A Time Budget For The Transgenic Supermouse

DIFFERENCES IN THE TIME ALLOCATION STRATEGY BETWEEN TRANSGENIC "SUPERMICE" AND NORMAL CONTROLS AND THEIR RELEVANCE TO THE PRINCIPLE OF ALLOCATION

BY

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Abstract

This study represents the behavioural component of a larger project investigating the life history tactics, physiological resource allocation and behavioural time budgeting of a genetically engineered animal. The "supermouse" is a transgenic strain (mMT-1/rGH) that has one chromosome genetically engineered with extra copies of rat growth hormone genes, each fused to a metallothionein-1 promoter. The GH transgenes are permanently incorporated into the genome of the mouse and are inherited as a block, in a Mendelian manner. "Supermice" exhibit an accelerated growth rate and reach body weights twice that of their normal siblings: both transgenic mice and normal mice are obtained by crossing transgenic males to normal females. Although there must be increased costs associated with achieving their higher growth rate, these mice show no increases in their specific feeding rates. Consequently there must be a reallocation of resources among various physiological and behavioural demands. The reality of such tradeoffs is known as the Principle of Allocation and predicts that reductions in behavioural activities might be one avenue for realizing extra growth. To test this, six components of the behavioural time budget (resting, locomotion, wheel running, feeding, drinking and grooming) were compared between transgenic and normal mice.

Infra-red videocameras recorded the activities of individual male mice in artificial enclosures over 24 hours. The time spent in each bout of activity was recorded and compared. Transgenic mice out-slept their normal counterparts by 126% (an increase of 3.4 h) and were only 53.83% as active in terms of locomotion and wheel running as normal mice. Pooling the data revealed that on average, large mice spent more time at rest and less time engaged in locomotion. Slight but significant decreases in time spent drinking and grooming were also found. Transgenic mice spent only 77.01% as much time drinking, and 69.01% as much time grooming as normal mice. No difference in the amount of time spent feeding was found.

Key Words: *Transgenic mice, Time budget, Principle of allocation, Growth hormone, GH, Activity, Behaviour, Evolution, Genetic Engineering, Ethology.*

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Preface

"Manipulating genes, chromosomes, and gametes rather than selecting of phenotype will be the next important phase of behaviour genetics. The conceptual framework of these experiments will be the use of genes as treatment affecting behaviour, rather than the determination of the heritability of traits."

"... *Drosophila* is the animal of choice from the genetic point of view, but its behavioural repertoire is limited. The mouse, with its numerous mutations and inbred strains, is the most suitable mammal."

- J.L. Fuller and W.R. Thompson, *Behavior Genetics* (1967).

Fuller and Thompson came to this conclusion back in 1967, before the start of the molecular revolution. Little did they realize that in less than 15 years their prediction would come true. In 1982 Palmiter and Brinster created a storm with the creation of the world's first transgenic mouse. Multiple copies of a rat growth hormone gene had been inserted into the DNA of a mouse and so for the first time in history the genome of a mammal had been successfully engineered. The foreign genes functioned in the transgenic mice in a natural context. The impact of this singular event on the field of behaviour genetics is only now beginning to be realized. Previously, very limited tools were available to the behavioural researcher. Analysis of mutants, most of which arose spontaneously in colonies, was one of the first approaches utilized. Over the years, a large number of behavioural mutants were isolated. The animal of choice was *Drosophila*, for a multitude of reasons including a short generation time, inexpensiveness, and responsiveness to mutagens. Its behavioural repertoire, however, is rather limited and as a species it is very far removed from the behaviourally richer and more interesting mammals

(Ehrman and Parsons, 1976). The most popular mammal studied was the house mouse, *Mus musculus*. Many studies were made concerning the effects of single gene mutations on mouse behaviour. Over 300 mutant genes occupying over 250 loci are listed for the mouse. A large number of these mutations affected behaviour (Green, 1966). The genetics of "waltzing," "twirling," "jerking," "squeaking," as well as susceptibility to sound induced seizures, were some of the most popular behaviours analyzed. In the 1970's newer techniques were developed which involved breeding recombinant inbred strains. Recombinant inbred strains are derived from the F_2 generation of a normal Mendelian cross in which a recombination of parts of chromosomes from the parental strains occurs. However, the tools of the behavioural geneticist were still limited to selection experiments, breeding experiments (diallel crosses), and strain comparisons. The most common complaint was that these types of studies limited the numbers of behaviours which could be studied to those in which mutations or recombination occurred, or those in which there were differences between strains.

With the advent of gene transfer technology behavioural genetics has entered a new phase. Molecular techniques now allow investigators to modify the genomes of inbred strains with cloned genes and monitor the effects. Researchers have already transferred a cloned copy of the gene believed to be responsible for species-specific components of the courtship song rhythms of *Drosophila simulans* into the genome of a *Drosophila melanogaster* mutant lacking this complimentary gene. These germline transformation experiments allowed them to map the genetic control of the song rhythm to a small amino acid encoding segment within the gene (Wheeler *et al.*, 1991).

The following study focusses on behavioural changes associated with a drastically altered phenotype, arising from a change in the genome of a mouse. It appears to be the first behavioural study of its kind using the transgenic mouse as a prototype for mammalian systems. There are several advantages to using

the transgenic mouse for this type of research. Foremost, a very specific alteration can be made in the genome of the mouse while maintaining a controlled genetic background which identical to that of the controls. Problems with inbreeding commonly associated with selection experiments are also avoided. In addition because only one gene was altered, the impacts of genetic alteration on the specific feature of interest is highlighted. Therefore linkages of the gene to other phenotypic and behavioural components are revealed. For these reasons it is my belief that the transgenic mouse will be useful in the future as a behavioural bioassay. As a result the focus in behavioural genetics will shift to experiments in which genes will be altered and their subsequent effects on behaviour will be studied, as Thompson and Fuller had envisioned, rather than simply attempting to identify genes associated with particular forms of behaviour.

Introduction

Organisms traditionally have been conceptualized as a "black box". Finite amounts of matter and energy enter the system and produce an output of progeny. Observed life-histories represent a compromise of allocation among limited resources. The system partitions the matter and energy between the conflicting demands of basal metabolism, defence, repair, storage, growth, reproduction and the costs of behavioural activity. Behaviour is crucial in animals because it represents the mechanism determining resource rates in the first place. Resources made available to one demand are therefore unavailable to others. The optimal strategy of allocating resources is the one which most effectively transforms the resource input into viable reproductive output and thus transmits the genes to future generations (Calow and Townsend, 1981; Pianka, 1983). This concept is referred to as the Principle of Allocation. The theory has been attractive to ecologists because of its inherent assumption that there exist fundamental and global rules which constrain organisms. This suggests that underlying allocation strategies and costs can lead to a general theory of organism design. However, organisms are much more complex than this. Organisms can control the rates and efficiencies of various processes like growth, reproduction and feeding (Tuomi *et al.*, 1983; Rollo, 1988). This superimposes a new level of complexity on the basic model. If the costs of any of the life history traits increase, organisms can sometimes compensate without a tradeoff by increasing the rate or efficiency of feeding or by utilizing accumulated reserves. This would imply that at least some organisms operate at submaximal rates (i.e. they do not maximize their rate of food intake). The lower the operating rate the greater the degree of compensatory scope (Rollo, 1988). However, if the cost becomes too great, tradeoffs will have to occur, so the end result is merely to shift allocation considerations upwards to a new resource ceiling.

Another level of complexity is that the allocation strategy (and consequently the life-history) of the organisms is usually not static and may change with age or environment. Some organisms, like insects pass through several life stages before reaching adulthood. The juvenile stages are devoted primarily to feeding, building up huge storage reserves and growth. Little or no energy is allocated to reproduction. When the adult stage is reached the opposite occurs. Growth and feeding slow or cease, and the storage reserves are often tapped in order to maximize reproductive effort. However, allocations in mammalian systems are not as strictly separated temporally, unlike the predicted strategies for organisms such as annual plants (Taylor and Gabriel, 1992). To further complicate matters, differences in allocation strategies may be present between the sexes as well. Clearly understanding an animal's tactics of resource allocation is an enormous challenge.

There has been considerable controversy in the literature over the various empirical approaches employed to study life-history theory. One of the most utilized methods involved analyzing phenotypic correlations between life-history traits on a series of individuals, populations or related species (Rollo, 1984, 1986; Jones 1985). The problem inherent with this method is that it assumes that there are no significant genetic differences which may alter phenotypic performance, and that resource levels are similar.

Alternatively another method utilized involved manipulating the life-history of the particular system under investigation (Partridge and Harvey, 1988). Calow and Townsend (1981) realized this and stated:

"In a relatively constant laboratory environment, animals could be experimentally induced to devote more (and less in a companion experiment) than the supposed 'optimal' amount to various activities such as growth maintenance,

and reproduction." Of course, experimental groups would be expected to suffer reduced reproductive success (fitness) compared to the control group."

One tactic involves providing "misinformation" to the organism via hormonal or pheromonal treatment. For example, the application of pheromones from a mature male induces young rodents to grow faster, block pregnancy, and accelerate the maturation of females (Atkinson, 1985) Other types of experiments involved exposing fruitflies repeatedly to mates in order to increase reproductive costs and subsequently analyzing the lifespan (Partridge and Farquhar, 1981; Fowler and Partridge 1989). These studies however attempt to accomplish a goal using indirect methods. The external environmental alterations are independent of the genetic background of the organism. This approach has been criticized by Reznick (1992), who feels that the major goal of estimating costs of components of life-history is to evaluate assumptions of evolutionary theories: consequently, only methods which involve measuring genetic contributions are important. It is only possible to predict with certainty the correlated response to selection on one trait by another by measuring the genetic correlation between them.

Genetic correlations among different components of life history accomplish this, but even this method has been challenged (Rose and Charlesworth, 1981). Partridge and Harvey (1988) have charged that difficulties can arise in the form of confounding results either as a result of an individual's phenotype, the environment, or both.

Ideally, the best method of measuring the costs of various components of life-history would involve *directly* genetically altering the cost of one of the traits and subsequently assessing its impact on the other traits.

The miracle of genetic engineering has now allowed the genomes of animals to be manipulated.

These so-called transgenic animals carry foreign genes in some, or all of their cells. Foreign genes are cloned and propagated in bacterial hosts and very often are modified. The coding region of one gene may be detached from its own promoter and attached to that of another. The most prevalent method of introducing foreign genes into the genome of an animal is via direct microinjection of genes, contained in a plasmid vector, into the pronucleus of a fertilized egg. The embryos are subsequently introduced into female recipients which have been made physiologically receptive. Implantation and gestation proceed normally. The most common outcome of DNA microinjection is the insertion of a single array of direct tandem repeats (head-to-tail) into one of the chromosomes (Palmiter *et al.*, 1982, 1983). Occasionally unusual transgenic animals are produced which carry foreign genes in only somatic or germline cells, or only in some somatic and germline cells (Wilkie *et al.*, 1986). However these "freaks", called mosaics, are not as useful to behavioural ecologists. Once foreign genes are integrated into one or rarely a few chromosomal sites, they are inherited in the typical Mendelian manner through the germline (Gordon and Ruddle, 1981), and may be outcrossed to various other strains with other characteristics of interest. Of interest to us are transgenic animals which have had foreign genes integrated at a single chromosomal site and are bred to produce a line. Such lines breed true and can provide unlimited numbers of transgenic animals. The mice can also be back-crossed to produce homozygotes for the transferred sequence (Gordon and Ruddle, 1981).

The line of mice utilized in this study possessed an enhanced growth rate twice that of normal, by virtue of extra copies of rat growth hormone genes inserted into their genome. Growth of vertebrates is mediated in part by a cascade of hormones. The hypothalamus secretes somatostatin or growth hormone releasing factor in response to neurotransmitters. These polypeptides cause the pituitary gland to inhibit or stimulate the synthesis and secretion of growth hormone (somatotropin). GH is thought to stimulate the liver to produce insulin-like growth factor-1 (somatomedin). IGF-1 appears to mediate growth by

activating receptors on peripheral tissues. IGF-1 works by allowing cells to pass from the G_1 phase of the cell cycle and enter the S phase where DNA synthesis occurs (Palmiter *et al.*, 1983; Gilbert, 1992). Mice with multiple copies of foreign GH fusion genes continually secrete GH such that serum levels of rat GH exceed physiological GH levels in normal mice by several orders of magnitude. It has also been shown that there is a lack of correlation between the level of expression and the number of copies of the foreign gene (Bishop and Al-Shawi, 1989).

Preliminary comparative studies conducted in our lab between the large transgenic mice and their normal siblings revealed that the consumption rate per gram of body mass was actually slightly less for transgenic mice than for normal mice. They were found to be twice as effective at converting food to body tissue as normal mice. This increased metabolic efficiency must have a tradeoff in some other life-history trait because the gain is obtained by diverting resources to growth (Kajiura, unpublished). This meant that of two siblings with identical genetic backgrounds and ages, one differed from another in terms of the cost of one of its life-history traits. This was an ideal empirical test for the Principle of Allocation which predicts that under these conditions, one or more tradeoffs have to be made.

The apparent lethargy of the animals hinted that behavioral trade-offs might occur. Many life history variables depend largely on behaviour (Sibly and Calow, 1983). Although activities such as foraging, finding mates, defending resources and sleeping occupy a large portion of an animal's time, behaviour as a life-history trait, has largely been ignored in studies dealing with the Principle of Allocation. This fact was recognized by Rollo (1984) and to date it remains to be the case. The overall question asked is what are the behavioural consequences (if any) of investing more energy in somatic growth at the expense of other life-history variables.

