

**EFFECT OF HABITAT FRAGMENTATION ON FOOD HABITS, INTESTINAL PARASITES AND ASPECTS OF REPRODUCTION AMONG *Praomys delectorum* SUB-POPULATIONS IN THE TAITA AND KYULU HILLS, KENYA //**

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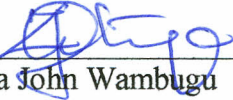
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**DECLARATION**

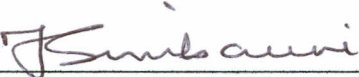
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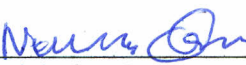
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## **DEDICATION**

This thesis is dedicated to Sister Rindi and my family for their perseverance, love and understanding and also to those who are eager to serve others selflessly. May their struggles always be rewarded.

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## ABSTRACT

Due to their short life histories, small mammals are useful indicators of environmental health and fauna diversity. Despite this, little or no investigations on the effects of habitat modification on small mammals' diets in Kenya has been done. This study investigated the effect of habitat fragmentation on food habits and litter size of *P. delectorum* in three sub-populations of the Taita Hills and the Kyulu Hills population. Response of the forest-dependant *Praomys delectorum* to anthropogenic disturbance in different forest patches of the Taita Hills suggests that it is an opportunistic omnivore as its population increases with increase in vegetation interdigitation. Food habits variation was assessed by comparing morphometry of the gastrointestinal tract while foetuses and placental scars were used as litter size indicators. The study also gave some information on intestinal parasites, and the histology of testes and ovary based on routine histological techniques. Total intestinal length was significantly correlated with body mass ( $r=0.624$ ,  $P<0.001$ ) and head plus body length ( $r=0.722$ ,  $P<0.001$ ), respectively. The linear response of total intestine length to head plus body length was greater ( $\beta = 0.642$ ,  $t = 5.951$ ,  $P < 0.001$ ) than that of body mass ( $\beta = 0.214$ ,  $t=1.983$ ,  $P=0.053$ ) suggesting it is a better covariate in removing size effect. A significant difference ( $F=2.883^*$ ,  $P= 0.043$ ) in the relative length of large intestine was noted among the sub-populations suggesting variation in food quality. There was no significant difference in litter size ( $F_{3,15} = 0.126$  <sup>ns</sup>  $P =0.943$ ) among the different sub-populations. Prominent nuclei of primary spermatocytes in the seminiferous tubules of both abdominal and scrotal testes were indicative of spermatogenesis though germ cells organization was clearer in scrotal testes. The ovary of female with vagina closed lacked corpora lutea which were nonetheless observed in the ovary of females with vagina open though developing Graafian follicles were observed in both. Thus vaginal condition is a good indicator of reproductive status in this species. Percentage prevalence of intestinal parasite conformed to the trends of anthropogenic disturbance among the Taita Hills sub-populations although Kyulu Hills population had the highest (63.16%) infestation. Information of this kind is essential in building a complete biological picture of *Praomys delectorum* like litter size and the intestinal parasites that infect it. *Praomys delectorum* display a digestive tract adaptation suggestive of an opportunistic feeder. This may have been due to change in food habits that could be associated with transformation of natural habitat into fragments.

**ABBREVIATIONS**

<b>BM</b>	-	Body mass
<b>C</b>	-	Caecum
<b>DPX</b>	-	Dextrane Plastiane Xylene
<b>FSH</b>	-	Follicle Stimulating Hormone
<b>GI</b>	-	Gastrointestinal
<b>GIT</b>	-	Gastrointestinal Tract
<b>HB</b>	-	Head plus Body length
<b>LH</b>	-	Luteinizing Hormone
<b>LI</b>	-	Large Intestine
<b>L.S.</b>	-	Longitudinal Section
<b>NE</b>	-	Number of Embryos
<b>RS</b>	-	Reproductive Status
<b>S</b>	-	Stomach
<b>SI</b>	-	Small Intestine
<b>TA</b>	-	Testes Abdominal
<b>TI</b>	-	Total Intestine
<b>TS</b>	-	Testes Scrotal
<b>T.S.</b>	-	Transverse Section
<b>VC</b>	-	Vagina Closed
<b>VO</b>	-	Vagina Open
<b>g</b>	-	grams
<b>cm</b>	-	centimetre

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background Information

Habitat fragmentation is among the most serious threats to biological diversity, as determined by a consensus of conservation biologists (Cameron, 1994). Effects of habitat fragmentation on biodiversity are very diverse with different authors measuring fragmentation in different ways and as a consequence drawing different conclusions regarding both the magnitude and direction of its effects (Lenore, 2003). Fragmentation is usually defined as a landscape scale process involving habitat loss and division of the natural habitat into progressively smaller patches of the total area isolated from each other by a matrix of habitat unlike the original (Lenore, 2003; Cameron, 1994).

Anthropogenic-induced deforestation and disturbance is almost phenomenal to habitat fragmentation. Since the beginning of the agrarian society, indigenous people have always harvested the forest to raise their crops. In the past, deforestation was at a low scale where the small patches burned or slashed would regenerate upon abandonment as opposed to the current trends where remnant forest patches are left in a sea of severely altered and degraded landscape (Laurance and Bierregaard, 1997; Laurance, 1991).

Habitat fragmentation not only reduces the area of habitat but also can isolate populations and increase edge effect. Understanding the possible consequences of habitat fragmentation has become a great concern to conservation biologists, since almost all natural habitats have become fragmented to an extent (Laurence, 1991).

## 1.2 Response of Small Mammals to Habitat Fragmentation

Small mammals, especially rodents, are opportunistic feeders capable of changing their feeding habits depending on availability of food. This is an evolutionary adaptation to regulate their density during post-disturbances and to restrict competition with others (Bekele and Leirs, 1997). Due to their sensitivity to change in the environment such as ground cover and food resource base, rodents are potentially useful indicators to changes in the local environmental conditions such as habitat modifications caused by man (Kuhnelt, 1976).

Researches on habitat fragmentation have identified several indicators as predictors of response of animals to habitat disturbances. Traits like population size, population fluctuation and storage, dispersal power, reproduction potential, annual survival, sociality, body size, trophic position, ecological specialization, microhabitat and matrix use, disturbance and competition sensitivity traits have been identified (Henle *et al.*, 2004).

*Mastomys*, one of the commonest and most widely spread rodents in Africa is known to flourish on land that has been recently burnt or cultivated (Mugatha, 2002). *Mastomys* is a common agricultural pest and important vector of diseases such as plague and lassa-fever. Extensive surveys have shown that it is commonly found in association with human populations (Delany, 1964). *Praomys jacksoni* was found in highest densities in presently grazed areas and in certain microhabitats of Mount Elgon, (Cameron *et al.*, 1996). Studies on ecosystem management practices and human plague problem in

Tanzania associated forest degradation with the wild rodent habitat degradation. This has resulted in creating the centripetal movement of the wild rodents to human settlements and their surroundings (Schemdoe, 2004).

Many species of rodents in Africa's forests are important consumers of seeds, seedling and insects (Delany, 1975; Kasenene, 1980 and 1984; Lwanga, 1994 and Basuta, 1979). The potential impact of rodents on the survivorship of seed and seedling population of trees is very great and is thought to play a major role on forest dynamics and regeneration (Struhsaker, 1997; Kasenene, 1980, 1984; Lwanga, 1994). If the biodiversity of our planet was to be effectively conserved in general and in particular tropical rain forests, then a far greater effort and commitment must be made towards establishing many large wilderness areas that are given protection (Struhsaker, 1999). The greater density of rodents due to logging can persist for decades and perhaps longer. The rodents in turn are likely to have a major negative impact on seed and seedling survival which will contribute to suppression of forest regeneration (Struhsaker, 1999).

### **1.3 Problem Statement and Justification**

Anthropogenic disturbance has resulted in isolation of *Praomy delectorum* into different sub-populations in forest habitats of the Taita Hills. *Praomys delectorum* population densities vary positively with forest disturbance. It is generally assumed that opportunistic species, particularly pest, would increase with deteriorating habitat conditions while specialized non-pest species decrease (Primark, 1993). The local extinction of individual species starting with the most vulnerable ones changes the

composition of trophic levels and that of the functional relations within ecosystems. Removal of particular key species can have cascade effects (Frankham *et al.*, 2002). Habitat fragmentation is known to cause such extinction. This study focuses on variation on morphometry of the GIT in the isolated *P. delectorum* sub-populations. Of particular interest are variations which could have resulted due to change in feeding habits in the different sub- populations in the Taita Hills and Kyulu population. A correlation between the total intestine length and body size was also quantified.

While a body of literature on laboratory experiments that are designed to find differences in intestine morphology due to differences in ambient temperatures and food (quality and quantity) has grown considerably, evidence of the validity of the results from natural population remains scarce (Korn, 1991). This study hypothesizes that the transition from high-energy protein grain and insect diet to low energy high cellulose diet of vegetation parts or vice versa could result in the modification of the alimentary canal.

The study also investigates the litter size by making a comparison of the Taita Hills sub-populations with the Kyulu population. There is little or no information on aspects of the reproductive potential of the *P. delectorum*. The histology of the testis and ovary plus the helminth infections of different groups were investigated. A study of this kind is essential in building a complete picture of any species' basic biological information.

Small mammals are integral components of the ecosystem and vectors of human diseases and can turn out to be pests especially when humans encroach into their natural habitats;

yet there is enormous deficiency of information on rodents inhabiting Kenyan forests. No surveys concerned with montane environment in East Africa have concentrated upon small mammals microhabitat requirements nor have any investigated the effect of habitat modification by humans upon small mammals (Cameron *et al.*, 1996).

#### 1.4 Research Questions

- i. How does the length of the gastro-intestinal tract (GIT) vary between the *P. delectorum* sub- populations in the Taita and Kyulu Hills forests?
- ii. What is the relationship between GIT length and body sizes in *P. delectorum*?
- iii. What is the relationship between the number of embryos observed in *P. delectorum* and population densities?
- iv. How does the intestinal parasite infection vary among the different sub-populations?
- v. What is the microscopic status of ovarian and testicular sections in animals described as vagina closed (VC) or open (VO), and testes abdominal (TA) or scrotal (TS), respectively?

#### 1.5 Null Hypotheses

- i. There is no variation in the mean length of the gastro-intestinal tract in the *P. delectorum* sub-populations.
- ii. There is no relationship between the GIT length and body size among the *P. delectorum*

- iii. Variations in population densities of the *P. delectorum* are not as a result of variations in the litter size.
- iv. Intestinal parasite infection does not vary among the different sub-populations of *P. delectorum*.
- v. There is no variation in the histology of ovaries from females VC and VO and histology of testes from males TA and TS.

## 1.6 Objectives

### 1.6.1 General Objective

To determine the extent of variation in food habits due to anthropogenic disturbance on the digestive tract morphometry , some aspects of reproduction and intestinal parasites infection in *Praomys delectorum*.

### 1.6.2 Specific Objectives

- i. To determine the mean length of the GIT in the isolated sub-populations of *P. delectorum*.
- ii. To determine the relationship between GIT length and body size in *P. delectorum*.
- iii. To characterize litter sizes among the *P. delectorum* sub-populations.
- iv. To quantify the infection of the *P. delectorum* by intestinal parasites among the Taita Hills sub-populations and kyulu population.
- v. To study the histology of testes TA and TS males and ovaries of VC and VO females.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Digestive Tract Morphological Adaptation

The acquisition and effective processing of food energy is critical to the survival and reproductive success of animals (Karasov, 1986; Derting and Bouge, 1993). Digestive tract morphology can affect digestive efficiency and is closely related to food habits. Differences in food habits among mammals are often reflected in the structure of their alimentary canal (Ellis *et al.*, 1994). Variation in intestine length within a species is expressed as adaptation to changes in quantity and quality of food in relation to energy demands of the animals (Myrcha, 1965; Green and Millar, 1987; Hansson and Jaarola, 1989). Changes in the morphology of the gastrointestinal tract may be subtle and could well involve changes in physical length and/or mass and also changes in the physical function (Cranford *et al.*, 2000).

There is accumulating evidence that the functional size of organs and aspects of the metabolic physiology of an individual may show great flexibility over time scales of weeks and even days depending on the physiological status, environmental conditions and behavioral goals. The flexibility is a way of animals to cope successfully with a much wider range of conditions occurring during various life cycle events than fixed metabolic machinery would allow (Piersma and Lindstrom, 1997). The structure of the gastrointestinal tract (GIT) is fairly homogenous among different orders of mammals (Chivers and Hladik, 1980) and the development of different parts of the GIT reflects adaptations to different food substances (Bruoton and Perrin, 1991; Wilczynska, 1999).

