11	16	2.61	4.85	6.70	0.308	3.60	3.64	4.54	0.217
C late	11	9	10	2.79	2.28	4.26	0.024	3.53	3.00	4.48	0.065
D late	12	14	9	1.79	2.64	2.35	0.345	3.67	2.69	2.61	0.086
D late: C early	9	5	7	4.42	2.68	3.42	0.453	3.04	4.30	2.89	0.199
D late: C late	18	19	15	5.94	6.44	7.21	0.541	3.54	3.32	3.44	0.713

*significant after sequential Bonferroni correction