Clarification of Contributions

The original idea for the study was proposed by my supervisor Dr. C. David Rollo as a component of a much larger investigative effort studying the life-history tactics of the transgenic mouse in an attempt to discover how various life-history traits interface. All experimental design, labour, analysis, and documentation of this project was conducted by myself under his guidance. The survey of the relatively immense literature on rodent behaviour and synthesis of its linkages to aspects of this project was carried out largely by myself.

Differences in the time allocation strategy between Transgenic "supermice" and normal controls and their relevance to the principle of allocation

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Introduction

Darwin's (1859) theory of evolution suggests that attributes of organisms which maximize fitness are selected while relatively less effective features are eliminated. This implies that organisms presently operate at near maximal rates and efficiencies. Organisms, however, must balance energy intake with energy expenditure for expensive processes such as somatic growth, reproduction, repair, basal metabolism, storage, defence, and behaviour. If resources or utilization efficiencies do not vary, any increase in one feature or process can only be made at the expense of another as a result of limited resources (Calow and Townsend, 1981). The requirements for such tradeoffs between various fitness components of an organisms design are the basis for the principle of allocation (reviewed by Rollo, 1986; Sibly and Calow, 1986). Radical changes in any one feature might place severe stress on the system resulting in maladaptation.

Experimental verification of this theory has been surprisingly difficult. There have been three traditional approaches for testing the theory. One method involves intraspecific or interspecific phenotypic comparisons among closely related (that is physiologically and morphologically similar) animals (Rollo, 1984, 1986; Jones 1985). The major problem inherent in this method is the assumption that there are no genetic differences changing phenotypic performance. In some instances this may not be true, and considerable caution must be exercised when interpreting the results. The second approach involves analyzing genetic correlations among different components of the life-history, either statistically or under directional selection (Reznick, 1985, 1992; Rose and Charlesworth 1981). Inbreeding and the fact that genetic correlations may change are two common problems with these methodologies. Finally the last approach involves experimental manipulations of the life history of the organism (Partridge and Farquhar, 1981; Fowler and Partridge; 1989). This approach represents a fixed environmental effect and is

independent of the genetic background. There is currently no unanimous consensus on the best approach. Some authors claim that experimental manipulations are most appropriate (*i.e.* Partridge and Harvey 1985; Lessells, 1991), whereas others claim that genetic correlations are most relevant (Reznick, 1985, 1992) The creation of transgenic mice in the early 1980's (Palmiter *et al.*, 1982) has provided a powerful new t8ool for the evolutionary ecologist concerned with the study of organism design. This new tool involves 8experimental manipulation of the genome itself (*i.e.* insertion of foreign genes, or changes in copy number or expression of a single gene or gene complex). Examination of associated changes (which we term the "transgenic" correlation structure) provides insights into both genetic and phenotypic aspects of organismal integration that have not been previously possible. In particular, a single aspect of the genetic program can be altered allowing its linkages to be quantitatively assessed.

The aim of transgenesis is to produce variants of species by incorporating new genes into their genome. The transgene compels the animal to operate in a manner that its evolutionarily determined design did not intend, an occurrence not possible in nature. By examining the disruption of various features and their interconnections the investigator can then assess the importance and interrelationship among life-history features of organisms. Transgenic mice carry a fusion gene which consists of a regulatory element from one gene ligated to the structural sequence of another. These genes were microinjected into the pronucleus of a fertilized egg, and subsequently inserted into the reproductive tract of a foster mother (Palmiter *et al.*, 1982,1983). The integration of the transgenes into the genome is an event unique to each embryo and as a result each founder animal has a different number of transgenes positioned in different locations within the genome. Long head-to-tail concatamers tend to form and integrate at one or rarely two sites within the host genome (*i.e.* in our mice all the copies of the transgene lie on a single chromosome). Once integrated, transgenes are transmitted to offspring by simple Mendelian inheritance.

These mice can also be back-crossed to produce homozygotes for the transferred sequences (Gordon and Ruddle, 1981). Although some anomalous transmission patterns have been observed, these are thought to be germline mosaics (Wilkie *et al.*, 1986). There is a general lack of correlation between the level of expression of the transgene and copy number (Palmiter *et al.*, 1983). Exogenous administration of GH in normal animals results in episodic elevations of GH in the system while in transgenic animals GH is elevated continuously. A wide variety of transgenic animals have been created, including fish, rabbits, sheep and pigs. Particular attention has been focussed on growth hormone with the hope that these heritable modifications will benefit agriculture (Guise *et al.*, 1988; Hammer *et al.*, 1985; Rexroad *et a.l*, 1989; Pursel *et al.*, 1989; Pursel *et al.*, 1990; Wall *et al.*, 1990).

The strain of mice used in this research carry a transgene containing the promoter of the mouse metallothionein-1 gene fused to a rat growth hormone gene (mMT-1/rGH). Growth hormone is the primary somatic growth factor in mammals (Golde, 1980). Rat growth hormone is structurally very similar to mouse growth hormone and has comparable activity. The MT-1 promoter is constitutively expressed in transgenic animals. It causes continuous transcription of the gene to which it is attached (Wall *et al*, 1990). As a result these mice display high levels of the fusion mRNA in their liver and high circulatory levels of growth hormone and insulin-like growth factor one (IGF-1). The latter is a member of the somatomedins a class of low molecular weight polypeptides. Transcription of IGF-1 in mice is under the control of GH. IGF-1 is thought to mediate growth by activating receptors on peripheral tissues. Because the foreign growth hormone is produced in animals from fetal stage onward, the immune system presumably recognizes rat GH as "self" and as a result transgenic mice display growth rates and an adult body mass twice that of normal mice (Palmiter *et al.*,1983). The body size has been shown to correlate with levels of circulating IGF-1 (Mathews *et al.*, 1988). It is therefore widely believed that GH influences growth indirectly by regulating serum IGF-1 levels (Palmiter *et al.*, 1982).

Increases in growth are not uniform among tissues or organs. Shea (1987) found significantly increased growth in the lungs, liver, heart, thymus, and kidneys but not the brain. While the body weight generally doubles, for most organs the percentage increase varies considerably. Mice artificially selected fo2r large body mass showed a similar pattern of increases in the mass of organs, with brain showing the smallest increase, and the muscle and the liver the greatest (Robinson and Bradford, 1969). The growth of organs is not proportional to overall weight gain. In fact, adult transgenic mice are shaped quite differently than normal controls (Shea, 1990). Wolf et al. (1991) reported a general increase in skeletal dimensions of transgenic animals carrying human GH genes compared to normal mice, although observed increases were not quite as dramatic as the increases in body weight and not all bones were affected to the same extent. Shea (1987) further reported that growth in transgenic mice neither starts earlier nor extends longer than growth among normal controls, it just occurs at a faster rate (i.e. the maturation schedule was unaffected). Wolf et al. (1991) confirmed these findings. Although GH has relatively far reaching and div2erse impacts on the phenotype, the results with "supermice" are similar to those obtained by traditional programs of artificial selection (e.g. MacArthur, 1949; Eklund and Bradford, 1977).

Anecdotal reports of lethargy of transgenic mice and pigs prompted the question as to whether or not there could exist trade-offs between levels of behavioural activity and growth. A series of classic experiments had found that in artificially selecting large and small lines of mice, the large mice tended to be docile while the small mice were hyperactive (MacArthur, 1949). This is very similar to what was seen in the transgenic animal system. Could there be possibly other changes in behaviour as well?

Most behaviours are mutually exclusive of one another. Therefore an important aspect of the ecology of any animal is the way it budgets its time among activities associated with maintenance, storage and productivity (Wolf and Hainsworth, 1971). This time budgeting strategy is the behavioural counterpart of the physiological resource allocation tactics. Together they define the

resource allocation framework within which the principle of allocation may hold. If the transgenic animals indeed are less active as a result of the energy demands from rapid growth it will show up in the time budgets. We documented and compared the behavioural time budgets with the intention of gaining insight into the integration of behaviour and physiology in these model animals.

It was hypothesized that the significantly higher growth rate of transgenic mice should place such a high energy demand on the system that, according to the principle of allocation the animals must compensate by reducing energy expenditure in other areas. Tuomi et al (1983) correctly pointed out that such tradeoffs might not be necessary if organisms could utilize stored reserves or increase energy intakes to cover costs. However a recent resource allocation study indicated that "supermice" do not alter their rate of food intake per gram of body mass, making it ideal to put the principle of resource allocation to the test (Kajiura, unpublished).

Methods

I. *Animals*

The original transgenic mice (*strain Tg [MT-1,GH] Bri2*) were donated by Dr. R.L. Brinster from the University of Pennsylvania in September, 1989. Since transgenic mice grow twice as fast as normal mice and reach body weights twice that of normal mice they were readily distinguished by 28 days of age. Mice heterozygous for the transgene were also crossed, producing mice homozygous for the transgene. Mice that did not inherit the foreign DNA were used as controls. Only male mice were used in the experiment to avoid variations in behaviour associated with reproductive cycles in females. A total of 42 mice were used (15 normal, 15 heterozygous transgenic, 18 homozygous transgenic, 2 normal spinner, and 2 transgenic spinner mice). In addition a small number of "spinner" mutants were obtained. These mice appeared to be opposite to transgenic animals in that they were hyperactive and had lower rates of growth and adult sizes.

II. *Apparatus*

Laboratory analysis of time budgets are best carried out in activity centers - artificial environments where animals have access to all relevant resources. Barnett (1966) described a "plus maze" in which a small mammal could live indefinitely and in which its activity could be recorded. These and other laboratory studies utilized sensors to record animal movements through an artificial habitat (Kavanau, 1962,1963; Collier *et al.*, 1990). The obvious advantage is the ease of data collection. However, while the amount of time spent eating, drinking and wheel running is easy to obtain, the amount of time spent sleeping, and grooming is impossible to accurately measure via such sensors. The following study utilized videocameras which recorded virtually every movement of the mice. The videotape provides a permanent

record of the activity which can be subsequently analyzed many different ways which is advantageous.

The enclosure was designed to accommodate animals for extended periods with minimal disturbance. It was constructed of 5 mm. thick, clear acrylic (l x w x h = 51 x 51 x 16 cm.) (*Figure 1.*). The central rectangular compartment was a choice box accessing four trapezoidal shaped compartments. One compartment contained a food dish, another allowed access to a standard rodent water bottle, the third contained some shredded nesting material and the fourth contained a 17 cm. diameter running wheel. Some glass marbles and a ping pong ball were also provided for behavioural enrichment. Woodchip bedding (Betachips, Hardwood Laboratory Bedding) covered the bottom of the cage.

The mice were fed standard rodent diet (Lab Diet Brand Animal Food, PMI Feeds Incorporated ® 5001) and tap water *ad libitum*. The colony and experimental room were maintained on identical 12:12 light:dark photoperiods at a temperature of 22 ± 2°C. The experimental room was isolated to prevent the animals from being disturbed for the 24 h filming periods.

The activity of the mice was recorded with a Panasonic® AG-6720 timelapse video recorder, two Panasonic® WV-BL200 cameras, and a Viacom® video switcher. Dim night-time illumination was provided by a blue 25 watt bulb to allow filming by the infra-red sensitive cameras. The cameras were vertically suspended over the cages from scaffolding (*Figure 2*). The video switcher allowed two cages to be monitored at the same time, by switching between cameras every three seconds.

III. *Experimental Runs*

Prior to each experimental run, two mice between the ages of 87 and 106 days of age were weighed

and placed in separate activity centers. A habituation period of 2 days was allowed. Preliminary studies found that this time was sufficient for the mice to acclimatize themselves to their new surroundings and settle into their typical circadian rhythm. Videotaping commenced on the third day approximately in the middle of the daytime photoperiod.

The two main categories of behaviour separated into activity and rest. Activity included movement, feeding , drinking, grooming, and excretion. The behaviour of the experimental mice was scored on the basis of rest (sleep and lack of activity) and five parameters of activity (described by Guillot, 1981). The time interval for each activity was recorded.

Resting: operationally defined as nonactivity in the nest or elsewhere in the cage. Thus, this category includes both sleeping and periods of immobility outside of the nest.

Locomotion: Characterized as physical movement; including exploratory activity, climbing, digging and nest building, but not wheel running The category *Total Locomotion* includes time spent in locomotion plus time spent running on a wheel.

Feeding: time spent eating and handling food.

Drinking: time spent drinking from the water bottle.

Wheel: time spent running on a wheel. Running on a wheel provides a means of sustained vigorous activity which generally is otherwise difficult to achieve for caged animals.

Grooming: time spent cleaning face and body; includes washing and scratching behaviour.

The activity centers, water bottles, and running wheels were washed with detergent and sterilized with bleach. A total of 42 mice were used in the study allowing the determination of the time budgets of fifteen heterozygous transgenic, eight homozygous transgenic, fifteen normal mice. In addition data were

obtained for two transgenic spinners and two normal spinners.

IV. *Statistical Analysis*

Overall total mean time (+/- standard errors), number of bouts and average length of bouts for each activity for each group were calculated resulting in an absolute time budget. Each measured behaviour was also expressed as a fraction of total activity in order to produce a relative time budget. Two-tailed student t-tests for each activity between each group were performed, as well as correlation and regression analysis using Excel 4.0 ® (Microsoft, 1992). The data for the groups were pooled and also analyzed on the basis of live body weight.