Vorontsov (1962) argued that the evolutionary transitional trends of gut morphology for rodent species indicates that changes from feeding on high-energy, high-protein and high-lipid diets of seeds and small invertebrates to a low-energy, high cellulose diet of vegetative parts of plants resulted in several evolutionary modifications in digestive tracts of muroid rodents.

Alimentary tract measurements have been used as indicators of dietary factors in small mammals (Schieck and Millar, 1985). Increase in energy demand had minimal effect on the meadow voles (*Microtus pennsylvanicus*) GIT tissue mass but resulted in a slight increase in length in all tissues except the small intestine (Cranford *et al.*, 2000). Under extreme environmental conditions (high dietary fibre), prairie voles (*Microtus ochrogaster*) exhibited increased GIT tissue mass and length (Hammond and Wunder, 1991).

## **2.2 Effects of Food Habits on GIT Morphology**

The tissues of the gastrointestinal tract have been identified as most responsive to changes in energy demands. This could be due to increased thermoregulatory costs (Gross *et al.*, 1985), low quality diet (Hammond and Wunder, 1991) or high reproductive expenditure (Barnett, 1973).

Although there seems to be very little research on the physiology of *Praomys delectorum* documented, numerous intra-specific and inter-specific research on the morphology and morphometric modification of GIT have been reported in other animals. Several species

of birds, fish, insects, mammals and reptiles can change the length of their gut in response to changes in food quality and quantity (Starck, 1994; Piersma and Lindstrom, 1997).

Ontogenetic changes in the gut morphology and digestive enzyme activity in the characid fish *Brycon guatemalensis* from the Costa Rican rain forest stream has been investigated (Drew *et al.*, 2004). *Brycon guatemalensis* consumes a terrestrial diet shifting from eating insects as juvenile to fruits and leaves as adults. The overall results support the view that *B. guatemalensis*, is specialized morphologically and biochemically to function first as a carnivore and then as a herbivore during its life history as the larger-sized fish have longer guts (Drew *et al.*, 2004).

Many researchers (Drobney, 1984; Pulliainen, 1976 and Kehoe *et al.*, 1998) point at the great dependence of birds' caeca lengths on the kind of food they feed on. Particularly with reference to representatives of the order Galliformes, a distinct positive correlation of length of those organs with the amount of fibre in their diet was revealed (Drobney, 1984). Miller (1974) suggested that even in water birds, caeca lengths are also related to the content of fibre in the diet. Relative small sizes of caeca of the black scoter may be related to the diet of this species (Dzialska-Szczepanczyk and Betlejewski, 2003). Taking more food may be connected to the increased demand of the birds' intestines. Such a hypothesis was formulated by Pulliainen (1976) studying wintering willow grouse (*Lagopus lagopus*). He stated that lighter females of this species and young individuals had relatively longer small intestines and caeca than heavier birds. No such regularity was found in the Mallard *Anas platyrhynchos* – the differences in the lengths and weights

of caeca between young individuals and adults of these species were statistically insignificant (Dzialska – Szczepanczyk, 2002).

Studies on the digestive tract morphology and food habits of six species of rodents were carried out by Wang *et al.* (2002). They observed that the strict herbivore *Microtus brandti* showed the longest large intestine and caecum, which was consistent with their prediction. The small intestine does not reflect the dietary fibre contents and thus is not a good indicator for food habits. The hindgut is more important for herbivorous than for omnivorous rodents and could be a relative reliable indicator of food habits. They also noted that the gut sizes of rodents change with seasons, food quality, temperature, reproductive status and other internal and/or external factors (Wang *et al.*, 2002).

Numerous researches on intraspecific morphology and morphometric changes on the GIT due to diet and other external factors have been done on small mammals particularly in the voles (*Microtus*). Food shortage is normally interpreted as relative lack of food in the environment but during some seasons, the rate of food assimilation can be more important (Wunder, 1978). The small rodents are likely limited by the ability of the digestive tract to process food fast enough to satisfy both thermoregulatory and reproductive requirements (Wunder, 1978; Peterson and Baumgardt, 1971). The number of surviving offsprings determines one aspect of how well a species is adapted to its environment, therefore traits which promote reproductive performance like nutrient needs should be selected for. The understanding of how animals adapt to increased nutrient needs is a question fundamental to animal ecology.

Intra-specific gut length variation relates to seasonal changes in quality and composition of food in some species. Such phenotypic plasticity is thought to adjust 'gut' structure and function to seasonal differences in nutrition and to maintain optimized gut function under differing feeding regimes (Starck, 2003). In mammals and birds, intestinal flexibility is based on a balance of cell proliferation and cell loss (Starck, 2003). Intra-specific comparison and the inferences drawn from them are more sensitive to small differences than are inter-specific ones; the Brambell method is less influenced by variability compared to the Leopold's methods (Freehling and Moore, 1987).

There have been contrasting views over whether the increase in digesta content in the alimentary canal is as a result of availability or quality of diet. McPherson *et al.* (1988) explored seasonal and habitat variation in the diet of pine voles (*Microtus pinetorum*) in four apple orchards. They reported that stomach content mass declined in winter and suggested that this was related to the winter decreases in food availability and they further suggested that if the mass of the stomach contents had increased, it would reflect an increase in the availability of winter forage and therefore an increase in food intake. However, Hammond (1993) suggested that higher stomach content mass due to increased food intake actually reflects a decrease in forage quality. This latter explanation may be more likely because research findings by Cranford *et al.* (2000) on meadow voles and other studies on voles' food intake and mass of gastrointestinal (GI) contents increased when the animals were fed on low quality diet (Hammond and Wunder, 1991). Increase

in content mass is sometimes accompanied by increase in length in some GIT tissues under conditions of high-energy demand (Hammond and Diamond, 1992).

Studies by Korn (1991) on the intestine length variation in small mammals reported significant differences in *Aethomys chrysophilus* a herbivore granivore species with relative longer intestines in the wetter seasons. In the European woodland rodents *Clethrionomys glareolus* and *Apodemus sylvaticus* reproductively active female have longer intestines than non-reproductive females and males due to higher energy demands. Pregnant or lactating females also accounted for significant differences between the sexes in *Aethomys chrysophilus* and *Aethomys namaquensis* since breeding females also tended to have longer intestines ( Korn, 1991).

### **2.3 Reproductive Response to Nutritional Value of Food**

Variation in the nutritional value of the diet, other than causing morphological change in the GIT due to energy demand of an animal and the body size, also affects fertility by influencing litter size in rodents (Starck, 1994; Waweru and Odanga, 2003). The type of food and its availability is a key environmental factor that influences fertility. Short-term increase in nutrients in larger mammals can stimulate ovulation (Scaramuzzi and Khalid, 2004). While the nutrition effects have been well described, the physiological mechanisms that underlie them are poorly understood (Scaramuzzi and Khalid, 2004). According to Scaramuzzi and Khalid (2004), glucose is a critical metabolic fuel that influences ovulation in mammals. They demonstrated that there is significant uptake of glucose by the ovary in both the follicular and luteal phases of the oestrus cycle and that

the administration of gluconeogenic amino acids to experimental animals increases insulin concentration and stimulate folliculogenesis (Scaramuzzi and Khalid, 2004).

Reproductive response of rodent 'mothers' to variation in dietary protein intake has been studied. The result demonstrated that typical levels of reproduction extended over a narrower range of dietary protein content for *Mastomys coucha* than *M. natalensis* females (Jackson and Van Aarde, 2003). Only *M. natalensis* females bred on 6% protein diets while on 20% protein diet the reproductive output of *M. coucha* was lower than a diet containing 10 – 15% protein. *Mastomys natalensis* responded to low protein diet by reducing litter size and litter mass (Jackson and Van Aarde, 2003).

In an intraspecific study on cotton rats *Sigmodon hispidus*, Derting, (1989) noted that the basal metabolic rate (BMR) was related to the reproduction rate and the author concluded that if food energy is unlimited, increases in basal metabolic rate are associated with increased reproduction rates, at least on a short-term basis. Therefore the hypothesis that increased rate of basal metabolism is associated with increased rates of reproduction must be accepted at the individual level.

#### **2.4 Food Habits and the Reproductive Potential of Murid Rodents**

In small mammals particularly rodents, selection generally favours the production of relatively many offspring's per litter (Eisenberg, 1981). Intraspecific litter size variation requires individual mothers to establish an optimal litter size in terms of fitness consequences (Charnov and Krebs, 1974). Because mammalian reproduction is

energetically very demanding (Loudon and Racey, 1987), litter size decisions are influenced by the maternal condition and environmental factors. Environmental constraints associated with the availability and quality of food during pregnancy and lactation may limit the acceleration in energy expenditure that occurs during reproduction, which may in turn negatively affect reproductive success of small mammals (Veloso and Bozinovic, 2000).

Ground water can affect reproduction in rodents by generating plant growth (rodent food) and by softening the earth so that rodents can dig burrows (Taylor and Green, 1976). Food quality appears to determine the reproductive performance of small mammals (McClure, 1987). Reproduction in *Mastomys natalensis* (and perhaps other rodent species) is related to rainfall. Breeding in *Mastomys natalensis* occurred at much the same time of the year as in *Arvicanthis niloticus* although it tended to start somewhat later, when cereals first became available. There was no detectable switch in diet in *Mastomys natalensis* when breeding ceased toward the middle of the dry season, although more insects and green stuff were eaten then, as opposed to weed seeds and cereals. *Rhabdomys pumilio* also bred when cereals were plentiful but a breeding population was discovered in a grass/clover ley where the animals were feeding mainly on clover, a crop noted for its high crude protein content (Taylor and Green, 1976).. *Otomys tropicalis* fed almost entirely on green stuff-predominantly grass and bred at all times of the year, but with more reproduction taking place during the rainy months, when vegetation was more lush (Taylor and Green, 1976). Studies on population dynamics of the grass rat *Arvicanthis niloticus* showed that seeds dominated the diet at the time of

maximum reproduction and when the seeds declined, reproduction also declined (Delany and Monro, 1986).

Female rodents use various compensatory mechanisms to cope with reproductive demands. For example, *Mus musculus* increases uptake at intestinal mucosa during lactation (Hammond and Diamond, 1992). Theoretically, an upper limit may eventually be reached beyond which food intake cannot meet energy requirements because of physiological and structural constraints. In such cases, individuals use different strategies that affect survival and growth rate including changes in the length of pregnancy and lactation and in litter size or body mass of the young (Iverson *et al.*, 1993).

## 2.5 Reproductive Activities

Documentation on breeding habits of *Praomys delectorum* in natural habitats or in captivity seems to be scarce. Other species of the genus studied show that breeding occurs throughout the year. *Praomys tullbergi* breeds throughout the year with one peak at the end of the main dry season and at the beginning of the wet season (February to April), and another during the secondary dry season that stretches between October and November, according to Happold (1987). Litter size is usually three to four (3 – 4) young although litter sizes of up to six have been recorded (Happold, 1987). Adult females may have several litters in rapid succession. *Praomys jacksoni* is thought to breed throughout the year in Uganda (Delany, 1975). Studies of captive *Praomys jacksoni* indicate that gestation lasts for a period of 26 – 27 days. The young usually number between four and five although a litter of one to eight has been reported for *P.*

*jacksoni* (Delany, 1975). Other research on *P. jacksoni* reported that litter size is between two and five and that the body size related positively to litter size (Waweru and Odanga, 2003).

*Praomys delectorum* appears to breed throughout the year. Other species of *Praomys* like *P. jacksoni* have been found to breed throughout the year with peaks during certain months of the year (Delany, 1975). Males of rodents with such breeding patterns have been found to show seasonal variation in the percentages of scrotal active testes. During regressed states, sperms and spermatids were completely absent in the seminiferous tubules (Ghobrial and Hodiab, 1982). Females also exhibited a definite peak in the reproductive activity. Corpora lutea were always present in ovaries of pregnant and post-paturient lactating females (Ghobrial and Hodiab, 1982).

The quality, quantity and vitamin content of food have been reported to greatly affect reproductive activities. Inadequate nourishment has been found to readily disturb the menstrual cycle in women and depress gonadotrophic stimulus to the testes whose output of the male hormone declined (Yapp, 1970). Seasonal variation in testicular weight has been attributed to the increase in the diameter of the seminiferous tubules and accumulation of spermatozoa (Ghobrial and Hodiab, 1982).

In sexually mature adult males of most mammalian species, germ cell production occurs in a highly regulated and organized way with the resultant spermatozoa having a uniform, and species-specific shape. In species such as the laboratory rat and mouse as well as in

farm animals, the maturing germ cells within the testicular seminiferous epithelium are organized into a series of characteristic cell associations of various maturational stages that occupy the entire cross-sectional area of seminiferous tubules (Morales, 2003).

## **2.6 The Endocrine Control of Reproduction in Mammals**

The evolution of viviparity and placenta led to the minute microlecithal egg. Hence, fertilization is necessarily internal and the external genitalia are suitably adapted (Vines and Rees, 1977). Sexual maturity is said to have been reached by an animal when its gamete producing organs, the gonads, and its accessory sex organs, the genitalia, are fully functional. Effects of nutrition and environmental factors on the fertility of mammals have shown that full reproductive activity is reached only when the nutritive environment is adequate and complete (Strecker and Emlen, 1953).

In mammals, endocrine control of reproductive physiology involves interaction of the pituitary gonadotrophins with testicular androgens in males and ovarian hormones in females (Purves and Orians, 1987). Between the seminiferous tubules lies supportive connective tissue which contains the interstitial/Leydig cells which are responsible for the synthesis and secretion of testicular androgens particularly testosterone. (Pocock and Richards, 2001). Small amounts of testosterone enter the seminiferous tubules where it binds to an androgen-protein secreted by the Sertoli cells and subsequently play a crucial role in the spermatogenesis (Pocock and Richards, 2001).

The endocrine control of reproductive physiology appears to be more complex in female mammals than in male mammals (Purves and Orians, 1987). The continuous trickle of follicles through the hormone-independent pre-antral stages ensures that at any one time there are several follicles that have completed their pre-antral growth and possess the appropriate receptors for gonadotrophins (Pocock & Richards, 2001). Once a primordial follicle has been triggered to recommence development, it undergoes conversion to pre-antral follicle. Towards the end of the pre-antral stage follicular cells acquire receptors for certain hormones. The granulosa cells develop receptors for estrogen and for pituitary follicle stimulating hormone (FSH), while the theca cells develop receptors for pituitary luteinizing hormone (LH). Further development depends upon the endocrine status of the body at the time. Pre-antral follicles that do not possess hormone receptors undergo a process of atresia. FSH and LH convert pre-antral to antral stage. During this time the granulosa and theca cell layers thicken. The granulosa cells start to secrete follicular fluid all around the oocyte and the fluid forms the antrum. A fully developed antral follicle is also known as a Graafian follicle (Pocock and Richards, 2001).

Under the influence of pituitary LH, the theca interna synthesize small amount of estrogen and the androgen testosterone. The granulosa cells appear to respond to FSH by converting androgen to estrogens. The estrogens produced by the follicular cells bind to the receptors and stimulate proliferation of further estrogen-sensitive granulosa cells. If ovulation is to occur, the granulosa cells acquire receptors for pituitary LH. LH receptors are synthesized in response to pituitary FSH and estrogen. Any follicles that do not have LH receptor at this time become atretic (Pocock and Richards, 2001). In the pre-

ovulatory state, LH stimulates the granulosa cells to start synthesizing progesterone instead of estrogen. At this time the granulosa cells lose their receptors for FSH and estrogen. Under the influence of LH, cells of the stalk (cumulus oophorus) dissociate and the follicles rupture. It is thought that the switch from estrogen production toward progesterone causes the rupture. After departure of the oocyte and follicular fluid, the remainder of the follicles collapse into the space. A blood clot forms and the follicle cells form corpus luteum (Pocock and Richards, 2001).

In other mammals other than man a luteotrophic complex, LH-prolactin, and possibly other hormones seem to be important in maintaining the corpus luteum. The corpus luteum secretes large amounts of progesterone and small amount of estrogen which maintain pregnancy (Pocock and Richards, 2001).

## **2.7 Intestinal Parasites of the Wild Animals**

Although wild animals are usually infected with several species of parasites, they seldom suffer massive deaths or epizootics because of the normal dispersal and territorialism of most species (Schmidt and Roberts, 2000). Very little can be done to control parasites of the wild animals. Although it is true that most wild animals tolerate their parasite burdens fairly well, the animals will succumb when crowded and suffering from malnutrition (Schmidt and Roberts, 2000). Another important aspect of animal parasitology is the transmission of parasites normally found in wild and domestic animals to humans, leading to zoonosis.

## 2.8 Rodents' Intestinal Parasites

Rodents harbour many intestinal parasites, some of which also infect other animals including man. Some of these parasites include *Trichuris muri*, *Hymenolepis sp*, *Trichostrongylus sp* and *Nippostrongylus sp*, *Angiostrongylus cantonensis*, *Strongyloides sp* and *Trichnella sp* (Schmidt and Roberts, 2000).

*Trichuris muris* is a nematode parasite that is related to the human whipworm *Trichuris trichiura*. Some 60 – 70 species of *Trichuris* have been described from a wide variety of mammals. The parasite is normally found in the large intestines and rectum of various mammals. *Trichuris muris* occur often in rats and mice (Cheng, 1986).

According to the American Liver Society (2004), the genus *Hymenolepis* contains in excess of 400 species virtually all of which are found in higher vertebrates. Two species of *Hymenolepis* are of particular interest. *Hymenolepis nana* which is a parasite of humans and rodents in particular, mice and *Hymenolepis diminuta* a parasite of rodents but has also been reported in humans on rare occasions. *Hymenolepis nana* can complete its life-cycle in the absence of an intermediate host. It presents a modification of the typical cyclophyllidean life-cycle pattern in that the parasite requires only one host in which to complete its development. The beetle *Tribolium confusum* and *Tenebrio molitors* are their common intermediate hosts (William and Landfair, 2004).

Hookworms belong to the order Strongylida which is a very large order that contains important pathogens of man and domesticated animals. The order contains the super

family Trichostrongyloidea that comprises intestinal nematodes, which are parasites of many domesticated animals and wild rodents. *Trichostrongylus sp* has been found in the small intestines of ruminants, rodents, pigs, horses, birds and humans. *Trichostrongylus axei*, is found in a wide variety of mammals. The eggs resemble those of hookworms but are usually larger (Schmidt and Roberts, 2000; Cheng, 1986). *Nippostrongylus brasiliensis* is a nematode parasite found in the gastrointestinal tract of rats with similar life cycle and morphology to the human hookworm. Transmission does not require an intermediate host. Infection of rats is generally accomplished by skin penetration by the infective filariform larvae (Miller, 1998 and Cheng, 1986).

*Angiostrongylus cantonensis* is a nematode parasite that was initially thought to be a parasite of rodents. However it was later found in humans in many countries. *Strongyloides* are among the smallest nematode parasites of humans. *S. ratti* is parasitic in rodents (Roberts and Janovy, 1996).

Domestic trichinosis involves *Trichinella spirallis* in the strict sense. It is epidemiologically most important to humans because of the close relationship among rats, pigs and people. Infected pork is the most common source of infection. Pigs become infected by eating trichinous meat in garbage or by eating rats (Schmidt and Roberts, 2000). In the life-cycle of *Trichinella spiralis*, the animal serves as both the definitive and the intermediate host with larvae and adults occurring in different organs (Cheng, 1986).

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Study Area

Taita Hills and the Kyulu Ranges are among the rain forests of the montane habitat in South East Kenya (Appendix 1). The Taita Hills, like other montane rain forests, have been subjected to heavy human activities leading to fragmentations into different forest patches (Rogo and Oguge, 2000). These hills rise abruptly from the Tsavo Plains to a series of ranges. Dry bush-land runs up into the lower slopes of the hills, grading into moist forest, farmlands or plantations. The forests of Taita Hills are experiencing different levels of habitat alterations with the indigenous trees being selectively harvested or cleared to give way to plantations and other forms of land use. The Taita Hills are divided into three distinct isolates: Mount Sagala, Mbololo and Dawida Massif. The main body of the hills known as Dawida Massif is made up of eight forest patches, among them are: Ngangao, Chawia, Yale and Macha forest patches (Oguge *et al.*, 2004).

Ngangao is one of the two larger patches with the least anthropogenic disturbance but with the lowest *Praomys delectorum* population density of an average of 20 animals per hectare (Oguge, pers. comm). Yale and Macha are smaller forest patches with an average density of about 30 animals per hectare and Chawia with an average density of 50 animals per hectare (Oguge, pers. comm).

The Kyulu Ranges are recent volcanoes of perhaps over 1000 years old (Bally, 1939). The high ranges of velvety green cones rising to an altitude of 2170 m creates a verdant mountainous contrast to the surrounding arid African Savanna (Beentje, 1990). The ranges run 64 Km in length and 16 km in width and are geographically equidistance from Mt. Kilimanjaro to the (South-east) Taita Hills (East) and highlands of Machakos (North-west) (Oguge *et al.*, 2004). The forest vegetation here is presumed to be pioneer (Beentje, 1990); standing at 1450-1750m above sea level. The southern portion is composed of mist forests (Beentje, 1990) while the northeast has a much drier vegetation type. The Kyulu Forest population assumed to be from an area that is ecologically stable was compared with sub-populations from Taita Hills.

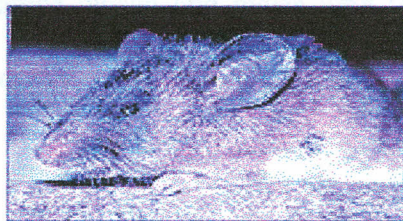
The forest patches of the Taita Hills are found between  $03^{\circ} 20'S$  and  $30^{\circ} 15' E$ . These forests patches are: Yale (2100 m;  $03^{\circ} 38' 28'' E$ ), Chawia (1600 m;  $03^{\circ} 28'S, 38^{\circ} 20'E$ ) and Ngangao (2150 m;  $03^{\circ} 22'S, 38^{\circ} 20'E$ ) (Oguge *et al.*, 2004). The animals from Kyulu Forest were collected in the mist forest at  $2^{\circ} 47.1'S 37^{\circ} 52.14'E$  at an altitude of 1700 m (Oguge *et al.*, 2004).

### 3.2 The Study Species

*Praomys delectorum* (Rodentia, Muridae) is a soft-furred small rodent belonging to the murid group. It has a head plus body length of 8 -14 cm and a body mass of 21-57 grams (Kingdon, 1974). *Praomys delectorum* is one of the eight species in the genus *Praomys*. *P. delectorum* is endemic in Kenya, Tanzania, Malawi and North Eastern Zambia (Ronald, 1997). In Kenya it is found in the rain forests of the montane habitat in

southeast Kenya (Oguge *et al.*, 2004) and is one of the most common rodents in the Eastern Arc forests.

**Plate I *Praomys delectorum* –East African Soft-furred Rat**



*Praomys delectorum* is distinguished by its soft brown fur, long tail and dirty white under belly. It is found mainly on the ground and is very rarely arboreal. The rodents feed on seeds, vegetation and insects and nest in short burrows where they make concealed runways (Ronald, 1997). The individuals collected from Kyulu forest and Taita hills had a head plus body length of 7.8- 11.2 cm and a body mass of 17.5-38g. These assumed to be mature and hence appropriate for use in this study.

### 3.3 Collection and Preservation of Animals

The animals were collected from the study areas using standard smaller Sherman's mammal live traps (foldable aluminium trap of 5.5x 7x 18 cm) by line transect. Upon capture, the animals were weighed (using Pesola balance in grams) and the live weight recorded. Euthanasia was performed on the animals, the head plus body (HB) length measured and reproductive status (RS) noted. The HB was taken as the distance between the tip of the snout and mid anus. The RS for the females were categorized as either vagina open (VO) or vagina closed (VC) depending on whether the vaginal opening was found perforate or imperforate at the time of capture. The males were categorized as

testes scrotum (TS) or testes abdomen (TA) depending on whether the testes had descended from the abdomen or had not descended into the scrotum. Females with vagina open and males with testes scrotal are regarded to be in active reproductive phase, while females with vagina closed and males with testes abdominal as either juvenile or reproductively inactive.

Body mass was also considered as a criterion in categorizing the animals as mature or immature. Some of the females with a body mass of 17.5 grams were found to be VO hence animals with a body mass of 17.5 grams were regarded as mature. Animals that had a body mass of 17.5 grams or more were selected for this study and their digestive tract was assumed to have been fully developed. The number of the captured animals from different patches was determined and sexes separated on the basis of their external genitalia. The animals were then fixed in formal saline buffered with Borax salt for 72 hours. They were then washed in water and preserved in 70% ethanol before being transported to the laboratory at Kenyatta University.