Results

Mice are nocturnal and usually initiated activity within half an hour before or after the dark phase commenced. During the light phase the majority of time was spent resting in the nest, with occasional daytime excursions to feed, drink, or eliminate waste. Some movement was observed while the mice were sleeping which presumably represented minor tossing and turning. Eating, drinking, grooming, wheel running and locomotion tended to occur in short intervals ranging from a few seconds to a minute or two, although longer bouts of patrolling behaviour were seen. Resting bouts were much longer in duration ranging from 20 minutes to several hours in length.

The total time for each of the scored behaviours was tallied over a 24 hour period and the means for each treatment were calculated to obtain an absolute time budget (*Table 1*). Analysis revealed significant differences between the overall means of the heterozygous transgenic and normal mice in all monitored activities with the exception of feeding. These differences in means were similar in comparisons between the homozygous transgenic mice and controls with the additional exception of drinking (no significant differences in means were found between homozygous transgenic mice and controls). The sample size for the homozygous group was very small ($n=8$) Since no significant differences in mean time were found between the heterozygous and homozygous groups, data for heterozygous and homozygous transgenic mice were pooled, increasing the sample size of the transgenic animals to 23. The number and average length of each activity bout was also calculated.

NOTE: The main thrust of life-history theory is to identify evolutionary trends and tradeoffs. Most studies involve correlations drawn within and between groups with regards to life-history variables. In

some instances trends may increase linearly within each group and also increase across groups. Conversely, trends may decrease linearly within groups yet increase linearly across treatments. The t-test is a valuable tool therefore in revealing differences in the means across groups regardless of whether or not general trends can be found within or among groups. The t-test is the key criteria to determine if the experimental gene had an impact on the organism. In this particular experiment comparisons were made between transgenic "supermice" and normal controls. In many cases the t-test revealed highly significant differences between the group means even though linear regression found non-significant relationships within treatments. Regression analysis within or among groups however, is useful and could provide further insights. For example many studies looking at impact of diet restriction on rodents yield increases in longevity positively correlated to body size within treatments, yet demonstrate negative correlation between longevity and body size across treatments. One possible explanation is that the within-group difference may be accounted for by some individuals possessing superior metabolisms. This method of identifying tradeoffs by comparison of phenotypic traits is currently the main thrust of life-history study and the extrapolation of data to elicit general trends is consequently required to generalize results to this theoretical framework.

The second concern is whether or not it is permissible to lump across-treatment data and perform linear regression. Are there really in essence only two points, in which case linear regression is not useful? A parallel study conducted by Kajiura (unpublished) indicated the "supermouse" and normal mice in fact represent a continuum of responses with respect to their net production efficiencies. Normal and transgenic animals fall along the same line with considerable overlap. On this basis it was deemed acceptable to perform both within and across-treatment regression, (i.e. the data set can be considered to represent a single continuum of response).

Mass. Male transgenic animals between the ages of 86 and 106 days weighed 52.9 ± 1.27g (pooled heterozygous and homozygous data) while normal males weighed 26.59 ± .38g ($p < .0005$). This amounted to an almost doubling of body mass by the transgenic mice (198.95%). The two normal spinners weighed an average of 27.7 ± 3.7g while the two transgenic spinners weighed 42.8 ± .7g.

Rest.

Immobility (I): The transgenic and normal mice spent identical amounts of time immobile outside of the nest (28 minutes and 28.71 minutes respectively).

Sleep (S) To gain a better estimate of sleep time and active time, periods of immobility of less than five minutes outside the nest, were scored as active time. The remaining time periods greater than five minutes were considered sleep, and the time remaining was considered activity. Re-analysis incorporating this change extracted that transgenic mice spent 997.05 ± 19.42 minutes sleeping versus 791.12 ± 17.28 minutes for normal mice. Transgenic mice outslept normal mice by 126.01%. This amounted to an overall 14.3% increase in time which is comparable to the increase in the amount of *Total Rest* ($p < .0005$).

Total Rest (S+I): Overall transgenic mice spent an average of 1025.05 ± 17.7 minutes immobile versus 819.83 ± 18.5 minutes for normal mice ($p < .0005$). This amounted to an increase of 3.4 hours or 14.25% of daily time which happens to be identical to the increase in estimated time spent sleeping. Calculations reveal that transgenic mice rested 125% more than normal mice. Correlation analysis of the pooled data revealed that body weight is a very good predictor of time spent resting. ($p < .0005$) (*Table 3*). Linear regression confirmed the positive relationship between mouse body weight and resting time ($r^2 = .55$) (*Figure 3*). The two normal spinners showed an extremely large variation in the

amounts of time they spent resting. One mouse rested for only 91.47 minutes while the other rested for 963.18 minutes. The transgenic spinners showed relatively less variation and rested for an average of 813.32 ± 205.72 minutes.

Locomotion.

Locomotion (L): Overall mean time spent moving throughout the activity center was significantly less for transgenic mice (269.62 ± 11.52 minutes for normal mice versus 181.58 ± minutes for transgenic mice ($p < .0005$)).Transgenic mice were only 67.35% as active as normal mice and spent 6.11% less time engaged in active locomotion through the activity center. Strong negative correlations between weight and locomotion ($p < .0005$) indicate that body weight is a good predictor of locomotion. This indicated that larger animals generally spent less time in high energy expenditure activities like moving throughout the nest (*Table 3*). Linear regression revealed a negative relationship between body weight, and time spent moving throughout the nest ($r^2 = .45$)(*Figure 4*). The two normal spinners showed a large vasriation in the amount of time spent in locomotion. The mouse which was the short sleeper, spent 12303.64 min (20.06 h) locomoting while the other mouse spent 297.36 minutes locomoting. The transgenic spinners spent an average of 434.75 ± 164.78 minutes locomoting.

Wheel Running (W): An 89.25 minute ($p < .0005$) reduction in time spent on the running wheel by the transgenic mice was seen. Transgenic mice were therefore only 21.98% as active on the running wheel as normal mice. This represents a decline of about 6.2% in daily time. Strong negative correlations between body mass and wheel running were noted ($p < .0005$) (*Table 3).* The larger animals generally spent less time in high energy expenditure activities like running on the wheel ($r^2 = .47$)(*Figure 5).* The normal and transgenic spinners spent virtually no time on the running wheel.

Total locomotion (L + W): Much stronger trends were found when wheel running and locomotion

were added together. A 177.28 ($p < .0005$) minute reduction in time spent engaged in locomotion and wheel running by the transgenic mice was found which represents a 12.31% reduction. In other word transgenic mice were only 53.83% as active as normal mice. Very strong negative correlations between body mass and total locomotion were found. Larger animals spent less time engaged in activities involving locomotion ($r^2 = .63$) (*Figure 6*).

Feeding. No significant differences in mean feeding time were found between normal mice and the transgenic animals (*Figure 7*). The overall feed time estimates obtained for both groups may be underestimated. Coprophagous behaviour in these animals is well documented and the overhead position of the camera combined with their similarities in body position of these two activities made distinguishing grooming from coprophagous feeding difficult.

Drinking. Transgenic mice spent only 77.01% as much time consuming water as normal mice which translates to a 2.95 minute or .2% ($p < .04$) decrease in overall time spent drinking. Linear regression revealed no significant relationship between body weight and drinking ($r^2 = .07$) (*Figure 8*).

Grooming. Transgenic mice spent only 69.6% as much time grooming as normal controls which translates to an overall decrease of 36.9 minute or 2.56% ($p < .002$). These estimates may be overestimated. As stated previously distinguishing actual grooming from coprophagous feeding was difficult and as a result may have been inadvertently included in the estimates. Nevertheless correlation analysis revealed that there was a significant, although weaker, negative correlation between body mass and grooming ($.02 < p < .01$) such that larger animals spent less time grooming (*Table 3*). Linear regression found a very weak negative relationship between body weight and grooming ($r^2 = .16$) (*Figure 9*).

The percentage of active time spent engaged in various activities was calculated producing a relative time budget (*Table 2*). This indicates whether or not there were any changes in the relative scheduling of the behaviour relative to the proportion of other activities. There were no changes in the relative amount of active time engaged in locomotion, drinking or grooming. The transgenic mice spent 11.76% ($p < .0005$) less of their active time engaged in wheel running (12.55% ($p < .0005$) less engaged in total locomotion) while they spent 10.17% ($p < .0005$) more of their active time feeding.

Discussion

The data yielded by second-to-second videotape analysis provide an intimate and detailed picture of the daily activities of the mice and the impact of the transgenes. Growth hormone is unique because it regulates the size of the organism (albeit indirectly) within the constraints of the genetic program. The "supermice" had multiple copies of the gene continuously being transcribed allowing us to experimentally ascertain possible behavioural tradeoffs as a result of excessive growth.

This experiment was designed to investigate the possible link between behaviour and growth in the tradeoff structure constituting the life-history tactics of the mouse. Small mammals require relatively large amounts of food daily to survive, thus subsequent caloric needs may become acute when a higher growth rate is superimposed on these already costly demands. Recent advances in genetic engineering provided us with mice possessing such a high growth rate.

Of course, there would be no need for tradeoffs if organisms increase their processing rates during periods of high supply or demand (Tuomi *et al.*, 1983). In this instance, this does not appear to be the case. Using mice from the same colony, a recent in-depth resource allocation study found that adult male transgenic animals consumed slightly less food per gram of body weight than their normal counterparts (Kajiura and Rollo, unpublished). Results of that experiment demonstrate that transgenic mice are twice as efficient at converting food to body tissue as normal mice. However this increased metabolic efficiency appears to be largely obtained by reductions in behavioural expenditures.

Behaviour pervades the entire biology of an animal as an expression of its morphology, physiology,

ecology, and distribution (King, 1968). Transgenic mice have a drastically altered phenotype and as such might be expected to reflect its higher growth rate in terms of changes in behaviour. One of the first behavioural observations we made upon initial receipt of the mice was their lack of excitability and apparent lethargy. Transgenic mice were extremely easy to handle compared to normal mice. This type of behaviour had been previously documented anecdotally and was also found in other animal systems transgenic for growth hormone (Pursel *et al.*, 1989). Our colony also produced mutants we termed "spinners," which continuously ran in circles. The spinners were very hyperactive and excitable. MacArthur (1949), artificially selecting for large and small body mass in house mice, obtained a line of small mice that were highly active and a line of large mice that were docile and phlegmatic. (In fact the first spinner mice were obtained in MacArthurs study). The spinner phenotype also supports the tradeoff between growth and behaviour since their hyperactivity was mirrored by growth and smaller adult sizes.

Not only were differences found in levels of excitability, but analysis of time spent resting revealed that normal animals spent 56.9% of their time resting under a 12:12 light-dark cycle compared to 71.18% for transgenic mice. The difference between these mice is perhaps better appreciated if only the available 2activity period is considered (i.e. the 12h scotophase). Of the available 12 hours, normal mice were awake for 11 hours, wheras transgenic mice were active for only 7 hours. This behaviour consumed less energy compared to other activities. More accurate electrographic studies of the CRI strain of *Mus musculus* obtained comparable estimates of sleeping under a similar photoregime (Van Twyver, 1969). Our study found that "supermice" rested for 3.4 hours longer. In fact when all the animals were analyzed as a group there was a strong positive linear relationship between live body mass and the amount of time spent resting. Sleep and non-activity in the nest are very low energy consuming activities (Guillot, 1986). Sleep may therefore set a ceiling on metabolic expenditures by limiting the amount of time available for

activity. Since the larger animals increased the amount of time spent in nonactivity this strongly suggests a possible behavioural shift. This is not a new idea. Zeplin and Rechtshaffen (1974) conducted a massive interspecific study between 53 mammalian species. They found a significant tendency for animals to sleep more as the metabolic costs of keeping awake increased and speculated that sleep may have the function of enforcing rest which may help maintain a balance between gains and losses in energy. Further, they stated that it is not unreasonable to think that it could be shortened, lengthened, or otherwise modified depending on the energetic requirements of a species. Webb (1971) expanded on the theory discussing the adaptive qualities of nonactivity. Sleep may in fact be the only real immobilizer for small mammals. Horne (1988) argued that relaxed wakefulness is an advanced form of behaviour beyond the ability of their cerebrum. When essential tasks are completed, the animals may be immobilized in order to conserve energy. Our data seems to bear this out. The amount of time the mice spent immobile without sleeping totalled to only 28 minutes for both transgenic and normal mice. The well developed cerebrum of advanced mammals may allow them to remain stationary and relax during wakefulness, and energy can be conserved in this way without having to resort to sleep.

Animals may obtain higher metabolic outputs if stored reserves are utilized, rather than the direct outputs of metabolic pathways (Peterman *et al.*, 1990). Sleep may allow accumulation of reserves to support greater short-term activity outputs. If growth is diverting away such reserves, even with greater sleep , this could explain the slow lethargic performance of the supermice.

It is not known if the length of sleep varies with the different phases of growth. It has been estimated that human infants sleep approximately 14.8 hours a day while adults sleep approximately 7-8 hours a day (Kleitman, 1963). Infants are in the most rapid part of their growth phase in human development when growth costs are highest. It seems possible that long periods of sleep may provide an

energy savings which can be diverted to other life-history traits, including growth.