The total number of animals used in the study was 73. The Chawia sample consisted of 20 animals: 12 were females and 8 males. The Yale sample had 18 animals, 10 female and 8 males. From the Kyulu group 19 animals were used of which 9 were females and 10 were males while Ngangao sample consisted of 16 animals with 6 females and 10 males. The gastrointestinal measurements were of individual rodents with a body mass  $\geq$  17.5 grams of the overall sample

### **3.4 Data Collection**

#### **3.4.1 Gastrointestinal Measurements**

The animals were dissected and from each, the digestive tract (stomach, small intestine, caecum, and large intestine) was removed and put in water for 1 hour to reduce brittleness. The digestive tract was then cleaned of the mesenteries through stripping the connective tissue and lipids. The Brambell method was employed in which the entire gastrointestinal tract (GIT) was laid in a trough filled with water and the pyloric sphincter end was pinned on paraffin wax adjacent to the zero mark of the fixed measuring tape. The other end (the anus) was pulled horizontally by a tensiometer (7 gm weight). The intestine was moved gently to ensure that it was not stuck at any point.

The total intestine length (TI) (small and large intestine with the caecum in place) was measured. The length of the small intestine (SI) and that of the large intestines (LI) were also determined and recorded before removing the caecum for measurement. The stomach length was measured by laying it on a tape without stretching it. The stomach outer curve from the cardiac sphincter to the pyloric sphincter was measured with the aid of a string. Caecum (C) curve measurement was taken from the ileocaecal valve to the spiral valve with the aid of a string.

#### **3.4.2 Number of Embryos/Foetuses**

The uteri from the dissected females were removed and checked for embryos/foetuses and placenta/embryo scars. To determine the number of placenta / embryo scars, a dissecting microscope was used to observe the uteri. Placenta or embryo scars are the

past-partum wounds or nodules found in the uterus. The number of embryos/foetuses and placenta scars was determined by counting and recorded. These were used as indicators for litter size.

### **3.5 Histological Procedure**

#### **3.5.1 Dehydration and Sectioning**

Some ovaries from females with vagina closed (VC) and from females with vagina open (VO) were selected at random for dehydration and sectioning. Testes from males with scrotal testes and with abdominal testes were also selected at random for dehydration and sectioning.

The tissues were dehydrated using increasing concentrations of ethanol starting with 70%, 90% then 100% for 2 hours each. The tissues were then placed in xylene for 30 minutes to remove the alcohol. These tissues were impregnated with paraffin wax in the embedding oven at a temperature of 56<sup>0</sup> C for one hour after which they were fixed on wooden blocks. The tissues were sectioned into 5µm thick sections using a microtome. The sections were mounted on slides using Mayer's egg albumin then dewaxed using xylene (Appendix 2 ).

#### **3.5.2 Staining Procedure**

The sections were hydrated by placing them for two minutes each in decreasing concentrations of ethanol starting from 100% then 90%, 70% and 50%. They were then stained with haematoxylin and eosin. The sections were then dehydrated by placing them

for three minutes each in increasing concentration of ethanol starting with 50% then 70%, 90%, and two changes of 100%. DPX was used as the mounting medium. The slides were then viewed under the light microscope using low power magnification (X 100), medium power (X 250 and X 400) and high power magnification (X 1000) using oil immersion (Appendix 2).

### **3.6 Intestinal Parasites and Microscopic Examination**

Portions of the digesta from the stomach, small intestine, caecum and large intestine from each animal were put in small Bijour bottles. The contents were mixed with 10% formal saline at a ratio of 1:10 (digesta content to formal saline). The digesta was then concentrated by centrifugation at 5000 revolutions per minute for 3 minutes. The supernatant was discarded. Two drops of the sediment were transferred to a slide and stained with Lugol's iodine and observed under low (X100) and medium power (X 250) using an optical microscope for ova and larvae. Any parasites seen during the extraction of the digesta were recorded for presence.

### **3.7 Data Analyses**

The raw data obtained was first analyzed for correlation using Pearson Correlation. The mean length of the digestive tract components relative to head plus body (HB) and relative body mass (BM) were compared using one way ANOVA. Where there was a statistically significant difference, multiple comparison of means was performed (Tukey HSD  $P \leq 0.05$ ). Head plus body length and body mass as independent variables were regressed against total intestine length to assess the linear response of total intestines on

BM and HB. The significance of  $\beta$  regression coefficient was tested using t- test for separate linear response of total intestine length to BM and HB and against both BM and HB. Means of the digestive tract components length adjusted for head plus body length and number embryos/ fetuses/ placenta scars were analyzed using one-way ANOVA and, where applicable, multiple comparisons of means was performed (Tukey HSD  $P \leq 0.05$ ).

## CHAPTER FOUR

## RESULTS

## 4.1 Morphometry of the GIT

## 4.1.1 Comparison Between Males and Females

. Since the number of males and females was not balanced when sampling the animals in the four study groups, statistical analyses to assess whether there was any variation of the GIT due to sex was necessary. There was no significant difference in the total length of intestines ( $df = 1$ ,  $F = 0.021^{ns}$ ,  $P = 0.884$ ) between males and females. No significant difference was noted in the sex interaction between forests ( $df = 3$ ,  $F = 0.042^{ns}$ ,  $P = 0.988$ ) (Table 1).

**Table 1: The mean ( $\pm$ SE) length (cm) of different regions of the GIT in *P. delectorum* from Chawia, Yale, Kyulu, and Ngangao forest patches comparing males and females within and between habitats.**

		Chawia	Yale	Kyulu	Ngangao
<b>GIT sections</b>	<b>Sex</b>				
<b>TI</b>	<b>Female</b>	56.63 $\pm$ 1.44	53.60 $\pm$ 1.58	60.39 $\pm$ 1.66	55.17 $\pm$ 2.03
	<b>Male</b>	57.19 $\pm$ 1.76	54.19 $\pm$ 1.76	60.40 $\pm$ 1.58	53.70 $\pm$ 1.58
<b>SI</b>	<b>Female</b>	47.56 $\pm$ 1.33	44.62 $\pm$ 1.46	50.33 $\pm$ 1.53	45.10 $\pm$ 1.88
	<b>Male</b>	48.25 $\pm$ 1.63	44.90 $\pm$ 1.63	49.85 $\pm$ 1.46	44.68 $\pm$ 1.46
<b>LI</b>	<b>Female</b>	8.77 $\pm$ 0.25	8.60 $\pm$ 0.27	9.67 $\pm$ 0.28	8.57 $\pm$ 0.35
	<b>Male</b>	8.75 $\pm$ 0.30	8.84 $\pm$ 0.30	10.10 $\pm$ 0.27	8.72 $\pm$ 0.27

SE- Standard error

Similar results were obtained for small intestine (sex  $F_{1,65} < 0.001^{ns}$ ; forest \*sex  $F_{3,65} = 0.69^{ns}$ ) and large intestine as the dependent variable (sex  $F_{1,65} = 0.993^{ns}$ ; Forest \*sex  $F_{3,65} = 0.166^{ns}$ ).

#### 4.1.2 Comparison Between Sub-populations

The mean length of total intestine, small intestine and large intestine (colon) were compared among the Taita Hills sub-populations i.e. Ngangao, Chawia, Yale and Kyulu population (Table 2). The F value was highly significant for all sections of the digestive tract components:- TI  $F = 7.467$ ,  $df = 3$ ,  $P \leq 0.05$ ; SI  $F = 6.009$ ,  $df = 3$ ,  $P = 0.001$ ; LI  $F = 9.430$ ,  $df = 3$ ,  $P < 0.001$ .

Multiple comparison of the means (Tukey HSD  $P < 0.05$ ) showed that the Kyulu population had a longer total intestine length (TI), which was statistically different from Ngangao and Yale (Table 2). The same trend was observed for the small intestine. Large intestines of the Kyulu group was statistically longer than for the other groups (Table 2).

**Table 2: Multiple comparison of means ( $\pm$ SE) for the different components of the digestive tract in centimeters from different forest patches compared**

Forest Patch	N	TI $\pm$ SE	SI $\pm$ SE	LI $\pm$ SE
Chawia	20	56.9 $\pm$ 1.2 <sup>AB</sup>	47.84 $\pm$ 1.14 <sup>AB</sup>	8.76 $\pm$ 0.19 <sup>A</sup>
Yale	18	53.9 $\pm$ 1.22 <sup>A</sup>	44.74 $\pm$ 1.07 <sup>A</sup>	8.71 $\pm$ 0.21 <sup>A</sup>
Kyulu	19	60.40 $\pm$ 0.71 <sup>B</sup>	50.08 $\pm$ 0.70 <sup>B</sup>	9.90 $\pm$ 0.17 <sup>B</sup>
Ngangao	16	53.88 $\pm$ 1.32 <sup>A</sup>	44.84 $\pm$ 1.24 <sup>A</sup>	8.66 $\pm$ 0.22 <sup>A</sup>

SE-Standard error

Within a category (column) means followed by different letters are significantly different (Tukey HSD,  $P < 0.05$ ).

The stomach length had a high positive correlation to the stomach curve (Pearson Correlation  $r=0.851$   $P<0.001$ ) as both parameters increase with increase of digesta but caecum length (the outer curve) did not show any significant difference among the groups ( $N=72$ ,  $df=3$ ,  $F=1.141^{ns}$ ,  $P = 0.339$ ).

#### 4.2 Relationship Between Body Size and the Intestine Length

Both the body mass (BM) and head plus body (HB) length had a significant correlation to the intestine length. A positive correlation of HB and BM to all components of the intestines was recorded (Table 3).

**Table 3: Pearson correlation between body mass and head body length against components of digestive tract ( $P<0.001$ )**

Aspects of body size	Total Intestine	Small Intestine	Large Intestine	Caecum	Stomach length	Stomach Curve
Body mass N = 56	0.624**	0.586**	0.579**	3.80**	0.289**	0.182
Head body length N = 64	0.722**	0.657**	0.749**	0.331**	0.286*	0.206

\*\* highly significant at  $P < 0.001$

The results in the table 3 above indicate that there was significant correlation between body mass and head plus body length against the components of the GIT.

All sections of the gastro-intestinal tract were then assessed against the two aspects of body size (BM and HB) to determine which related more to their variations.

**Figure 4.1** Scatter gram showing relationship between total intestine length against body mass

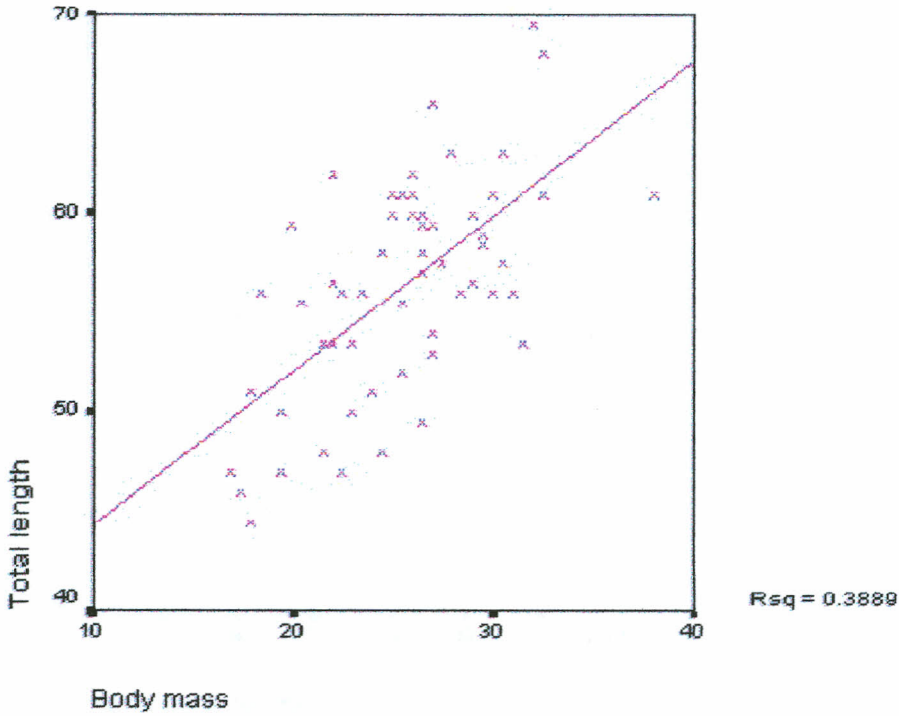
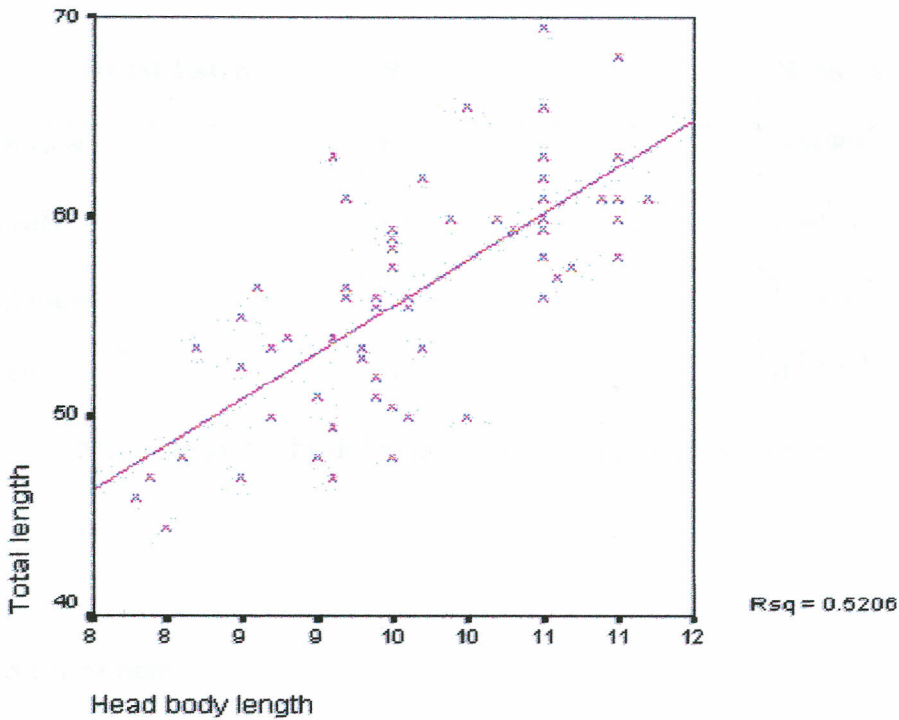


Fig. 4.1 shows that only 38.89% of the total intestine length variation can be explained or accounted for by the fitted regression. When the  $\beta$  of the best-fit line was tested for significance using ANOVA with the total intestine length as the dependent variable and HB and BM as the independent variable, the t value at the subscribed P value of 0.05 was more for the HB than BM. The t value for test of significance of  $\beta$  regression coefficient using t-test was greater for HB ( $\beta = 0.722$ ,  $t = 8.205$ ,  $P < 0.001$ ) than for BM ( $\beta = 0.624$ ,  $t = 5.862$ ,  $P < 0.001$ ).

The linear response of total intestine against both body mass and head body length showed that the  $\beta$  value was not significant ( $\beta = 0.268$ ,  $t = 1.983$ ,  $P > 0.05$ ) for the body mass but that of head body was significant ( $\beta = 0.642$ ,  $t = 5.951$ ,  $P < 0.001$ ).

**Figure 4.2 Scatter gram showing relationship between total intestine lengths against head plus body length**



This shows that 52.06% of the variation of the total length can be explained or accounted for by the fitted regression.

The differences in the GIT length could be due to overall size (body mass and head plus body length) as well as the difference in shape. Since the head plus body length appears to contribute more to variation in the digestive tract components, the GIT length was worked out relative to head plus body length. There was no statistically significant difference in the GIT sections relative to body mass. Comparison of large intestine length relative to head plus body length had statistically significant differences between groups ( $F_{3,60}=2.883^*$ ,  $P<0.05$ ). Animals from Yale had the longest relative large intestine (0.9772) and those from Chawia the shortest (0.9089) while Kyulu (0.9321) and Ngangao (0.9436) had intermediate means. (Table 4)

**Table 4: Multiple Comparison of means relative length of large intestine (cm) (Tukey HSD P=0.05)**

Forest Patch	N	Relative LI means
Chawia	15	0.9089 <sup>A</sup>
Kyulu	19	0.9321 <sup>AB</sup>
Ngangao	14	0.9436 <sup>AB</sup>
Yale	16	0.9772 <sup>B</sup>

Means followed by different letters are significantly different

#### 4.3 Litter Size

. The number of embryos/foetuses/placenta scars observed ranged from 3 to 6 from a sample of nineteen animals. The remaining sixteen females were five with VC and eleven with VO.

**Table 5: The distribution of embryos and placenta scars in animals of the different forest patches**

Forest Patch	No. of animals	Embryo/foetus		Placenta scars	
		Left horn	Right horn	Left horn	Right horn
Kyulu	1	3	0	-	-
	2	2	2	-	-
	1	0	4	-	-
	1	1	4	-	-
	1	3	1	-	-
Ngangao	1	2	2	-	-
	1	1	3	-	-
	1	0	4	-	-
	1	-	-	3	2
Yale	2	1	3	-	-
	1	0	4	-	-
	1	2	1	-	-
	1	3	2	-	-
Chawia	1	0	3	-	-
	1	6	0	-	-
	1	-	-	2	1
	1	-	-	2	3

In the Kyulu group all the females used to assess litter size had foetuses. Only two had foetuses in one horn of the uterus. One had three in the left uterine horn and the other had four in the right uterine horn. In the other three, the distribution was as follows: Two in the left horn two in right horn, one in the left horn four in the right horn and three in the left horn one in right horn. Of the Ngangao group 3 females had fetuses and in one of them the four foetuses were in the left horn of the uterus. One female from Ngangao group had five embryo scars, three in the left uterus two in the right uterus. In the Yale group all the females used had foetuses and only one had foetuses in the right horn of

uterus. Two females in the Chawia group had foetuses while the other two had placenta scars. One had three foetuses in the right horn of the uterus and the other had six foetuses

in the left horn of uterus. Embryos/placenta scars were observed in only one horn of the uterine in one animal while five animals were found with embryos in only one horn of the uterine. Seven animals had embryos/ foetuses in both the left and right uterine horns while in six animals, placenta scars were observed in both the right and left horns of the uteri (Table 5).

Placenta scars/embryo scars were observed in seven females with a mean of 4.14 while embryos/foetuses were observed in twelve females with a mean of 4.083. The total litter means calculated from embryos/foetuses and placenta scars was 4.1 (range of 3-6). The highest number of almost mature foetuses observed was six and were in the left horn of the uterus from the Chawia sample. These had distinct features exactly like those of the adult and could probably have been born soon had the mother not been caught though one was slightly smaller than the other five. The female that was used to assess the litter size had 4 pairs of mammae, two pairs in the region of thorax and 2 pairs in the rear abdominal region. The mean litter size was 4.25 for both the Chawia and Ngangao groups for the same number of animals. In Yale and Kyulu groups the mean was 4.00 from five and six females respectively used to assess the litter size. No group exhibited statistical significant difference in the foetuses means ( $F_{3,15} = 0.126^{ns}$ ).

## 4.4 Histology of the Testes and Ovaries

### 4.4.1 Testes

From Kyulu Forest, all the males (10) had scrotal testes while in the Ngangao group only three had scrotal testes and the other seven had abdominal testes. Five males in Yale group were scrotal testes while three were abdominal testes. Six males from the Chawia group were scrotal testes and two were abdominal testes (Table 6).

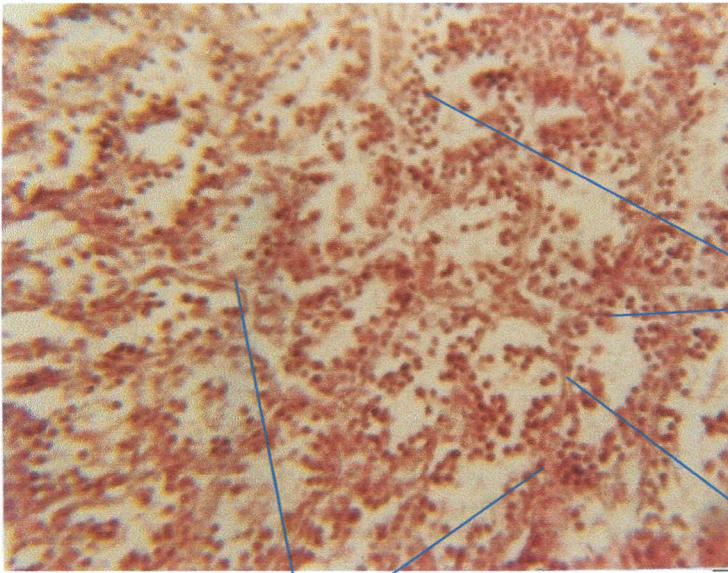
**Table 6: Distribution of testes abdomen and testes scrotum among the forest patches.**

Forest Patch	TA	TS
Kyulu	-	10
Ngangao	7	3
Yale	3	5
Chawia	2	6

Microscopic observations of the TA and TS males differed with respect to lumen diameter of the seminiferous tubules and markedly with the number of interstitial cells between the tubules. In both TA and TS males, prominent nuclei were observed in primary spermatocytes. Spermatogonia and primary spermatocytes were identified in both, though there were more primary spermatocytes in the testes scrotum. No spermatids or spermatozoa were observed in both preparations (Plates I and II).

Plate II

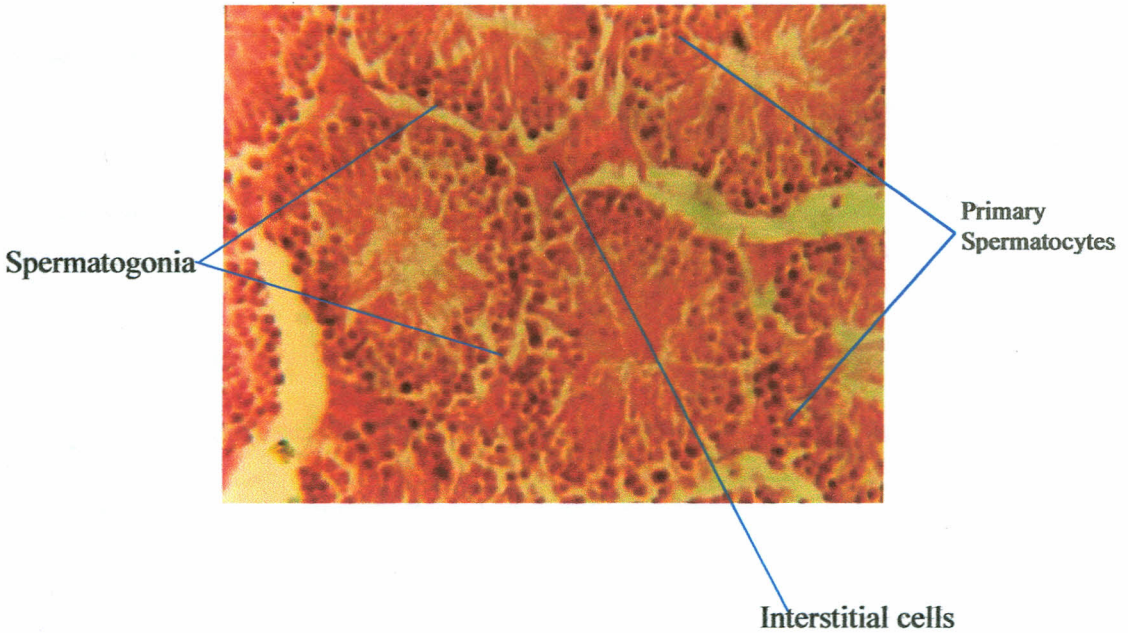
T.S. of testes (TA) showing spermatogonia and actively dividing primary spermatocyte in the seminiferous tubules *P. delectorum* males (Magnification X100)



Spermatogonia

Primary  
SpermatocytesInterstitial  
cells

**Plate III T.S. of testis (TS) showing spermatogonia and actively dividing primary spermatocytes in the seminiferous tubules *P. delectorum* males magnification X 100**



#### 4.4.2 Ovaries

In the Kyulu Forest group, nine females had vagina open (VO) and two were vagina closed (VC). Only one female in Yale group had vagina closed while the other nine had vagina open. Of the 12 females from Chawia, eight were vagina open and four were vagina closed. Five females from Ngangao were vagina open while one was vagina closed (Table 7).

**Table 7: Distribution of females from different forest patches with vagina closed (VC) and those with vagina open (VO)**

<b>Forest Patch</b>	<b>VC</b>	<b>VO</b>
<b>Kyulu</b>	2	9
<b>Ngangao</b>	1	5
<b>Yale</b>	1	9
<b>Chawia</b>	4	8

Ovarian follicles at different stages of development were observed in the ovaries of both vagina open and vagina closed females (Plate III and IV) but corpora lutea and pre-ovulatory Graafian follicles were only observed in the ovary of the females with vagina open.