The system may monitor GH levels. It has been shown that GH produces a dose-dependent increase of REM sleep in rats, cats and humans (Drucker-Colín, 1977). GH may therefore play a part in the triggering mechanism of sleep. If this is true, it would be expected that a GH inhibiting factor such as somatostatin would produce alterations in sleep patterns or a decrease in the amount of sleep. A dose of 10 μg of somatostatin in normal and hypophysectomized mice resulted in a an overall reduction of sleep. However the studies were obscured by the fact that somatostatin induced motor excitation and prolonged spells of compulsory scratching and circular movements which made it difficult to determine whether this peptide altered some basic sleep mechanisms. (Havlicek et al, 1975; Rezek et al, 1975; Drucker-Colín and Valverde-R, 1981). The reported hyperactivity and particularly its linkage to "circular movement" is very reminiscent of our own small "spinner" mice. It is highly significant that we obtained only about five *transgenic* spinner animals over the years compared to nearly 100 normal spinner mice. Moreover the *transgenic* spinners had reduced "symptoms" (i.e. they were not nearly as hyperactive as the normal spinner mice). It therefore seems likely that the spinner genes are in fact recessive, but are widespread in mice. They appear to show low penetrance at high titres of growth hormone. As growth hormone levels drop via selection, inbreeding, genetic variation, or experimental manipulation the spinner genes appear to be expressed. In fact they may be adaptive in offsetting the lethargy associated with large size as shown by the less hyperactive transgenic spinners. Futher support comes from our analysis of the spinner animals. Although one normal spinner showed amounts of sleep comparable to normals (963.18 mins), the other mouse only slept 91.47 minutes! Similarly, one *transgenic* spinner slept like other transgenics, but the other only slept 607.6 minutes. Clearly, spinner genes are associated with mice with insomnia and pehaps disturbed rhythms.

Locomotion is the means by which animals escape predators and acquire resources and mates. Such

activity is energy demanding and may be sensitive to energy deficits. Two types of locomotion were differentiated: general locomotion and active locomotion (wheel running). The availability of the running wheel allowed the mice to express even higher levels of locomotive activity. Rodents in the wild frequently engage in such vigorous motor activity during foraging or to escape predators. Both types of behaviour are energy expensive. Guillot (1986) estimated that locomotion used three times as much energy as resting, among mice. The transgenic animals showed significant decreases in locomotion as well as wheel running. There was an inverse relationship between body size and wheel running and a similar relationship with locomotion. It might be argued that since larger mice sleep longer they have less time available to engage in other activities. Naturally, we should see reductions in time spent engaging in other activities. However, when wheel running alone was analyzed as a proportion of total locomotion (Locomotion + Wheel Running), transgenic mice showed an almost three-fold decrease in time spent actively running on a wheel (12.17% of active time for transgenic mice versus 29.79% for normal mice). The relative time budget revealed similar trends. Transgenic mice spent 11.76% less time ($p < .0005$) running on a wheel compared to normal siblings.

Several lines of evidence suggest that there are tradeoffs between lowered energy intake or increased costs and amounts of energy expended in active locomotion. Modest reductions in food intake over a 5 week period resulted in a disruption of behaviour in house and deer mice. Deer mice exhibited a gradual and sustained reduction in the amount of running activity while house mice exhibited temporal shifts in behaviour (Blank and Desjardins, 1985). When energy intake is limited, behaviour patterns are altered to affect conservation. In many cases the literature on feeding behaviour is contradictory. Short-term reductions in food intake, may initially increase motor activity. To improve foraging efficiency more time and energy may be temporarily allocated to locomotory activity. If unsuccessful or if costs exceed returns, subsequent reductions in motor activity may follow. Food restriction studies differ from one another with respect to the severity and length of the food restriction period. As a result, the energetic costs vary from

study to study (Duffy *et al*, 1989). One must therefore interpret results cautiously. Similar trends are seen in studies involving obese strains of mice. Mice with hereditary obese-hyperglycaemic syndrome (obese, *ob/ob*) show a decrease in voluntary activity which precedes the development of obesity. Comparison of the activity rates of nonobese animals and young obese animals of the same weight demonstrate that inactivity is not the result of the animal being overweight, but vice versa (Mayer, 1953; Mayer, 1954). Among rats, bilateral lesions of the ventromedial nuclei of the hypothalamus produce hyperphagic obese animals which show markedly less activity than normal animals.(Schacter, 1971). There appears to be a linkage between growth rate, resting and locomotor activity. Strongest support comes from a study of activity levels of three inbred strains of mice by Theissen (1961) who found that there was an inverse relationship between activity and body weight.

Kajiura's (unpublished) resource allocation study pointed out that larger transgenic mice do consume more food per mouse than smaller normals, but they actually eat slightly less food on a per gram basis. This poses an interesting question as to whether or not transgenic animals have the ability to increase their rate of food intake. A study using mice from the same colony demonstrated that after 12 hours of food deprivation transgenic animals had the same ability as normal controls to double their short term rate of food intake to compensate (DelCotto and Rollo, unpublished). It appears then that transgenic animals have the capacity to double their rate of food intake, but they do not deploy this ability to pay for their extra growth. Our study found no difference in the amount of time spent feeding between groups which is consistent with the mass budget calculated by Kajiura (unpublished). Possibly, feeding regulation is targeted to the existing body mass of animals and not the growth rate of these tissues. There may be no regulatory physiological linkages between feeding rate and growth rate, allowing them some independent selection genetically. While exogenous administration of GH in pigs (Campbell *et al.*, 1988) and

endogenous production of foreign GH result in appetite suppression (Pursel *et al.*, 1990; Wall *et al.*, 1990), studies involving mice selected for large body size have found that a higher growth rate accompanies this increase in body size (Eklund and Bradford, 1977).

An alternative and more likely explanation of what may be occurring is that the high foreign GH levels may lower endogenous GH production. Endogenous production of mGH is known to be severely reduced in transgenic mice carrying human GH transgenes, presumably due to feedback inhibition of human growth hormone or IGF-1 levels (Palmiter *et al.*, 1983). It appears that the metallothionein-1 promoter of the transgene is activated in the liver and as a result large amounts of foreign GH are made in those tissues, rather than in the pituitary gland where endogenous GH is usually secreted (Palmiter *et al.*, 1982,1983). If the regulatory mechanisms linking feeding rate to growth rate take place in the pituitary or surrounding tissues, feeding rate may be reduced. The extrapituitary GH would therefore bypass the feeding regulatory mechanism while feeding back on endogenous GH production.

Further support for this theory comes from a study by Miller et al. (1988) who found lower circulatory levels of endogenous porcine GH in transgenic pigs engineered with human growth hormone. Our laboratory previously obtained similar results injecting rainbow trout with foreign GH (Jobin, 1988). It is expected that in a properly regulated system, higher growth rates should be linked to higher feeding rates. The suppression of appetite by exogenous GH suggests a regulatory disruption. Increased growth without corresponding increases in the intake of energy appear to be the primary cause for chronic stress in our mice and possibly other transgenic animals.

It is interesting that feeding behaviour was resistant to change while resting locomotion and wheel running were much more adaptable. A recent long-term study explored how the costs of access to food,

water, a nest box and a running wheel affected the behaviour of rats. It was found that running behaviour declined more rapidly than feeding or drinking as costs increased (Collier *et al.*, 1990).

Slight decreases in time spent drinking and grooming by the transgenic mice were also noted. The reduction in the percentage of daily time spent engaging in these activities may simply be the result of the increased amount of time spent resting. The relative time budget confirmed this by indicating no changes in the relative amount of active time spent engaged in either of these activities (*Table 2*). Curiously, we found a slight decrease in water consumption between normal mice and heterozygous mice, but not between normal mice and homozygous mice. In the case of homozygous mice, the small sample size may have obscured statistical resolution.

We therefore speculate about the existence of a general trend. It would seem that when energy intake is reduced or when more energy is shunted into a life-history feature such as growth, the net amount of energy left in the system available for other features is reduced. One method by which the allocation system may conserve energy expenditure is by shifting the time allocation strategy, which could result in altering behaviour patterns such as increasing resting and minimizing locomotion. This suggests that behaviour may be sensitive to modifications in energy availability and lends support to the principle of allocation. The principle of allocation states that an organism has a limited amount of time, matter and energy available to devote to activities such as foraging, maintenance and reproduction. If any of the costs for these conflicting demands changes without a corresponding increase in energy intake, a shift in resource allocation strategy is predicted. The behavioural changes exhibited by the transgenic mice seem to support the realty of such unavoidable tradeoffs and also the idea that mice have evolved behavioural responses to counter energy shortages in the system.

It is remarkable that we found such dramatic behavioural changes with the mice since they were at the tail-end of their growth phase when energy demands for growth are declining. Of course they do have increased maintenance costs associated with a larger body size. We therefore predict that even greater behavioural differences might be observed in younger transgenic mice during their earlier, more rapid growth phase. With our strain of homozygous transgenic mice we appeared to reach a plateau in terms of body size. Although these mice could be recognized by early elevations in growth rate, their rival adult sizes were no more than 5 to 10 g greater than heterozygous transgenics. If we could somehow increase body size even further (if the genetic constraints allow it!), more dramatic changes might become apparent. Recently mice have been developed containing insulin like growth factor-I fusion transgenes, but these grow less quickly than GH mice (Mathews *et al.*, 1988). Assuming that growth is bottlenecked by a lack of sufficient IGF-1 genes in GH mice, and lack of sufficient GH in IGF-1 mice, crossing these strains might produce offspring with multiple copies of both GH and IGF-1. The effectiveness of both might be increased. Alternately, higher growth rates may be constrained by the diet. Recent studies involving transgenic pigs have found that increased levels of dietary protein, particularly lysine resulted in the animals gaining weight faster (Pursel *et al.*, 1989; Wall *et al.*, 1990) Our lab is currently studying the effect of varying high protein and high carbohydrate diets on our line of mice to determine whether or not there is a dietary bottleneck inhibiting higher growth. Preliminary results with high protein diets (40% protein) have yielded significant increases.

In any case the sparse literature on the lifehistory of transgenic mice combined with our data suggest that the transgenic mouse is a truly maladapted organism. The overexpression of GH has been associated with several pathological changes including shortened lifespan, glomerulosclerosis, hepatocellularmegaly, and infertility (Doi *et al.*, 1983; Brem *et al.*, 1989; Bartke *et al.*, 1988; Quaife *et al.*, 1989; Naar *et al.*, 1991). Pursel et al. 1989 reported similar results among transgenic pigs in addition to lethargy, lameness

and uncoordinated gait. Many of the pathologies are prevalent in natural populations at lower incidences and less severity (Pursel *et al.*, 1989). It is believed that the anomalies are not artifacts resulting from ectopic expression of a heterologous GH gene, but the result instead from the chronic elevation of circulating GH (Quaife *et al.*, 1989). In fact, a study of a line of mice selected for large body mass found a decrease in mean lifespan comparable to that of our own transgenic mice. In addition there was a trend for increased incidences of tumours in the high growth line (Eklund and Bradford, 1977).

Interestingly enough, strains of mice carrying ovine GH transgenes (mMT/oGH), and strains which are transgenic for GH releasing factor (mMT/hGRF) are generally fertile. Although both of these strains of mice are larger than controls, the increased growth is significantly less than the increased growth observed in our transgenic mice expressing GH. A possible explanation of the trend is that these strains allocate less energy to somatic growth and more to reproduction than their transgenic GH cousins (Orian *et al.*, 1989; Hammer *et al.*, 1985). No mention was made regarding the activity levels of these mice, but we would suspect that they would be very similar to normal controls. The strain carrying ovine GH did show some physiological pathologies including lesions in the liver. This indicates that even modest increases in growth (average 30%) can have effects on the health (Orian *et al.*, 1989). This suggests there may be greater DNA damage or somatic damage accompanying accelerated growth. These problems hint at possible longterm tradeoffs as a result of the increased growth rate rather than artifacts which occur due to the unusual nature of the animals.

Growth may be pathological when morphological increases are not accompanied by commensurate improvements in functional efficiency (Goss, 1978). Presumably this lends support to the argument that growth rate may be optimized rather than maximized in animals. The very existence of transgenic mice indicate that physiologically, mice have the capacity to at least double their body size. However, when

this happens, a whole range of problems become apparent as we have indicated. It appears then that mice have an optimal growth rate fine tuned to their life-history and physiological circumstances. It can be expected that both optimum and maximum growth rates are fixed or constrained genetically. The maximal rate is fixed by developmental and physiological constraints while the optimal rate is determined by active control via GH, IGF-1 and other growth factors. The old adage that mice are the size they are for a good reason may hold true.

Many molecular biologists have claimed that elimination of the side effects associated with over expression of the transgene in domestic animals can be achieved by regulation of transgene expression during the rapid growth phase (Pursel *et al.*, 1989). The data yielded by our transgenic mice indicates that this may be only a partial solution. Unless food intake is increased, maintenance of the elevated growth rate without the associated problems may not be possible. Perhaps by crossing the "supermouse" with a line of hyperphagic mice a line of supermice with large appetites to match, may be produced. This seems an easier much more practical solution and the implication for agriculture are obvious. It seems therefore, that if the advances of modern molecular biology are ever to be effectively applied, an ecological overview is required. Life history traits are not independent of one another. Consequently simple alterations of the genome may not be enough to "improve" a species. Intimate knowledge of the inter-relationships between lifehistory features is essential. By understanding these relationships we can have a basis for predicting directions and also magnitudes of expected differences in life-histories under selective regimes or as a result of genetic manipulations (Taylor and Gabriel, 1992). Evolutionary ecology will therefore become much more important as molecular biology advances

In conclusion, it is our hope that the wide range uses of the transgenic mouse system in investigating otherwise untestable hypotheses will not be overlooked by fellow evolutionary ecologists. We are indeed

on the verge of a new era and the transgenic mouse may be a bridge allowing us to span the gulf between ecology and molecular biology.