**Plate IV L.S. of ovary (VO) showing corpus luteum and developing ovarian follicles (*P. delectorum*) Magnification X 40**



Pre-ovulatory  
Graafian follicle

Granulosa cells

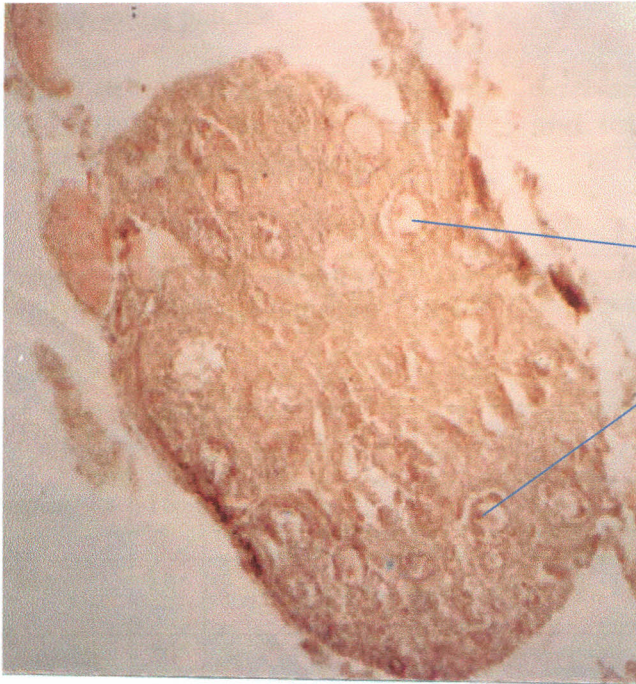
Follicular fluid

Secondary Follicle

Corpus  
luteum

The ovary was chiefly made up of follicles at all stages of development plus corpora lutea.

**Plate V L.S. of female ovary (VC) showing developing ovarian follicles of *P. delectorum* (Magnification X100)**



**Developing  
follicles**

In the ovary of the female VC, the primordial follicles were not clearly distinguishable, but growing secondary follicles were irregularly scattered within the ovarian cortex. The growing follicles were at early and intermediate developmental stages.

#### 4.5 Intestinal Parasites

All the animal groups were found to have the ova and adult of *Hymenolepis sp.*

*Trichuris muris* ova were only observed in the digesta of the Kyulu animals while *Trichostrongylus sp* (Hookworm) larvae and eggs were identified in all the animals from the four forest patches.

**Table 8:** Number of *Praomys delectorum* infected by *Hymenolepis sp*, *Trichostrongylus sp* and *Trichuris sp* and total percentage infection in different samples of the sub-populations

Forest Patch	N	<i>Hymenolepis</i> ova/adults	<i>Trichostrongylus sp</i>	<i>Trichuris sp</i> ova	Total No.of animals infected	% Infection
Kyulu	19	5	1	6	12	63.16
Ngangao	16	1	3	-	4	25.00
Yale	18	4	4	-	8	45.83
Chawia	20	5	5	-	10	50.00

Two animals from Chawia had *Trichostrongylus sp* (rodent hookworms) larvae as well as ova. Although not included in the table, an Ascaroide-like nematode was observed in the stomach of one animal in the Ngangao sample. Some animals had multiple infestations. In Kyulu one animal had both hookworm and *Hymenolepis sp* and four had *Trichuris sp* and *Hymenolepis sp*. One animal from Yale and two from Chawia had both hookworm and *Hymenolepis sp* ova. The animals with multiple infestations were considered for each type of parasite (Table 8).

## CHAPTER FIVE

### DISCUSSION

#### 5.1 Summary of the Major Findings

Anthropogenic disturbance on natural habitat may disrupt food habits of animals. For an omnivorous rodent, the change in feeding habits may result in the animal tending to be more carnivorous or more herbivorous. Analysis of the GIT morphometry in *Praomys delectorum* sub-populations suggests such an alteration in feeding habits. The head plus body length was found to be a better aspect of body size in removing the effect of the body size. The Chawia sub-population with shorter relative length of the large intestines suggest a diet with less fibre compared to that of Yale sub-population with longer relative large intestines (Table 4).

The variation in the diet of *Praomys delectorum* had no significant change in the litter size. The Yale sub-population inferred to be feeding on the less nutritive fibrous food had the lowest litter size mean. The variation in food quality may not have been significant enough to cause variation in litter size.

The ovaries of the VO females were found to have pre-ovulatory Graafian follicles and corpora lutea while from the VC females to have developing Graafian follicles only. This suggest that the condition of the vagina could be used as a good indicator of the reproductive status in *Praomys delectorum*. The testes of both TA and TS males were found to have spermatogonia and primary spermatocytes. This was an indicator of

spermatogenesis in progress. The testes of TS males had wider semiferous tubules and more interstitial cells compared to TA males.

The *Praomys delectorum* population was found to be infested with *Trichuris muris*, *Hymenolepis sp* and *Trichostrongylus sp*. The *Trichuris muris* ova were only observed in the digesta of the Kyulu group. This suggests that *Praomys delectorum* in the Taita Hills could be a strain that develops immune response to the *Trichuris muris* or the Taita Hills population was free of *Trichuris muris* (Table 8).

## 5.2 GIT Morphometry and its Correlation to Body Size

The findings from this study show that there are intraspecific variations in the morphometry of the GIT in *Praomys delectorum* sub-populations. There was statistically significant longer total intestine and small intestine of the Kyulu sub-population as compared to Yale and Ngangao sub-populations (Table 2). The large intestine of the Kyulu sub-population was also longer than that of Yale, Chawia and Ngangao sub-populations. While there was no significant difference in the body mass between the groups, there was a significant difference in head plus body length of the Kyulu population as compared to Yale, Chawia and Ngangao (Table 2).

Although the body mass did not show significant difference, body mass mean trends were the same as those of head plus body length in which the Kyulu sample varied significantly from the other three samples (Yale, Chawia, Ngangao). The Kyulu sample had a longer head plus body length followed by Chawia, then Ngangao with Yale having

the shortest. The same was observed for total intestine and small intestine with the Kyulu sample having the longest, followed by Chawia while Yale recorded the shortest. The means for large intestines differed slightly from the observed trends with the Kyulu sample having a significantly longer large intestine followed by Chawia and Ngangao (Table 2). As the body size increases the constituent organ sizes also seem to increase.

Food affects body growth in terms of body mass as well as the head plus body length. Once an animal has reached its sexual maturity the head plus body length tends to remain constant. The body mass may however, show seasonal variation due to accumulation of fat, reproductive status or short-term environmental factors like the amount of water or digesta in the body. The body mass did not significantly vary among the samples and accounted less to variation of the digestive tract components. Studies on *Elephantulus myurus* show that they had remarkably constant body mass throughout the year. This indicated that body mass was, unlikely, the determining factor in the variations of the digestive tract and there was no significant correlation between the adults' body mass and any digestive tract parameter (Woodall, 1987).

When the total intestine was regressed against both the body mass and head plus body length the  $\beta$  value for the body mass was not significant. The scatter gram for total intestine length against body mass and head plus body length revealed that the percentage of the total variation of the total length of intestine that is accounted for by the fitted regression coefficient of determination ( $R^2$ ) is greater for the head plus body length ( $R^2=0.5206$ ) than for the body mass ( $R^2=0.3889$ ). The digestive tract measurement of *P.*

*delectorum* varied more as a function of head plus body length than with body mass. As change of organ size relates to change in body size, functional interpretation of differences in size of organs should be based on size-adjusted ratios.

Since the strength of correlation between head plus body length to sections of the GIT was higher than that of body mass in this study the HB length was used to remove the effect of body size. This approach was used by Korn (1991) although the rationale for using the HB was not given. While Korn used Leopold's method in measuring the GIT length, Brambell method was used in this study (Appendix 3).

### **5.3 Effects of Food Habits on the GIT Morphometry**

When the components of the digestive tract were size adjusted, it is only the large intestine that showed statistically significant difference between the sub-populations (Table 4). Being an omnivore, the food consumption of *Praomys delectorum* is likely to vary due to temporal change in abundance and availability of types of food. This means that the analysis of digesta may yield an incomplete and biased picture on the fibre content of the food. It may not be possible to associate the gross digestive tract morphology to crude trophic classes but variation in morphometry of the digestive tract is likely to be associated with food quality in *P. delectorum*.

The prediction that with a decline in food quality animals should have larger digestive chambers was supported by the response of the prairie vole to change in food quality (Sibly, 1981). *Praomys delectorum* are small herbivore, granivore, insectivore rodents

and quality and quantity of their food may vary tremendously with availability. With such variation of food habits digestive tract modifications may be necessary for most efficient use of the available food resources. It is well established that with an increased passage rate there is a corresponding decrease in digestibility (Moe, 1981; Van Soest *et al.*, 1983). Therefore, gut capacity is critical in determining how much of the potentially digestible materials in the forage will be assimilated and how much will be excreted (Demment and Van Soest, 1985). This plasticity in the GIT is an important adaptation to compensate for greater food intake resulting from increased energy needs or reduced diet quality (Gross *et al.*, 1985).

The large intestine increases in size earlier than the small intestine. This may be due to the large intestine being more responsive to changes in metabolic requirements and dietary changes than small intestines (Woodall, 1987). Tissue, especially that of small intestines, is metabolically expensive to maintain in comparison to other body tissues (Webster, 1981). Thus the animals may be expected to maintain a minimum amount of gut tissue necessary to meet daily energy needs. In mammals, intestinal flexibility is based on a balance of cell proliferation and cell loss process assumed to be energetically expensive (Starck, 2003). Response to variation in diet of the *Praomys delectorum* therefore is expected to start with a tissue that is more responsive to metabolic requirement and less expensive to maintain. Modification of the digestive tract due to energy demand must be hinged on a “trade off” in which the animal uses comparatively less energy in the adjustment than it gains. As the Yale sub-population adapts to the assumed less nutritious available food the physiological adjustment should normally start

with less metabolically expensive tissue to maintain the large intestine. Hence the Yale sub-population was found to have longer large intestine as an adaptation to more fibrous, less nutritive food.

Conclusions inferred from the comparison of the GIT morphometry can only generalize on the types of food in terms of the amount of fibre content. The results therefore compare the sub-populations with significant difference, the Yale and Chawia sub-populations. The intermediate means in the large intestine length of Ngangao and Kyulu samples could therefore suggest that the sub-populations in the comparatively least disturbed Ngangao forest and more stable Kyulu ecosystem were feeding on food with intermediate fibre content. Intestinal length variation in small mammals due to changes in food quality was reported for *Abrothrix andinus* by Bozinovic *et al.* (1988) from South America, for *Microtus agrestis* by Hansson and Jaarola (1989) from Europe, by Gross *et al.* (1985) for *Microtus ochrogaster* and Green & Millar (1987) for *Peromyscus maniculatus*.

Fragmentation of natural forest habitat creates an ecotonal area between the forest inclusion and the clear-cut areas. Forest edges are transition zones between the wide climatic fluctuations of the external (usually deforested) environment and the relatively stable environment of the forest interior. The areas around the forest patches form the edges with vegetation intergradation. Different forest-dependent species of animals may be affected differently by these edge vegetations hence the edge effect. The response of forest-dependent species like *P. delectorum* to these ecotonal areas exhibits an edge

effect which seems to be positive. The edge to interior ratio increase with decrease in the size of habitat fragment.

The forests of Taita Hills are experiencing different levels of habitat alterations with the indigenous trees being selectively harvested or cleared to give way to plantations and other forms of land uses (Oguge *et al.*, 2004). This has led to random fragmentation of the original continuous forest into forest patches of varying sizes. Yale Forest is smaller in size compared to Chawia and could have a more significant “edge effect”. This implies that other than the anthropogenic effect in their natural habitats, more animals are most likely to extend their home range into the surrounding edge areas compared to those in Chawia. A positive “edge effect” for species like *Microtus* that clearly prefer high to low-cover habitat (Birney *et al.*, 1976) suggest that forest-dependent rodents like *P. delectorum* could also be affected positively.

Introduction of exotic plants in the surrounding area is likely to provide the rodents with easy-to-get food though not necessarily nutritious. Ecosystem modification practice in Usambara, Tanzania, like introduction of fodder grasses such as Guatemala grass (*Tripsacum laxum*), and Elephant grass (*Penisetum purpureum*) was linked to rodent harbourage. Though Guatemala grass was ranked first since it is green throughout the year and therefore could provide rodents with good shelter and food in both wet and dry seasons (Shemdoe, 2004). Adaptation to feeding on plant material, though of lower nutrient content compared to invertebrates and other animal food ensured that the rodents had food throughout hence could breed continuously. Herbivorous species of rodent

were found to breed throughout the year although more reproduction took place during the rainy months when vegetation was most lush (Taylor and Green, 1976). The Yale sub-population was most likely favoured by the availability of vegetative food, which supported breeding throughout the year. Yale population density was second to that of Chawia. The Chawia sub-population was deduced from the GIT comparative analysis to be feeding on food with less fibre. Such food is assumed to be more nutritive and is likely to support a higher population.

This study reveals that there was no significant difference in caecum length between the groups. Caecum and large intestine are better indicators of fibre content of food hence its quality. Variation in diet is expected to significantly affect the caecum size since most of the microbial digestion of cellulose takes place in the caecum (McBee, 1970, Cranford *et al.*, 2000). The variation in the diet of *P. delectorum* may not have been so significant to cause variation in the caecum morphometry.

The stomach length of the *P. delectorum* sampled correlated positively to the stomach curve (Pearson correlation  $r=0.851$   $P<0.001$ ) as expected. Research by McPherson *et al.* (1988) which explored seasonal and habitat variations in the diet of pine voles (*Microtus Pinetorum*) suggested that decline in stomach content in winter related to decline in food availability. However, Hammond (1993) suggested that the stomach content mass reflects forage quality. The Yale population, though smaller in body size (BM and HB) as compared to the other three sub-populations, had stomach length and outer curve only second to Kyulu sample for the unadjusted measurements. It seems plausible to infer that

longer large intestine relative to body length in the Yale sample suggest a more herbivorous mode of feeding comparative to other sub-populations.

#### 5.4 Change of Food Habits and Litter Size

In this study there was no significant difference between sex ( $F_{1,65} = 0.021^{ns}$ ) and interaction of sex among the forests patches ( $F_{3,65} = 0.042^{ns}$ ) for the total intestine length as well as for the different components of the intestines. Therefore, any variation in the intestine length could not be associated with compensatory gut changes due to reproductive energy demand. Many studies of mammalian reproductive energy have confirmed the traditional view that reproduction is energetically the most demanding activity of the life for a female mammal (Bronson, 1989). Females use various compensatory mechanisms to cope with reproductive demands. For example *Mus musculus* increases uptake of intestinal nutrients by increasing the mass of the intestinal mucosa during lactation (Hammond and Diamond, 1992). Such a response has an upper limit beyond which food intake alone cannot meet energy requirements because of physiological and structural constraints. In such cases, individuals use different strategies that may affect survival and growth rate including changes in length of pregnancy, lactation period and in litter size or body mass of the young (Iverson *et al.*, 1993, Mattingly and McClure, 1985).

Intraspecific variation in relative intestine length in *Aethomys chrysophilus* was associated with mainly due to the presence of reproductively active females in the sample (Korn, 1991). In the European Woodland rodents *Clethrionomys glareolus* and

*Apodemus flavicollis* reproductively active females, due to higher energy demands, have longer intestines than non-reproductive females and males (Myrcha, 1964, 1965). Pregnant or lactating females also accounted for the significant difference between the sexes in *Aethomys chrysophilus* and *A. namaquesis* since breeding females also tend to have longer intestines (Korn, 1991). Although the effects of food quality and quantity, water stress, temperature and breeding condition can be separated easily in the laboratory, they may function synergistically or antagonistically in the natural environment. A comparison of the *Praomys delectorum* females and male intestine length within the population and mean difference between sexes among the forests was carried out to elucidate that variation did not occur as a result of the breeding females.

The difference between the estimated litter size from the number of embryos and that estimated from the number of placental scars was insignificant and so either of the two methods could be taken as a reliable estimate of litter size for *Praomys delectorum*. Intraspecific differences in litter size could reflect variation in ecological conditions like food availability and quality. Studies by Ghobrial and Hodieb (1982) suggested that the food quality was most likely to be the main factor affecting litter size. Availability of food and its quality could still influence the litter size by resorption of the embryo / foetuses even after conception.

It is known that nutrients enhance growth and reproduction. The most important nutrients like the essential amino acids, certain polyunsaturated fatty acids and a variety of minerals are more abundant in seeds and animal food (Bronson, 1985). Based on the

availability of such key nutrients, a partitioning process must dictate among reproductive and non reproductive needs. The acquisition of these nutrients may not vary in an omnivorous rodent like *P. delectorum* as it has a wider variety of food compared to herbivorous rodents. Inextricably, variations in the feeding habits may arise due to habitat disturbances making the rodents to feed on less nutritive food. The Yale sub-population which reflects a more herbivorous mode of feeding had the lowest litter size with a mean of 4.00 while Chawia sub-population deduced to be feeding on a more nutritious diet had a mean of 4.25 similar to that of Ngangao. Chawia is a large patch with heavy anthropogenic disturbance while Yale is a small disturbed patch.

## **5.5 Histological Comparisons**

### **5.5.1 Testes**

Proper functioning of the mammalian testis is dependent upon an array of hormonal messengers acting through endocrine, paracrine and autocrine pathways ( Holcraft and Braun, 2004). Within the testes, the primary messengers are the gonadotrophins FSH and LH, and androgens. Abundant evidence indicates that the role of the gonadotrophins is to maintain proper functioning of testicular somatic cells ( Holcraft and Braun, 2004).

Abdominal testes (TA) in males is an indicator that they are either juvenile or are not in the reproductively active stage. Males with scrotal testes (TS) imply that the males are mature and could be reproductively active. Microscopic observations showed that the lumen diameter of seminiferous tubules of TA males were smaller than those of TS males and the interstitial cells were more in the scrotal testes TS (Plates I and II). Reproductively active males are expected to have wider lumen of seminiferous tubule for

temporary storage of spermatozoa and more interstitial cells for secretion of testicular androgens. Testosterone, one of the testicular androgens, plays a crucial role in the spermatogenesis and maintenance of male secondary characteristics (Purves and Orians, 1987).

The presence of spermatogonia and primary spermatocytes could suggest that the TS males may have been preparing for breeding while the TA males were maturing or recovering from a regressive phase (Plates I and II). Even in those rodents that breed throughout the year, some reproductively mature males lack spermatids and spermatozoa when they are in the reproductively regressed state (Ghobrial and Hodieb, 1982). Hence the TA males were most likely reproductively inactive or were juveniles. This may have been the reason why both the spermatids and spermatozoa were not observed. However, the presence of primary spermatocytes in the first meiotic division was indicative that spermatogenesis was in process. The more interstitial cells in the scrotal testes TS compared to abdominal ones suggest a high ability to produce androgens which invariably imply a higher potential in the stimulation of spermatogenesis.

In the Kyulu sub-population all the males had scrotal testes TS while in Ngangao the ratio of TS to TA males was 3:7, the ratio in Yale of TS to TA males was 5:3 while Chawia had a ratio of 3:1. These ratios may not significantly cause variation in the population growth as one reproductive male could mate with several females. However, the large number of interstitial cells in the TS males suggest higher production of androgens. Androgens, mainly testosterone, are an absolute requirement for

spermatogenesis. This therefore suggests that Chawia, with a higher ratio of TS: TA of the Taita sub-population is likely to have had more fertile males followed by Yale and Ngangao with the least at the time of the collection of animals.

### 5.5.2 Ovaries

In this study, the reproductive status of vagina open (VO) was used as an indicator of those females that were reproductively active while those with vagina closed (VC) could have been juvenile or reproductively inactive. Examination of the ovary from the VO females revealed several corpora lutea with one being prominent and this is consistent with the facts that each ovary can release more than one ovum (Pocock and Richards, 2001). From the sample of females used in the assessment of litter size for those where the embryos were used, only six had one embryo in one horn indicating single ovulation. The other six had two or more embryos indicating multiple ovulation from the ovary. The highest number of foetus observed were in one horn suggesting that simultaneous ovulation by both ovaries does not affect the litter size.

Under the influence of the pituitary LH, the theca interna produces small amounts of estrogen which stimulate the proliferation of granulosa cells. In the ovaries of VC and VO females there were several secondary follicles which seem to have been in the proliferation phase. Based on the fact that rodents like *P.delectorum* exhibit multiple ovulation, it then follows that most of the secondary follicles have oestrogen and pituitary FSH receptors. This is because the pre-antral follicles that lack hormone receptors undergo atresia (Pocock and Richards, 2001).

Although both ovaries of VC and VO females had follicles at different developmental stages, pre-ovulatory Graafian follicles were only observed in the ovary of the VO females. The Pre-ovulatory ovum has a higher number of granulosa cells and larger vacuoles of follicular fluid than secondary follicles (Plates III and IV). Lack of corpus luteum in the ovaries of the VC females can only be interpreted to mean the females had not yet ovulated.

In the rodents, primarily the number of Graafian follicles that mature determines litter size. In the pre-ovulatory state, the granulosa cells lose the receptors for FSH and estrogen and acquire those of LH which stimulate them to secrete progesterone and bring about ovulation. The follicles that remain after ovulation form the corpus luteum that secretes large amounts of progesterone and a small amount of estrogen that maintain pregnancy (Pocock and Richards, 2001).

The reproductive potential of the species is the possible number of offsprings that a typical female can produce during her life. This is determined by the length of the gestation period, litter size, length of time between delivery and the next conception and the reproductive life of the female. Rodents typically have short gestation periods with high litter size and an ability to fall pregnant again within a few days of delivery. The particularly high litter size of *P. delectorum*, coupled with other reproductive aspects is without doubt, why, like other murid rodents, they can be a major agricultural pest (Eisenberg and Isaac, 1970).

## 5.6 Parasite Population

Animals from Kyulu were found to harbour all the three species of intestinal parasite that is *Hymenolepis*, *Trichostrongylus sp* and *Trichuris sp*. No *T. muris* eggs were found in the Taita Hills sub-population. The Kyulu population, with the highest prevalence of parasites (63.16%) is considered to be from a more stable ecosystem while Ngangao with the least anthropogenic disturbance had the lowest prevalence (25%) but with the lowest population density per hectare of *P. delectorum*. Chawia and Yale sub-populations had 50% and 45% percentage infection respectively and are from the more disturbed habitats.

Of the Taita hills sub-population Yale and Chawia with more anthropogenic disturbance had more animals with intestinal parasites compared to Ngangao. This could be attributed to interaction of the rodent with domestic animals and domestic waste. Virtually all the *Hymenolepis sp* are found in higher vertebrates. Two species of *Hymenolepis sp*, *H. nana* and *H. diminuta* are parasitic to both rodents and humans. The other parasites *T. muris* and *Trichostrongylus sp* are related to some intestinal parasites in man but are not known to be zoonotic. The Kyulu population surrounded by savannah grassland may have a wider range of movement. This could make them to interact with more wild animals some of which have common intestinal parasite.

The sub-populations of Taita Hills with more prevalence of intestinal parasites could be expected to be affected negatively in their population growth. However it is known that wild animals tolerate their parasite burdens fairly well although the animals eventually

succumb when crowded and suffering from malnutrition (Schmidt and Roberts, 2000). If the parasites were a contributory factor in the population variations, then we could expect Ngangao population to harbour more parasite than Chawia and Yale as it had the lowest population density per hectare.

The findings from this study revealed that no *Trichuris muris* ova were found in the Taita Hill sub-populations. This could only suggest that either the Taita Hill sub-populations were not infected or are a resistant strain to *Trichuris muris*. Research work on mice revealed that there is a strain of mice that, upon infection with the caecum nematode *Trichuris muris*, launch an immune response and in doing so expels the parasite before patency. There are other mouse strains which do not develop the protective response resulting in chronic infection and the presence of adult worms (Garside *et al.*, 2000).

Kyulu forest is surrounded by savannah grassland hence the *P.delectorum* could have a wider home range where they were more likely to interact with a wider variety of wild animals. This could have increased the chances of infection by a wider variety of intestinal parasites.

## CHAPTER SIX

### CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 Conclusions

The statistical difference in the length of large intestines of *P. delectorum* from the various forest patches suggests that the Yale group was foraging on food with more fibre compared to Chawia sub-population. This variation in the length of the large intestine may serve as an indicator to show that habitat fragmentation can alter food habits in rodents. The longer large intestine of Yale group compared to Chawia sub-population indicates that Chawia animals were feeding on less fibrous food, most likely, seeds and animal material. This assumed more nutritious food could be among other factors that contributed to the high population density in Chawia. The Yale sub-population deduced to be feeding on comparatively more fibrous food could be favoured by the greater 'edge effect' of the smaller forest patch that allows more animal to move to the surrounding areas. Ngangao sub-population from a comparatively least disturbed forest patch compares well with Kyulu population from a stable ecosystem with respect to food habits. The head plus body length accounts more to the variation of the digestive tract morphometry. This makes it a better covariate in removing the effect of body size.

The litter size did not differ significantly among the different groups of *P. delectorum*. The variation in the types and preferences of food in the different groups of *P. delectorum* did not affect the litter size.

Vaginal condition is a good indicator of the reproductive condition in the *P. delectorum*. Those females that had VC are reproductively inactive while those with VO were reproductively active.