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Integration, Implications and Conclusion

"The Mouse That Roared"

The aim of this study was to characterize the effect of increased growth costs on the behaviour of a mammal utilizing a new empirical method for testing life-history theory; the transgenic mouse. The results of the study provide evidence to support the idea that behavioural shifts can occur in organisms to help offset energetic costs associated with a high growth rate. To date this is the first study to arrive at such a conclusion. This implies that the resource allocation system is adaptable and can respond to changes in the cost structure by shifting energy flow pathways.

I have no wish to overstate the importance of behaviour as a life-history variable, merely I wish to prevent it from being overlooked in empirical studies and theoretical models. In the past, far too many studies have looked at life-history traits in isolation (for instance studying the relationship between growth and reproduction). Separate life-history traits have no independent existence in nature (Tuomi *et al*, 1983). Organisms are tightly integrated, precisely controlled systems which have evolved as a unified organization (Calow and Townsend,1981). Life-history traits are intercoupled and are dependent on the physiological organization of the individual organism. The way in which all of these life-history features interface is still unclear. It is our hope that the transgenic mouse will help us in determining how the genotype of an animal can influence these traits.

An ongoing longterm study has indicated that the average lifespan for our strain of normal female mice in our strain is 665 days while the lifespan for female transgenic mice is 307 days, or only 46.2% ($p<.0005$) as long. Lifetime reproduction for transgenic mice was also compromised. More than half

of the transgenic females were sterile. Those that reproduced produced on average 1.3 litters versus 5.3 litters, ($p < .0005$) for normal mice. Both of these decreases have been casually noted in the literature (Brem *et al*, 1989; Naar *et al*, 1991; Bartke *et al*, 1988). Clearly extra-normal growth has a direct cost in terms of fitness. Fitness is ultimately measured in terms of the phenotype's relative success in converting resources to reproductive products. Growth can be viewed as the developmental means of achieving the reproductive state. Increased growth usually *increases* fitness in several ways: Large animals may have a competitive advantage during resource acquisition and may be less vulnerable to predation. In addition large animals may have an easier time maintaining homeostasis. However maximizing somatic growth appears to have the opposite effects and in addition, this study indicates the costs of maintaining the higher growth rate can adversely affect fitness directly by lowering reproductive rate (Calow and Townsend, 1981).

Are these the only other tradeoffs made with a high growth rate? Most likely not. Two other possible avenues of energy reduction are being investigated. Comparison of the respiration rates and core body temperatures between normal and transgenic mice will be conducted during the next phase of the project. Both are known to occur in other systems, or in particular circumstances. It is very clear that the relationship between the various life-history traits is very complex.

This leaves us on the verge of many new and exciting discoveries about how life-history features interface with organism design and it is our hope that the transgenic mouse will play a useful role allowing in achieving these goals. Molecular biologists have referred to the transgenic mouse as "the mouse that roared". I do not believe however, that they fully realized how loud a roar this "*...wee timorous little beastie..*" will have on the future of biology. As the science of organism design evolves and spurred on

by the discoveries made using this tool, greater interdisciplinary study will occur and finally swing the pendulum back from reductionism in biology towards a more holistic approach.

Appendix

Tables

Table 1: The absolute time budget for normal mice and transgenic "supermice".
Values represent total mean time for each activity (mins) and expressed as a percentage of 24 hours +/- SE.

Variable	Normal	% of 24h	Transgenic	% of 24h	Difference	% T / N	t-test
Mass (g)	26.59		52.9		-26.31	198.95	p<.0005
SE	0.38		1.27				
Locomotion	269.61	18.72	181.58	12.61	88.03	67.35	p<.0005
SE	11.52	0.80	10.13	0.70			
Wheel	114.40	7.94	25.15	1.75	89.25	21.98	p<.0005
SE	15.02	1.04	6.27	0.44			
Resting	819.83	56.93	1025.05	71.18	-205.22	125.03	p<.0005
SE	18.50	1.28	17.70	1.23			
Feeding	97.55	6.77	111.95	7.77	-14.40	114.76	NS
SE	5.02	0.35	6.88	0.48			
Drinking	12.83	0.89	9.88	0.69	2.95	77.01	p<.04
SE	1.10	0.08	0.85	0.06			
Grooming	121.40	8.43	84.50	5.87	36.90	69.60	p<.002
SE	0.01	0.00	0.01	0.00			

Table 2: The relative time budget for normal and transgenic "supermice".
Values represent the percentage of active time each behaviour occupies.

Variable	Normal	Transgenic	Difference	t-Test
Locomotion	41.74	41.15	0.59	NS
SE	1.69	1.83		
Wheel	17.12	5.36	11.76	p<.0005
SE	2.06	1.21		
Total Locomotion	58.70	46.15	12.55	p<.0005
SE	1.69	1.81		
Feeding	15.19	25.36	10.17	p<.0005
SE	0.86	1.35		
Drinking	2.01	2.31	0.30	NS
SE	0.20	0.20		
Grooming	18.77	19.01	0.24	NS
SE	1.22	1.29		

Table 3: Correlation matrix for pooled normal and transgenic data. Mass measured in grams. All other variables measured in seconds. n=38.

	Mass	Resting	Locomotion	Feeding	Drinking	Wheel	Grooming
Mass	1.00						
Resting	0.74	1.00					
Locomotion	-0.67	-0.82	1.00				
Feeding	0.23	-0.05	-0.18	1.00			
Drinking	-0.27	-0.28	0.10	0.14	1.00		
Wheel	-0.68	-0.79	0.45	-0.10	0.37	1.00	
Grooming	-0.40	-0.65	0.51	-0.15	-0.05	0.29	1.00

Table 4: Correlation matrix for normal mice. Mass measured in grams. All other variables measured in seconds. n=15.

	Mass	Resting	Locomotion	Feeding	Drinking	Wheel	Grooming
Mass	1.00						
Resting	0.31	1.00					
Locomotion	-0.41	-0.47	1.00				
Feeding	-0.17	0.08	-0.27	1.00			
Drinking	-0.03	0.29	-0.35	0.11	1.00		
Wheel	-0.14	-0.70	-0.15	-0.09	0.13	1.00	
Grooming	0.32	-0.36	0.20	-0.23	-0.62	-0.09	1.00

Table 5: Correlation matrix for transgenic mice. Mass measured in grams. All other variables measured in seconds. n=23.

	Mass	Resting	Locomotion	Feeding	Drinking	Wheel	Grooming
Mass	1						
Resting	-0.05	1.00					
Locomotion	-0.09	-0.71	1.00				
Feeding	0.07	-0.54	0.06	1.00			
Drinking	0.19	-0.21	-0.08	0.30	1.00		
Wheel	-0.07	-0.42	-0.01	0.27	0.32	1.00	
Grooming	0.21	-0.54	0.30	0.02	-0.07	-0.12	1

Figures

Figure 1. Schematic diagram of the artificial enclosure. Constructed of 5mm. clear acrylic (l x w x h = 51 x 51 x 16 cm). The cover was constructed from 1 cm grid construction mesh attached to a wooden frame. A hole in the side of one compartment allowed access to an exterior water bottle supported by a retort stand.

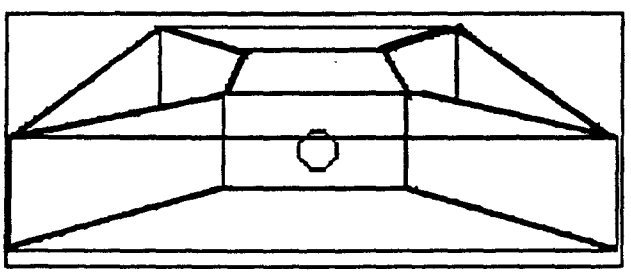
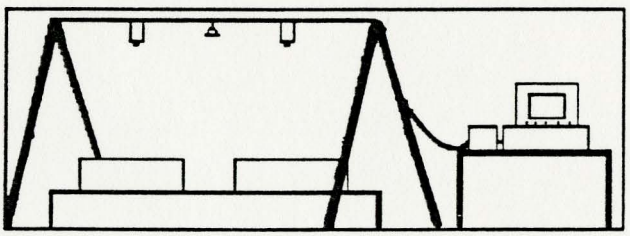


Figure 2. Diagram of the experimental setup. Two infra-red videocameras were suspended over the artificial enclosures from wooden scaffolding. A video switcher allowed two experiments to be conducted simultaneously by recording the inputs from the two cameras on one videotape.



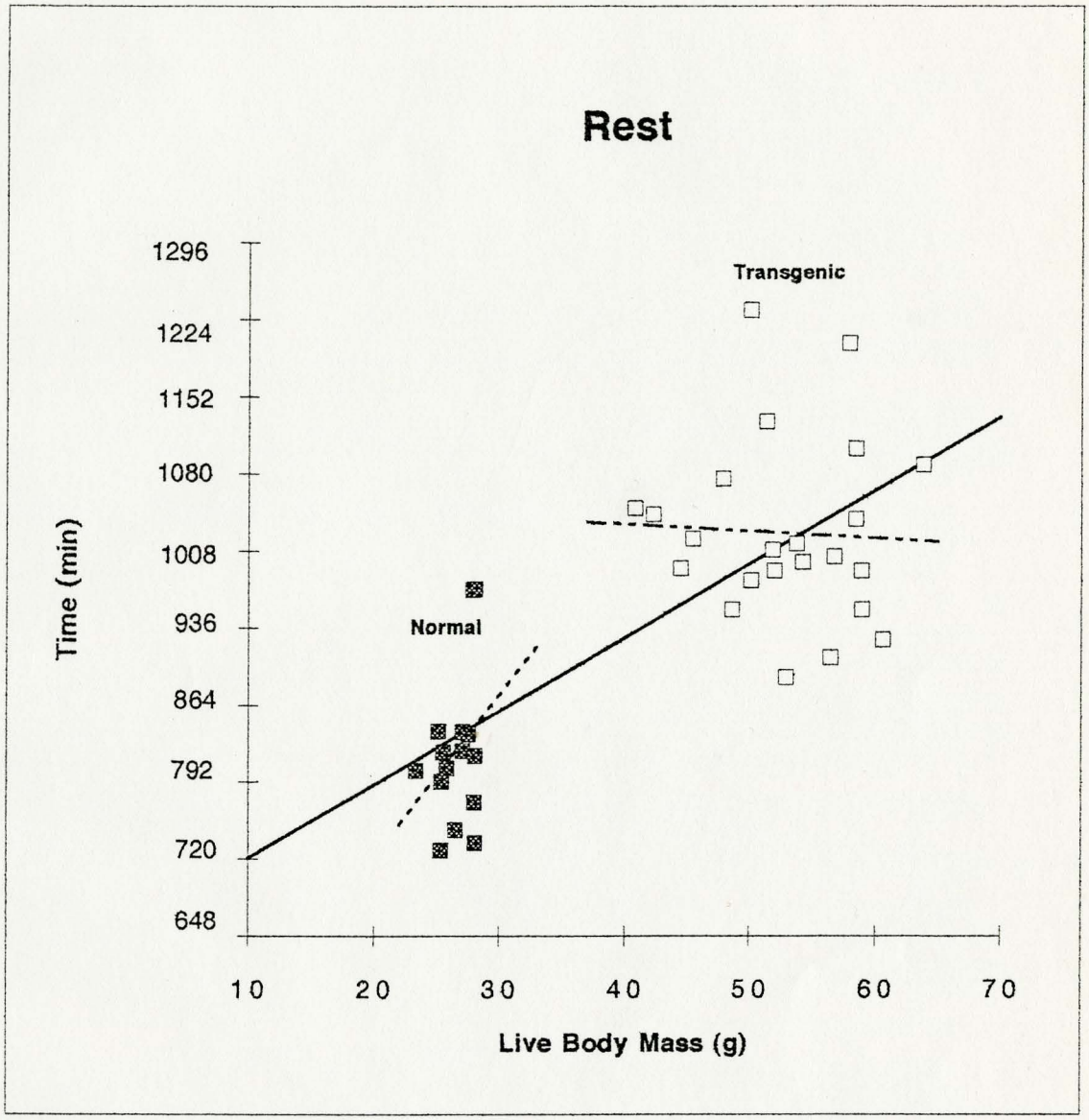


Figure 3. Graph of total time spent at rest (min) vs live body mass (g). Standard errors are omitted to avoid visual confusion.

Regression equation (pooled data) y= .00476 x + 652.38 (r^2 = .548, p<.0005, n=38)

Regression equation (normal data) y= .0105 x + 419.52 (r^2 = .0941, n.s. , n=15)

Regression equation (transgenic) y= -.00045 x + 1059.05 (r^2 =.002, n.s. , n=23)

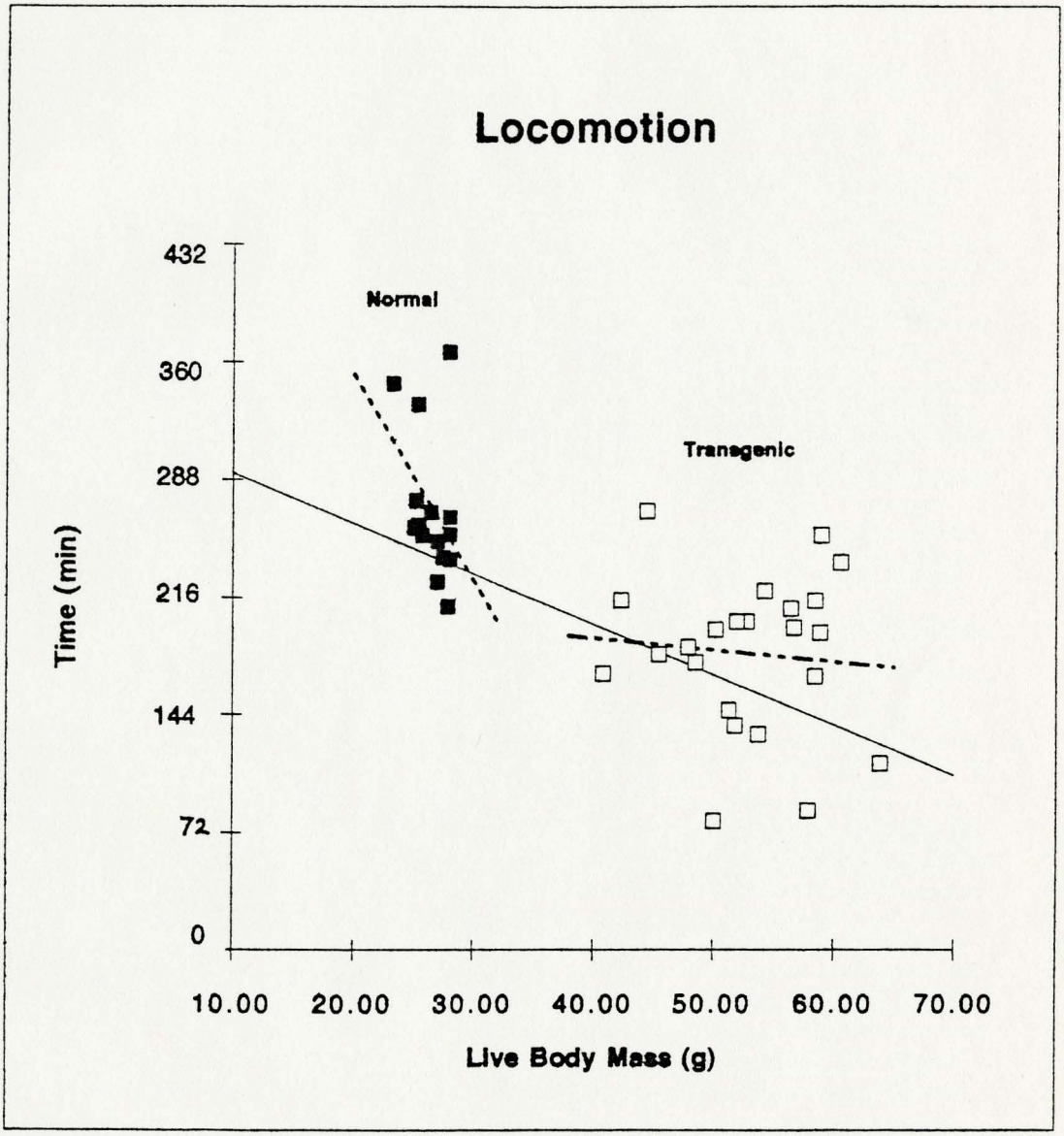


Figure 4. Graph of total time in minutes spent in locomotion, over 24 hours, versus live body mass, for pooled normal and transgenic mouse data. Standard error bars are omitted to avoid visual confusion.

Regression equation (pooled data) y= -.00214 x + 347.45 (r^2 = .452, p<.00005, n=38)

Regression equation (normal data) y= -.00873 x + 603.95 (r^2 = .169, n.s. , n=15)

Regression equation (transgenic) y= -.000509 x + 220.35 (r^2 = .00847, n.s. , n=23)

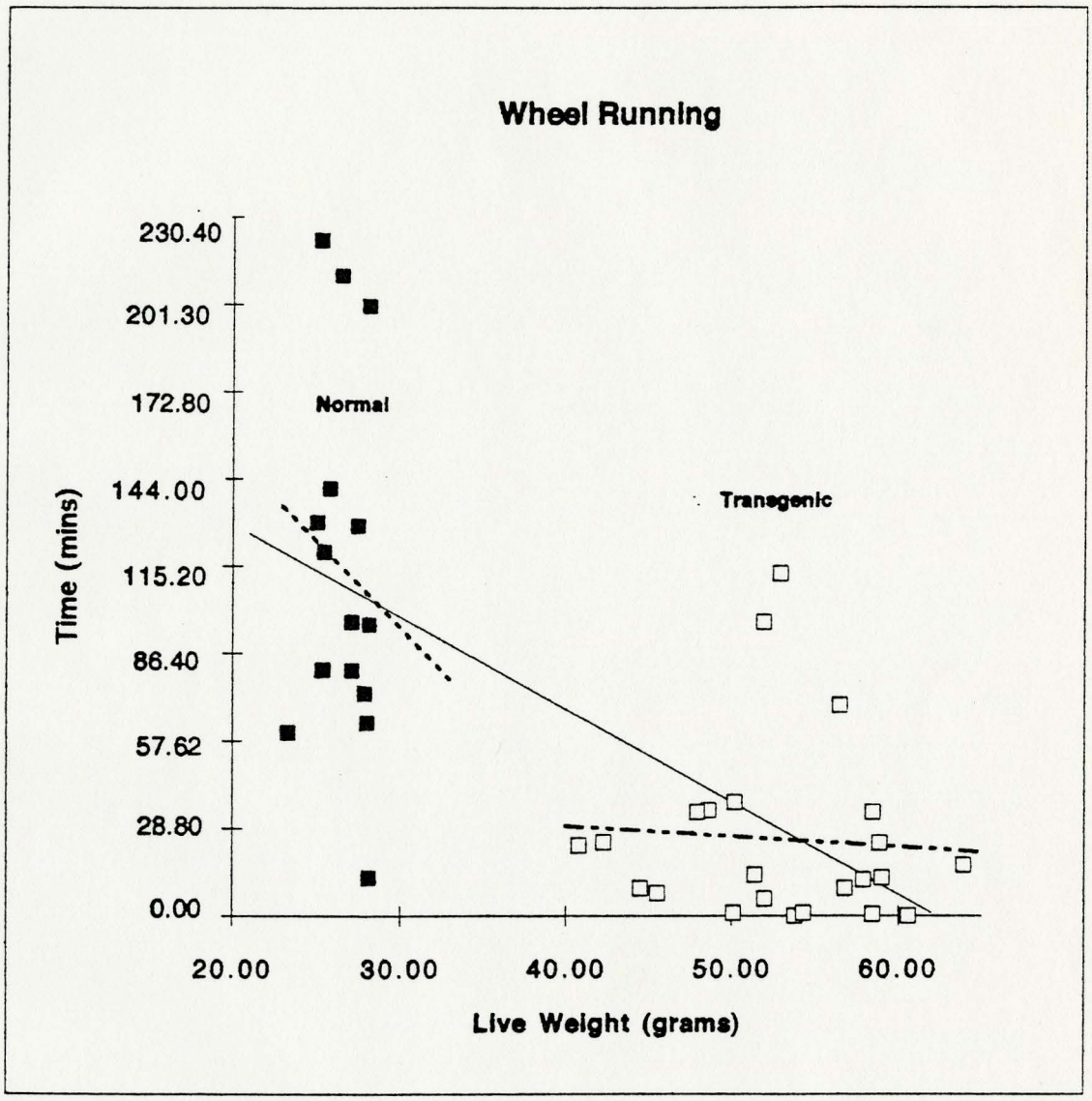


Figure 5. Graph of total time in minutes running on a wheel, over 24 hours versus live body mass, for pooled normal and transgenic mouse data. Standard error bars are omitted to avoid visual confusion.

Regression equation (pooled data) $y = -.0021 X + 190.07$ ($r^2 = .47$, $p < .0005$., n=32)

Regression equation (normal data) $y = -.004 X + 266.72$ ($r^2 = .02$, n.s. , n=15)

Regression equation (transgenic) $y = -.0002 X + 42.82$ ($r^2 = .005$, n.s. , n=23)

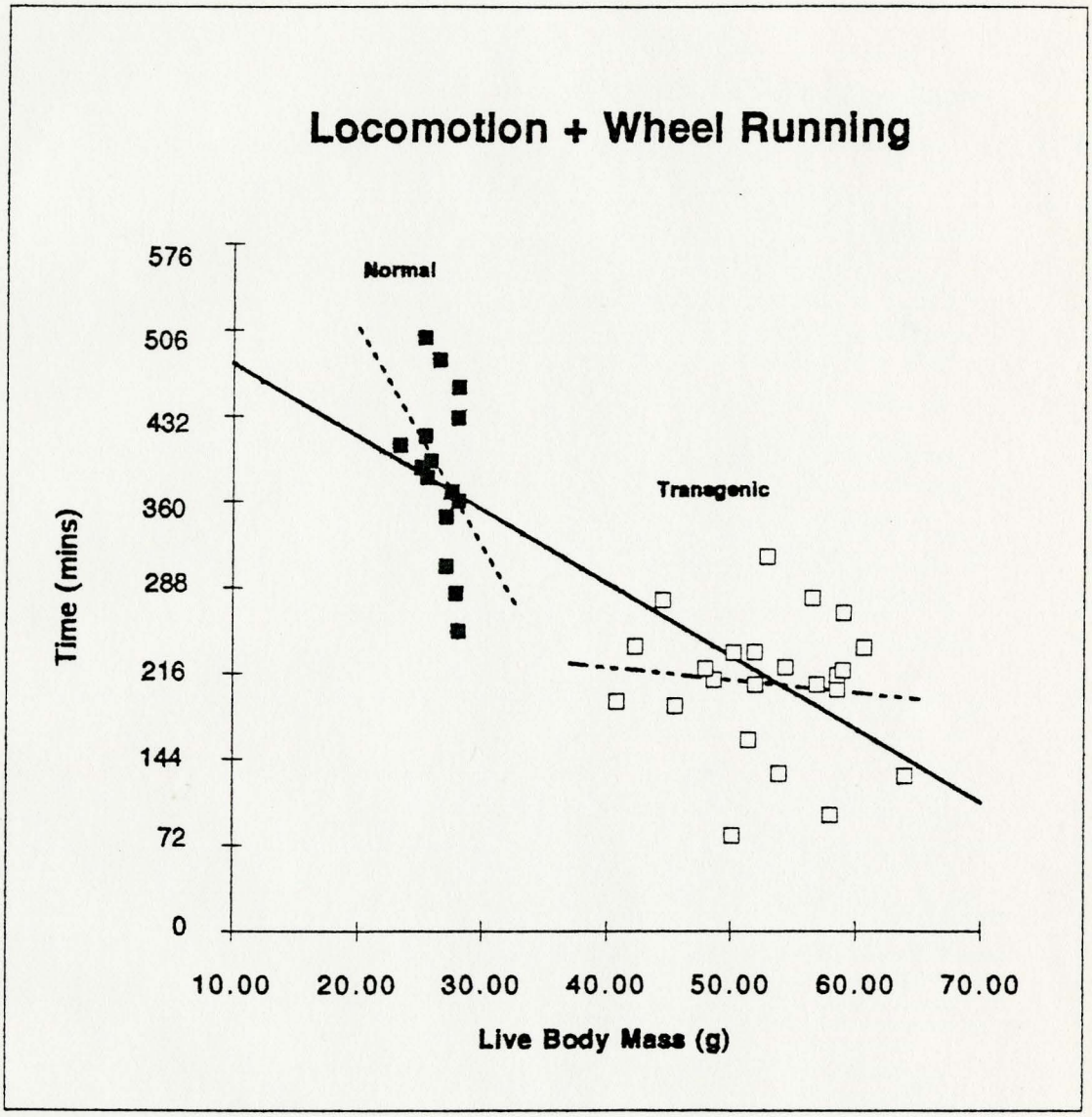


Figure 6. Graph of total time spent in minutes in locomotion and wheel running , over 24 hours, versus live body mass for both normal and transgenic mice. Standard errors are omitted to avoid visual confusion.