The prevalence of the parasites increased with increase in anthropogenic disturbance among the Taita Hills sub-populations. The Taita Hills sub-populations were either not infected by the *T. muris* or are resistant to it as no ova were found in their digesta.

## 6.2 Recommendations

- a. An in-depth study on the ecology and other biological aspects of *Praomys delectorum* is necessary. Of importance are investigations into the *P.delectorum* preys and its predators in stable and unstable ecosystems.
- b. Other aspects of reproductive potential like length of gestation period, length of time between delivery and the next conception, the reproductive life of females need to be researched on. This would help to formulate a complete picture of the species reproductive potential.
- c. It is also important to investigate which of the intestinal parasites of *P.delectorum* could infect domestic animals and other zoonotic pathogens for which they are likely to act as reservoirs.
- d. *Praomys delectorum* being an omnivore may have a significant effect on regeneration of forest. To elucidate this it is important to determine its feeding habits especially on the types of the trees seeds it feeds on.

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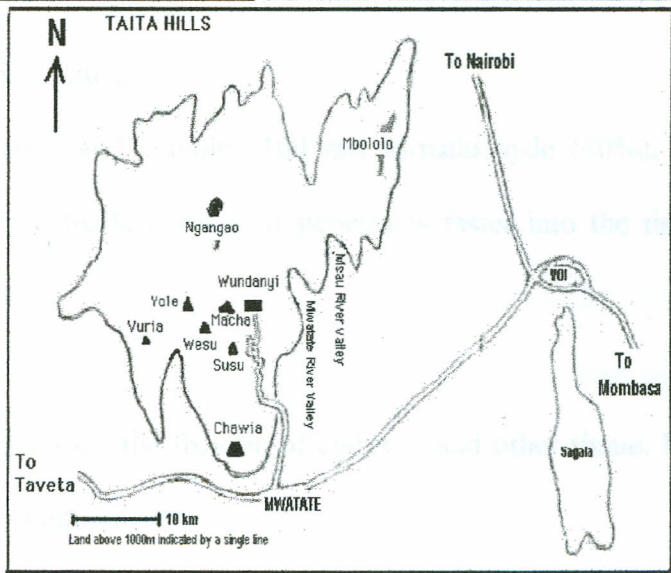
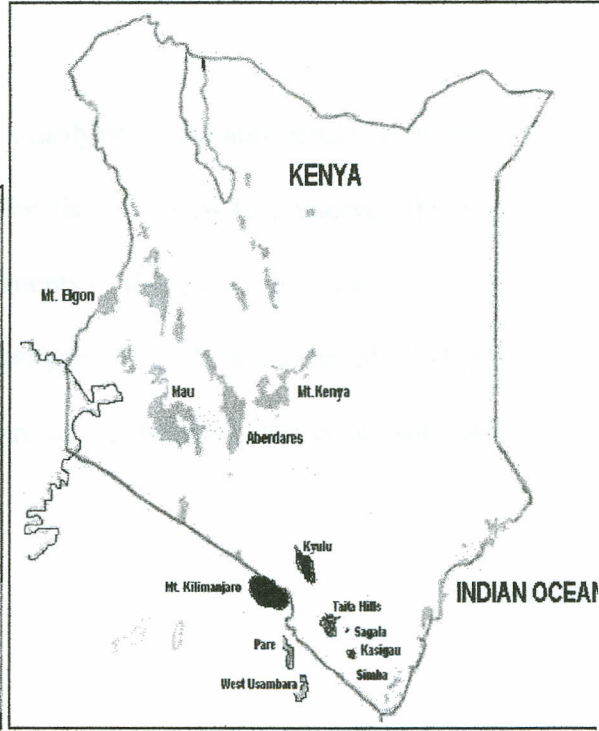
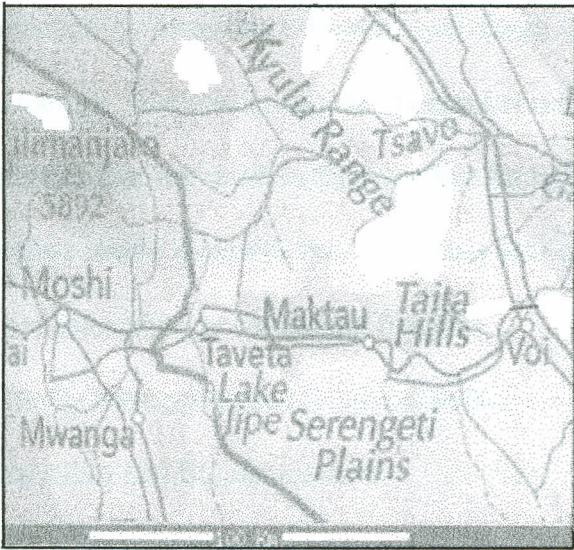
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APPENDICES

Appendix 1

Map of Kyulu and Taita Hills inset map of Kenya



## Appendix 2

### ROUTINE HISTOLOGICAL TECHNIQUES

The technique is applied to study the micro-anatomy of normal tissue (histology) or diseased tissue (histopathology)

#### 1. FIXATION

The tissues are put in appropriate fixative to stop autolysis by the autogenous enzyme and putrefaction by invasion of bacteria and fungi. The fixative helps to preserve after death the structure, relationship and the chemical constituents of the tissues and cells.

Substances that are used as fixatives like formaldehyde (HCHO) and Ethanol( $C_2H_5OH$ ) are called simple fixatives but when they are carefully combined with other substances they are referred to as compound fixatives.

Commonly used compound fixatives are like:

Formal saline and Bouin's solution

Formal saline fixation period is 3 hours.

It is a micro anatomical fixative and contains 100 mls formaldehyde (40%), 9gms of sodium chloride and 900mls of distilled water. It penetrates faster into the tissue than formaldehyde.

Bouin's solution is recommended for the fixation of embryos and other tissue. Period of fixation required is from 3 to 24 hours.

It contains 75ml of saturated aqueous picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid.

## 2. DEHYDRATION

Dehydration is the removal of water molecules from the tissue so that it can be miscible with other substances like xylene in later treatment. Substances like Ethanol, Acetone and Dioxane can be used to dehydrate tissues. Using Ethanol, water from the tissue is removed very gradually because sudden changes to the tissue structure can lead to denaturation. To ensure that all water has been removed from the tissue it is advisable to put a layer of anhydrous copper sulphate in the last alcohol bath covered with filter paper. If a blue tinge develops then the dehydration is not complete because there are residue water molecules.

The tissue is put in increasing concentration of ethanol starting with 50% then 70% followed by 90%, 100% and eventually another 100% for 2 hours in each.

## 3. CLEARING

Clearing also called de-alcoholization is the removal of alcohol from tissue after dehydration. Clearing is done with a medium which is miscible with ethanol and the embedding medium (paraffin wax). Simultaneously the tissue becomes transparent, raising the refractive index; hence the use of the term clearing. Examples of clearing agents are:

- i) Xylene –15-30 minutes (hardens tissue)
- ii) Benzene –15 –30 minutes (carcinogenic)

- iii) Toluene –15-30 minutes (expensive)
- iv) Chloroform- 6- 24 hours (poisonous)
- v) Cedar wood oil –48 hours (very slow)

#### **4. IMPREGNATION**

To prepare a tissue for sectioning, it must be held in hard substance i.e. paraffin wax so that when fixed to a wooden block, it can be clipped on to a microtome for sectioning. The tissue pieces are first put through at least two baths of molten wax in the embedding oven. The temperature in this case should be maintained at  $56^{\circ}\text{C}$  for one hour. Longer exposure of tissue to molten wax or higher temperatures will result to 'cooking' which will denature the tissue structures.

#### **5. EMBEDDING**

Embedding is sandwiching the impregnated tissue in a mould of paraffin wax. The wax holds the tissue and stops it from collapsing during sectioning. Molten wax for embedding is poured into Leukhand boxes, plastic trays or paper boats containing the section to be embedded. The face of the section should face upside down. The following substances are used for embedding,

- i) Paraffin wax
- ii) Gelatine
- iii) Celloidin

## 6. ATTACHING BLOCK TO WOODEN HOLDER

The block tissue is attached to a wooden holder the part which is attached to the microtome during sectioning. The base of the block is heated with a spatula and while molten, it is mounted on the surface of the piece of wood. It sticks readily.

## 7. SECTIONING

Thin piece of tissue are required if the microstructures are going to be seen under the microscope because the latter works on the principle of a light transmission. These thin pieces are called sections. The equipment used to section tissue is called microtome. Suitable thickness is between  $5\mu\text{m}$  and  $7\mu\text{m}$  (Microns).

## 8. MOUNTING SECTIONS

After the tissue sections have been cut, they are mounted on slide in preparation for staining. A solution called Mayer's egg Albumin smeared on the surface of the slide to hold the section firm onto the slide.

### **Mayer's Egg Albumin**

Combine equal parts of glycerine and egg white. Mix well, filter and add one crystal of thymol.

## Procedure

With the help of camel brush, put the sections/section ribbon on a clean slide and apply one to two drops of acid alcohol. Holding the slide in a slanting angle float the sections on a warm water in bath at  $37^{\circ} - 40^{\circ}$  C. After the section has stretched and all the furrows have been flattened. Smear clean slide with Mayer's egg Albumin and allow the slide to dry.

To mount the section on the slide with Mayer's egg Albumin scoop the section/ Section ribbon from water at angle. Allow the sections to dry on a hot plate at a temperature of  $40^{\circ}$  C until all water evaporates.

## 9. BIOLOGICAL STAINING

Haematoxylin is a dye derived from ether extraction of wood called *Haematoxylon comprecianum*. It has an affinity of cell nucleus. The nucleus is stained blue.

Eosin is an acid which has an affinity of cell cytoplasm. It stains the cell cytoplasm yellowish.

### Staining Procedure

1. Dewax with xylene for 5 minutes
2. Hydrate with descending concentrations of Ethanol
  - 100% ethanol - 2 minutes
  - 90% ethanol - 2 minutes
  - 70% ethanol - 2 minutes
  - 50% ethanol - 2 minutes
  - Tap water (running)- 5 minutes

3. Stain in solutions of haemotoxylin stain for 20-30 minutes.
4. Wash thoroughly in running tap water for 6 minutes. The current should not be very strong otherwise the section will be washed away.
5. Differentiate in solution (ii) acid alcohol until only the cell retain the stain (one dip in the bath is enough) N.B Care so that you do not wholly decolorize the slide.  
Wash it in tap water immediately.
6. Put section in tap water for 5-10 minutes. It develops a nice blue colour.
7. Counter- stain in eosin for 3 minutes
8. Wash excess eosin in tap water.
9. Dehydrate with ascending concentration of ethanol.  
  
50% ethanol- 3minutes  
  
70% ethanol- 3minutes  
  
90% ethanol- 3minutes  
  
100% ethanol- 3minutes  
  
100% ethanol- 3minutes
10. Clear using xylene – 2 minutes and again in Xylene for 2 minutes
11. Mount in D.P.X and carefully place the cover slip taking care not to trap air bubbles
12. Label the slides and allow them to dry in an incubator at 37<sup>0</sup>C for 12 hours.  
  
Store the preparation in a cool dry place if they are not to be examined then.

## Appendix 3

### LEOPOLD AND BRAMBELL METHODS OF MEASURING INTESTINES

#### 1. Leopold method

Involves removing the gastrointestinal tract and cutting all the mesenteries. The gut is then arranged in a straight line without stretching and the length measurement are taken.

This is called the convectional method of determining the length of intestines by straightening.

#### 2. Brambell method

The apparatus for measuring intestinal length consist of a trough and an instrument for pulling the gut to a predetermined tension. The trough is made of polyvinyl chloride (PVC) pipe of 5cm in diameter and a length of 120cm. (the length could vary depending on the need) .

The pipe is cut into two halves length wise. The open semi circular ends as sealed. The trough is then fixed on wooden block for stability. The bottom of the trough is covered by 1cm layer of melted paraffin wax. A tape measure is mounted on the inside wall above the paraffin wax layer. Water is put in the trough so that the intestines can float. After removing the mesenteries one end of the intestines is fixed adjacent to the zero mark of the tape measure. The other end of the intestine is attached to the tensiometer and pulled horizontally to a predetermined tension. For rodents like mice 7 g tension using a 10 g Pesola spring scale is recommended (Freehling and Moore 1987).

This method was first used by Brambell in 1965. The apparatus and methods were developed independently by Freehling and Moore in 1987.

Extrinsic variation in measurement of soft elastic organ such as the intestine is unavoidable. Brambell's method has been found to minimize such variability (Freehling and Moore, 1987).