Regression equation (pooled data) $y = -.00426\ x + 537.63$ ($r^2 = .635$, $p < .0005$, $n=38$)

Regression equation (normal data) $y = -.0127\ x + 879.65$ ($r^2 = .15$, n.s. , $n=15$)

Regression equation (transgenic) $y = -.000741\ x + 263.17$ ($r^2 = .603$, n.s. , $n=23$)

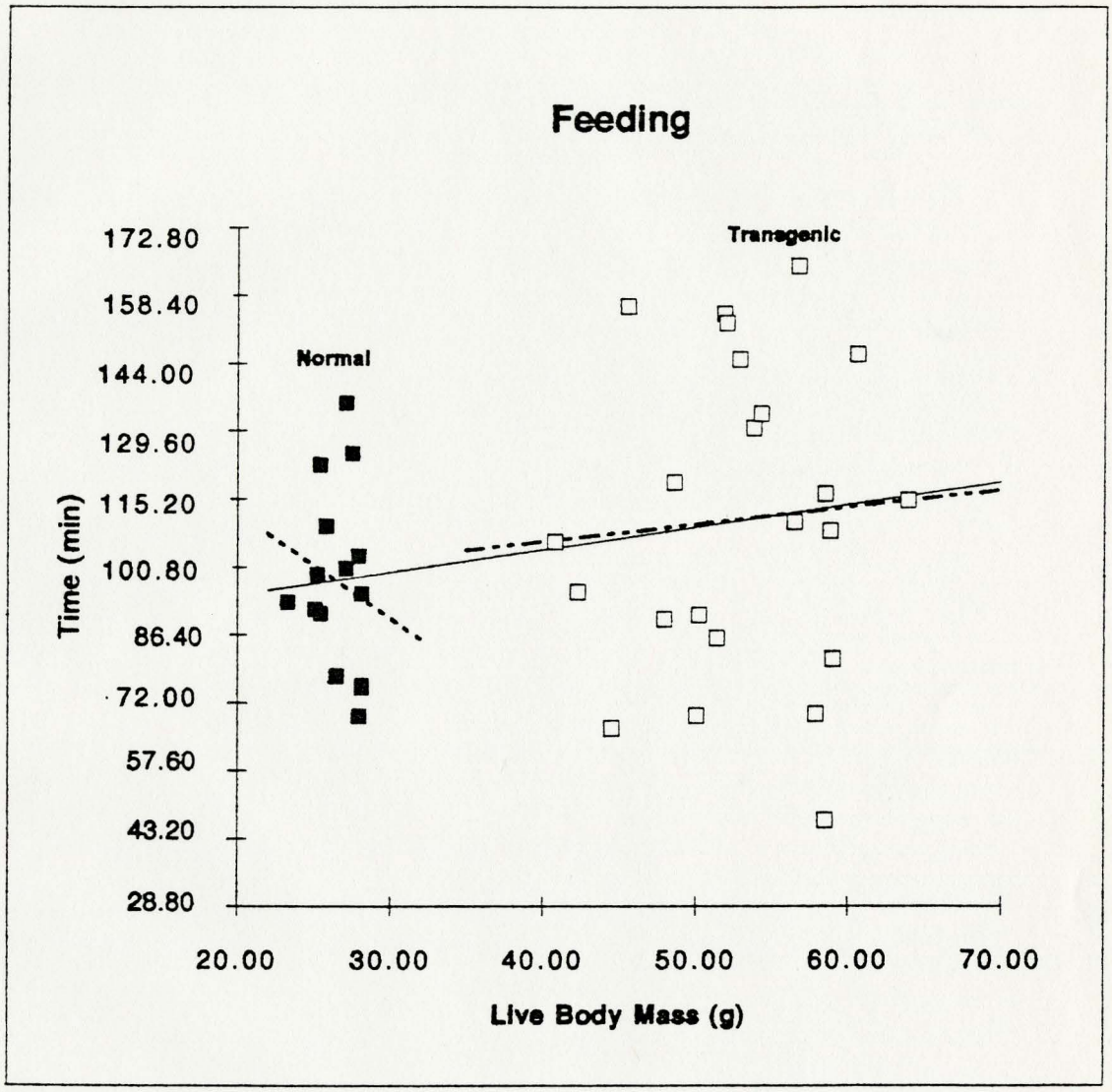


Figure 7. Graph of total time spent feeding, over 24 hours, versus live body mass, for pooled normal and transgenic mouse data. Standard error bars are omitted to avoid visual confusion.

Regression equation (pooled data) $y = .0003 x + 85.2$ ($r^2 = .053$, n.s. , n=38)

Regression equation (normal data) $y = -.0016 x + 157.7$ ($r^2 = .028$, n.s. , n=15)

Regression equation (transgenic) $y = .0003 x + 91.37$ ($r^2 = .005$, n.s. , n=23)

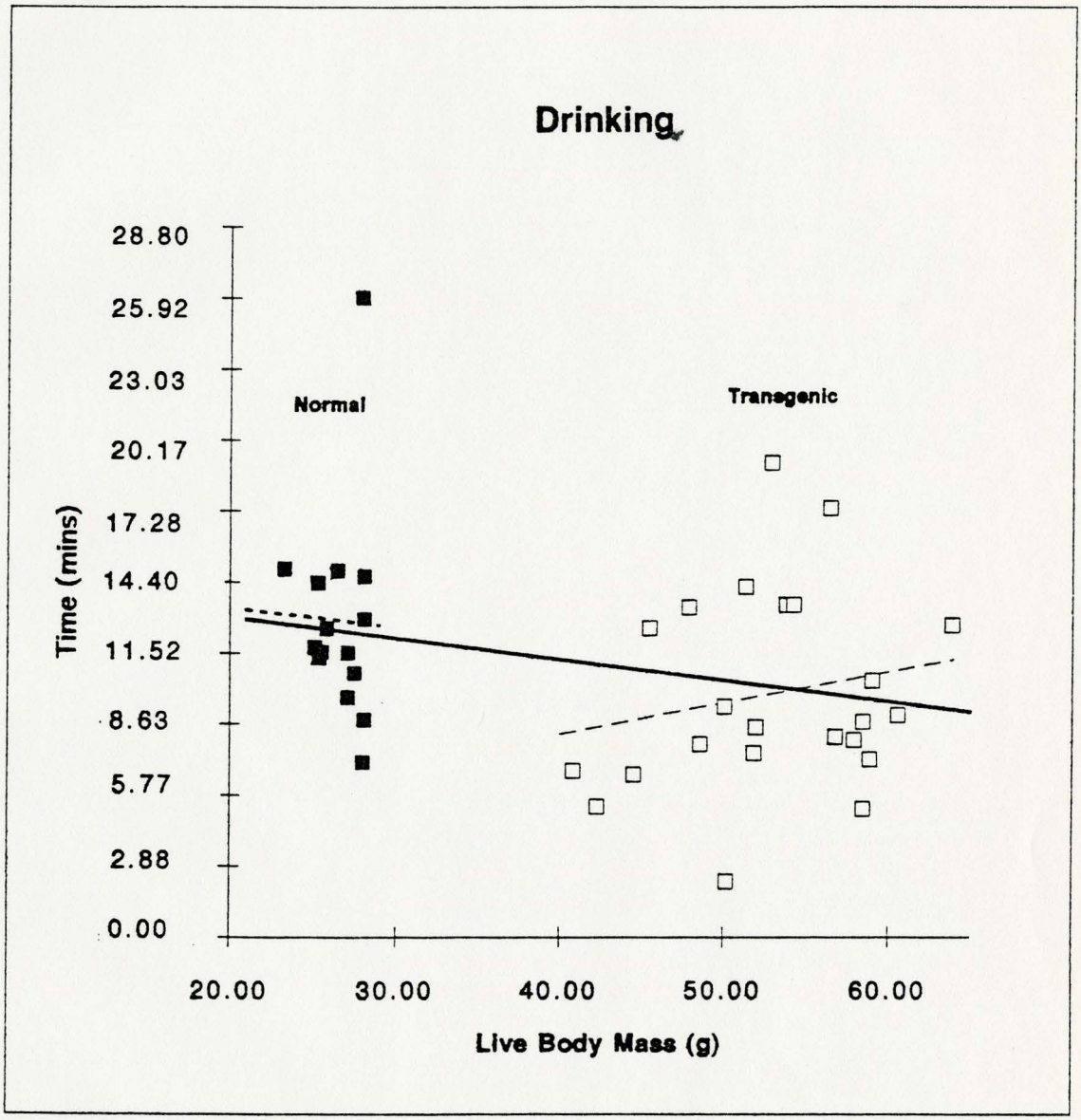


Figure 8. Graph of total time in minutes spent drinking, over 24 hours versus live body mass, for pooled normal and transgenic mouse data. Standard error bars are omitted to avoid visual confusion.

Regression equation (pooled data) $y = -5.91 \times 10^{-5} X + 14.67$ ($r^2 = .074$, $p < .04$, $n = 32$)

Regression equation (normal data) $y = -5.38 \times 10^{-5} X + 14.9$ ($r^2 = .001$, n.s. , $n = 15$)

Regression equation (transgenic) $y = 8.70 \times 10^{-5} X + 3.25$ ($r^2 = .04$, n.s. , $n = 23$)

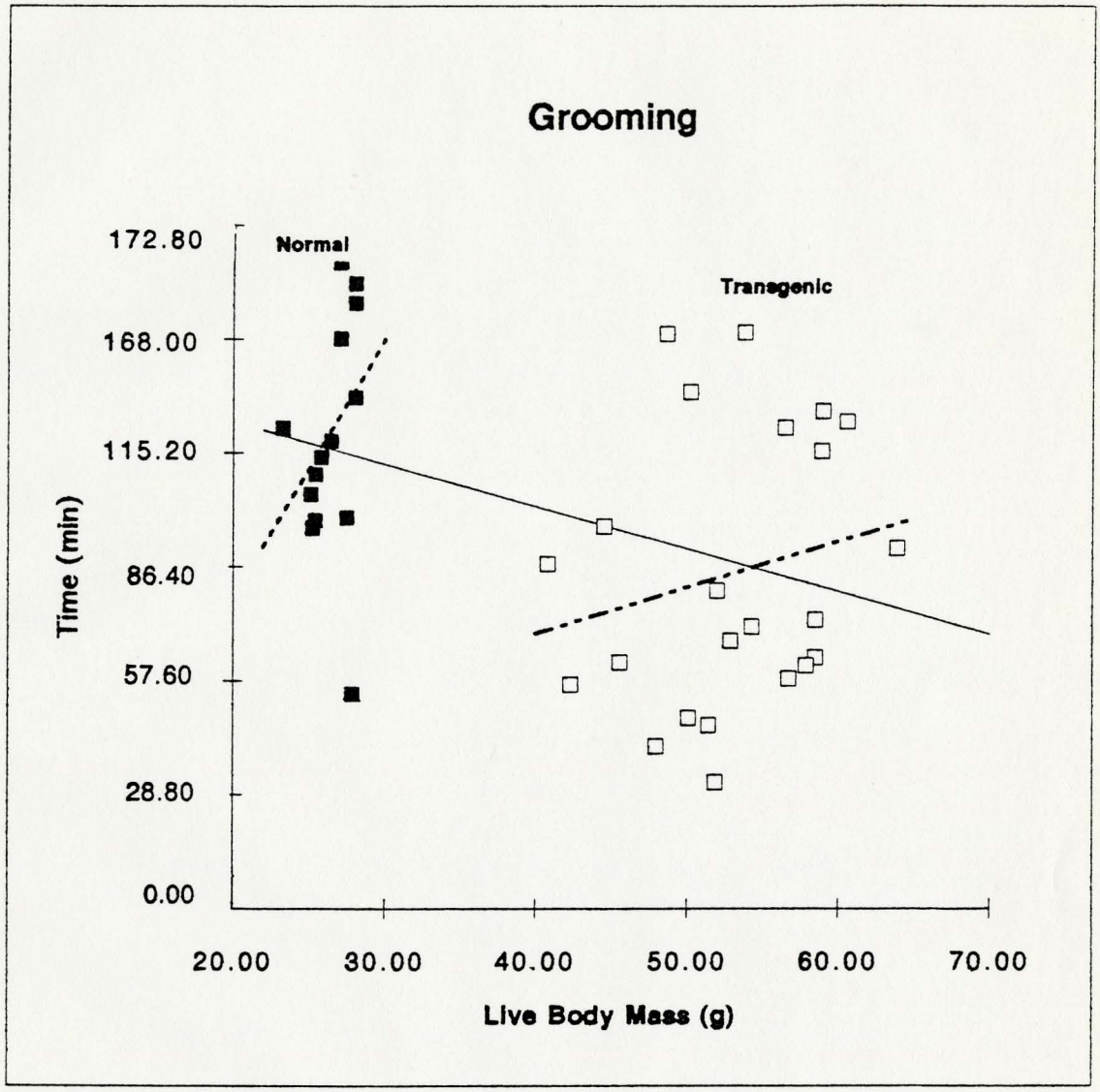


Figure 9. Graph of total time in minutes spent grooming, over 24 hours versus live body mass, for pooled normal and transgenic mouse data. Standard error bars are omitted to avoid visual confusion.

Regression equation (pooled data) $y = -.00075 x + 144.73$ ($r^2 = .16$, $p < .002$, $n=32$)

Regression equation (normal data) $y = .0046 x - 53.1$ ($r^2 = .10$, n.s. , $n=15$)

Regression equation (transgenic) $y = .00081 x + 22.97$ ($r^2 = .04$, n.s. , $n=23$)

Bibliography

Atkinson, D. 1985. Information, non-genetic constraints, and the testing of theories of life-history variation. In: *Behavioural Ecology: Ecological Consequence of Adaptive Behaviour.* Boston. Massachusetts. ed. R.M. Sibly and R.H. Smith. pg. 99-104.

Barnett, S.A., A.L. Cockcroft and J.L Smart. 1966. An artificial habitat for recording movement. J. Physiol. 187:15-16.

Bartke, A., R.W. Steger, S.L. Hodges, T.A. Parkening, T.J. Collins, J.S. Yun and T.E. Wagner. 1988. Infertility in transgenic female mice with human growth hormone expression: evidence for luteal failure. J. Exp. Zool. 248: 121-124.

Bishop, J.O. and R. Al-Shawi. 1989. Gene expression in transgenic animals. In: *Evolution and Animal Breeding.* C.A.B. International. Wallingford. United Kingdom. ed. W.G. Hill and T.F.C. Mackay. Pg 249-260.

Blank, J.L. and C. Desjardins. 1985. Differential effects of food restriction on pituitary-testicular function in mice. Am J. Pathol. 248:181-189.

Brem, G., R. Wanke, E. Wolf, T. Buchmüller, M. Müller, B. Brenig and W. Hermanns. 1989. Multiple consequences of human growth hormone expression in transgenic mice. Mol. Biol. Med. 6:531-547.

Campbell, R.G., N.C. Steele, T.J. Caperna, J.P. McMurtry, M.B. Solomon and A.D. Mitchell. 1988. Interrelationships between energy intake and endogenous porcine growth hormone administration on the performance, body composition, and protein and energy metabolism of growing pigs weighing 25 to 55 kilograms live weight. J. Anim. Sci. 66:1643-1655.

Calow, P. and C.R. Townsend. 1981. *Physiological Ecology: An Evolutionary Approach to Resource Use.* Sunderland, Massachusetts. Sinauer.

Collier, G.H, D.F Johnson, K.A. CyBulsk and C.A. McHale. 1990. Activity patterns in rats (*Rattus norvegicus*) as a function of the costs of four resources. J. Comp. Psychol. 104 (1):53-65.

Doi, T., L.J. Striker, C. Quaife, F.G. Conti, R. Palmiter, R. Behringer, R. Brinster and G.E Striker. 1988. Progressive glomerulosclerosis develops in transgenic mice chronically expressing growth hormone releasing factor but not those expressing insulin-like growth factor-I. Am. J. Pathol. 131(3):398-403.

Drucker-Colín, R.R., C.W. Spanis and J.A. Rojas-Ramírez. 1975. Investigation of the role of proteins in REM sleep. In: *Neurobiology of Sleep and Memory.* New York. Academic Press. ed. R.R. Drucker-Colín and J.L. McGaugh. pg 303-319.

Ehrman, L. and P.A. Parsons. 1976. The genetics of behavior: Drosophila. In: *The Genetics of Behavior*. Sunderland. Massachusetts. Sinauer Associates Inc. pg 161-184.

Eklund, J. and G.E. Bradford. 1977. Longevity and lifetime body weight in mice selected for rapid growth. Nature 265:48-49.

Fowler, K. and L. Partridge. 1989. A cost of mating in female fruitflies. Nature 338:760-761.

Fuller, J.L. and W.R. Thompson. 1967. *Behavior Genetics*. New York. John Wiley and Sons.

Gilbert, S.E. 1992. *Developmental Biology*. Sunderland, Massachussetts. Sinauer Associates.

Golde, D.W., J.E. Groopman, H.R. Hershman, and A.J. Lusis. 1980. Growth Factors. Ann. Int. Med. 92:650-662.

Gordon, J.W. and F.H. Ruddle. 1981. Integration and stable germline transmission of genes injected into mouse pronuclei. Science 214:1244-1246.

Green, E.L. 1966. Breeding systems. In. *Biology of the Laboratory Mouse*. New York. McGraw-Hill.

Guillot, A. 1981. Étude compartmentale des périodes d'activité ultradiennes des souris OF1 placées en cages expérimentales de dimensions différentes. C.R. Soc. Biol. 175:295-302.

Guise, K.S., A.A.R. Kapuscinski, P.B. Hackett Jr. and A.J. Faras. 1988. Gene transfer in fish. In: *Transgenic Animals: Proceedings of the Symposium on Transgenic Technology in Medicine and Agriculture*. Toronto. Butterworth-Heinemann. ed. N.L. First and F.P. Haseltine. pg 295-306.

Hammer, R.E., R.L. Brinster, M.G. Rosenfeld, R.M. Evans and K.E. Mayo. 1985. Expression of human growth hormone-releasing factor in transgenic mice results in increased somatic growth. Nature: 413-416.

Hammer, R.E., V.G. Pursel, C.E. Rexroad, R.J. Wall, D.J. Bolt, K.M. Ebert, R.D. Palmiter and R.L. Brinster. 1985. Production of transgenic rabbits, sheep, and pigs by microinjection. Nature 315:680-683.

Havlicek, V., M. Rezek and H. Friesen. 1976. Somatostatin and thyropin releasing hormone: central effect on sleep and motor system. Pharmacol. Biochem. Behav. 4:455-459.

Horne, J.S. 1988. *Why We Sleep*. New York. Oxford University Press.

Jobin, R.M.J. 1988. Effect of growth enhancement on rainbow trout: an ecological assessment of the potential impact of genetic engineering on organism design. M.Sc. thesis. McMaster University.

Jones, W.T. 1985. Body size and life-history variables in heteromyids. J. Mamm. 66:128-132.

Kavanau, J.L. 1962. Automatic multichannel sensing and recording of animal behaviour. Ecology 43:161-166.

Kavanau, J.L. 1963. Continuous automatic monitoring of the activities of small captive animals. Ecology 44:9

King, J.A. 1968. Psychology. In: *Biology of Peromyscus (Rodentia)*. Lawrence, Kansas. American Society of Mammalogists. ed. J.A King. pg 496-542.

Kleitman, N. 1963. *Sleep and Wakefulness*. Chicago. University of Chicago Press.

Lessells, C.M. 1991. The evolution of life histories. In: *Behavioural Ecology: An Evolutionary Approach*. London. Blackwell Scientific Publishing. ed. J.R. Krebs and N.B. Davies. pg. 32-65.

MacArthur, J.W. 1949. Selection for small and large body size in the house mouse. Genetics. 34:194-209.

Mathews, L.S., R.E. Hammer, R.L. Brinster and R.D. Palmiter. 1988. Expression of insulin-like growth factor I in transgenic mice with elevated levels of growth hormone is correlated with growth. Endocrinology 123:433-437.

Mayer, J. 1953. Decreased activity and energy balance in the hereditary obesity-diabetes syndrome in mice. Science 117:504-505.

Mayer, J., N.B. Marshall, J.J Vitale, J.H. Christensen, M.B. Mashayekhi and F.J. Stare. 1954. Exercise, food intake and body weight in normal rats and genetically obese adult mice. Am. J. Physiol. 177:544-548.

Miller K.F., D.J. Bolt, V.G. Pursel, R.E. Hammer, C.A. Pinkert, R.D. Palmiter and R.L. Brinster. 1989. Expression of human or bovine growth hormone gene with a mouse metallothionein-1 promoter in transgenic swine alters the secretion of porcine growth hormone and insulin-like growth factor-I. J. Endocrin. 120:481-488.

Naar, E., A. Bartke, S.S Majumdar, F.C. Buonomo, J.S. Yun and T.E. Wagner. 1991. Fertility of transgenic female mice expressing bovine growth hormone or human growth hormone variant genes. Biol. of Repr. 45:178-187.

Orian, J.M., C.S. Lee, L.M. Weiss and M.R. Brandon. 1989. The expression of a metallothionein-ovine growth hormone fusion gene in transgenic mice does not impair fertility but results in pathological lesions in the liver. 1989. Endocrin. 124:455-463.

Palmiter, R., R.L. Brinster, R.E. Hammer, M.E. Trumbauer, M.G. Rosenfeld, N.C. Birnberg and R.M. Evans. 1982. Dramatic growth of mice that develop from eggs microinjected with metalothionein-growth hormone fusion genes. Nature 300:611-615.

Palmiter, R., G. Norstedt, R.E. Gelinas, R.E. Hammer and R.L. Brinster. 1983. Metallothionein-human GH fusion genes stimulate growth in mice. Science 222:809-812.

Partridge, L. and P. Harvey. 1985. Costs of reproduction. Nature. 316:20.

Partridge, L. and P. Harvey. 1988. The ecological context of life history evolution. Science 241:1449-1455.

Partridge, L. and M. Farquhar. 1981. Sexual activity reduces lifespan of male fruitflies. Nature 294:580-582.

Perrigo, G. 1987. Breeding and feeding strategies in deer mice and house mice when females are challenged to work for their food. Anim. Behav. 35:1298-1316.

Pianka, E.R. 1983. *Evolutionary Ecology*. New York, New York. Harper and Row.

Pursel, V.G, C.A. Pinkert, K.F. Miller, D.J. Bolt, R.G. Campbell, R.D. Palmiter, R.L. Brinster and R,E Hammer. 1989. Genetic Engineering of Livestock. Science 244:1281-1288.

Pursel, V.G., D.J. Bolt, K.F. Miller, C.A. Pinkert, R.E. Hammer, R.D. Palmiter and R.L. Brinster. 1990. Expression and performance in transgenic pigs. J. Rep. Fert. Suppl 40:235-245.

Quaife, C.J., L.S. Mathews, C.A. Pinkert, R.E. Hammer, R.L. Brinster, and R.D. Palmiter. 1989. Histopathology associated with elevated levels of growth hormone and insulin-like growth factor I in transgenic mice. Endocrin. 124:40-48.

Rexroad, C.E., R.E. Hammer, D.J. Bolt, K.E. Mayo, L.A. Frohman, R.D. Palmiter and R.L. Brinster. 1989. Production of transgenic sheep growth regulatory genes. Mol. Reprod. and Develop. 1:164-169.

Reznick, D. 1992. Cost of reproduction - an evaluation of the empirical evidence. Oikos 44:257-267

Reznick, D. 1992. Measuring costs of reproduction. Trends Ecol. Evol. 7:42-45.

Rezek, M., and V. Havlicek, K.R. Hughs and H. Friesen. 1976. Cortical administration of somatostain (SRIF): effect on sleep and motor behavior. Pharmacol. Biochem. Behav. 5:73-77.

Robinson, D.W. and G.E. Bradford. 1969. Cellular response to selection for rapid growth in mice. Growth 33:221-229.

Rollo, C.D. 1984. Resource allocation and time budgeting in adults of the cockroach *Perplaneta americana*: the interaction of behaviour and metabolic reserves. Res. on Pop. Ecol. 26:150-187.

Rollo, C. D. 1986. A test of the principle of allocation using two sympatric species of cockroaches. Ecology 67 (3):616-628.

Rollo, C. D. 1988. Compensatory scope and resource allocation in two species of aquatic snails. Ecology 69: 146-156.

Rose, M.R. and B. Charlesworth. 1981. Genetics of life history in *Drosophila melanogaster*: I sib analysis of adult females. Genetics 97:173-186.

Rose, M.R. and B. Charlesworth. 1981. Genetics of life history in *Drosophila melanogaster*: II exploratory selection experiments. Genetics 97:187-196.

Schachter, S. 1971. Some extraordinary facts about obese humans and rats. Am. Psychol. 26:129-141.

Sibly, R. and P. Calow. 1983. An integrated approach to life-cycle evolution using selective landscapes. J. Theor. Biol. 102:527-547.

Sibly, R. and P. Calow. 1986. Why breeding earlier is always worthwhile. J. Theor. Biol. 123:311-319.

Shea, B.T., R.E. Hammer and R.L. Brinster. 1987. Growth allometry of the organs in giant transgenic mice. Endocrinology. 121:1924-1930.

Shea, B.T., R.E. Hammer, R.L. Brinster and M.R. Ravosa. 1990. Relative growth of the skull and postcranium in giant transgenic mice. Gen. Res. 56:21-34.

Taylor, B.E. and W. Gabriel. 1992. To grow or not to grow: optimal resource allocation for *Daphnia*. Am. Nat. 139:248-266.

Theissen, D.D. 1961. Mouse exploratory behaviour and body weight. Psychol. Rec. 11:299-304.

Toates, F.M. 1980. *Animal Behavior: A Systems Approach.* Toronto. John Wiley and Sons.

Tuomi, J., T. Hakala and E. Haukioja 1983. Alternative concepts of reproductive effort, costs of reproduction, and selection in life history evolution. Am. Zool. 23:25-34.

Wall, R.J., D.J. Bolt, W.I. Frels, H.W Hawk, D. King and V.G. Pursel. 1990. Transgenic farm animals: Current state of the art. AgBiotech News and Information 2:391-395.

Wheeler, D.A, C.P. Kyriacou, M.L. Greenacre, Q. Yu, J.E. Rutila, M. Rosbash and J.C. Hall. 1991. Molecular transfer of a species-specific behaviour from *Drosophila simulans* to *Drosophila melanogaster*. Science 251:1082-1085.

Wilkie, T.M., R.L Brinster and R.D. Palmiter. 1986. Germline and somatic mosaicism in transgenic mice. Develop. Biol. 118:9-18.

Wolf, E., K. Rapp and G. Brem. 1991. Expression of Metallothionein-human growth hormone fusion genes in transgenic mice results in disproportionate skeletal giantism. Growth, Develop. and Aging 55:117-127.

Wolf, E., R. Wanke, W. Hermanns, G. Brem, F. Pirchner and I. von Butler-Wenken. 1991. Growth characteristics of metallothionein-human growth hormone transgenic mice as compared to mice selected for high eight week body weight and unselected controls: I. Body weight gain and external body

dimensions. Growth Develop. Aging 55:225-235.

Wolf, L.L. and F.R. Hainsworth. 1971. Time and energy budgets of territorial hummingbirds. Ecology 52:980-988.

Zeplin, H. and A. Rechtshaffen. 1974. Mammalian sleep, longevity, and energy metabolism. Brain Behav. Evol. 10:425-470.