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Effect of habitat fragmentation on some aspects of reproduction among *Praomys delectorum* Sub-Populations in the Taita and Kyulu Hills, Kenya

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Abstract

In small mammals particularly rodents, selection generally favours the production of relatively many offsprings per litter. Intraspecific litter size variation requires individual mothers to establish an optimal litter size in terms of fitness consequences. Because mammalian reproduction is energetically very demanding, 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. This study investigated the effect of habitat fragmentation on litter size of *Praomys delectorum* in three sub-populations of the Taita Hills and the Kyulu Hills population. The presence of foetuses and placental scars were used as litter size indicators. The histology of testes and ovaries, based on routine histological techniques was studied to ascertain the reproductive status of the animals. There was no significant difference in litter size ($F_{3,15} = 0.126^{ns}$ 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 plugged vagina lacked corpora lutea which were nonetheless observed in the ovary of females with perforate vagina though developing Graafian follicles were observed in both. Thus vaginal condition is a good indicator of reproductive status in this species.

Keywords: *Praomys delectorum*, litter size, placental scars, foetuses, spermatogenesis.

Introduction

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).

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).

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). 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.

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 (Happold, 1987). Litter –size is usually three to four 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* 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 (2 – 5) 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 P*raomys* 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 Hodieb, 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 Hodieb, 1982).

The quality, quantity and vitamins 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 Hodieb, 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 a 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 *et al*., 2003).

There is little or no information on aspects of the reproductive potential of *P. delectorum*. In this study therefore, investigations on litter size have been carried out by making a comparison of the Taita Hills sub-population with the Kyulu Hills population. The histology of the testis and ovary of different groups has been studied. A study of this kind is essential in building a complete picture of any species' basic biological information.

Materials and Methods

Study Area

Taita Hills and the Kyulu Ranges are among the rain forests of the montane habitat in South East Kenya. The Taita Hills, like other montane rain forests, have been subjected to heavy human activities leading to fragmentations into different forest patches (Rogoand 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 *P. 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 forest patches of the Taita Hills are found between 03^0 20'S and 30^0 15 `E. These forest patches are: Yale (2100 m: $03^{0`}38^{0}28^{0`}$E), Chawia (1600 m; $03^0$28`S, $38^0$20`E) and Ngangao (2150 m; $03^0$22`S,$38^0$20`E) (Oguge *et al.*,2004). The animals from Kyulu Forest were collected in the mist forest at $2^0$47.1`S $37^0$52.14`E at an altitude of 1700 m (Oguge *et al*., 2004).

Collection and Preservation of Animals

The animals were collected from the study areas using standard small 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. Euthansia was performed on the animals and 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 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.

Categorization of Experimental Animals

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 scrotum were regarded to be in active reproductive phase, while females with vagina closed and males with testes abdominal as either juvenile or reproductive inactive.

Body mass was also considered as a criteria 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. The number of the captured animals from different patches was determined and sexes separated on the basis of their external genitalia.

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. The number of embryos/foetuses and placenta scars was determined by counting and was recorded. These were used as indicators for litter size.

Histological Procedure

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 56^0C 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.

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.

Results

Distribution of Foetuses and Placental scars

Litter size was assessed by number of embryos / foetuses or placenta scars. Placenta or embryo scars are the past-partum wounds or nodules found in the uterus. The number of embryos/foetuses/placental scars observed ranged from 3 to 6 from a sample of nineteen animals. The remaining sixteen females were five VC and eleven VO.All the females that were used to assess the litter size had four pairs of mammae; two pairs in the region of thorax and two pairs in the rear abdominal region.

In the Kyulu group all the females used to assess litter size had foetuses. Only twohad foetuses in only one horn each of the uterus. One female had three foetuses in the left uterine horn and none in the right uterine horn. In the other three, the distribution was as follows: Two animals had two foetus each in the left horn and two each in right horn. One animal had one foetus in the left horn and four in the right horn and the other had three in the left horn and one in right horn.

Of the Ngangao group three females had foetuses. In one of them, all the four foetuses were in the right horn of the uterus. One female from the group had five embryo scars, three in the left uterus two in the right uterine horn. In the Yale group all the females had foetuses and only one had the four foetuses in the right horn of uterus. Two females in the Chawia group had foetuses while the other two had placental scars. One had all the three foetuses in the right horn of the uterus and the other had six foetuses in the left horn of uterus.

Embryos/placental scars were observed in both horns of the uterus in one animal from Ngangao and two females from Chawia.Six animals were found with embryos/foetuses in only one horn of the uterine. Ten animals had embryos/ foetuses in both the left and right uterine horn(Table 1).

Placental scars/embryo scars were observed in three females with a mean of 4.33while embryos/foetuses were observed in 16 females with a mean of 4.125. 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 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 a significant difference in the foetuses means ($F_{3,15} = 0.126^{ns}$).

Table 1: The distribution of embryos/foetuses and placental scars in animals from differentforest patches

Forest	No.of animals	Embryo/foetus		Placental scars	
		Left uterine horn	Right uterine horn	Left uterine horn	Right uterine 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

Histology of the Testes and Ovaries

Testes

From Kyulu Forest, all the males (10) were testes scrotum (TS) while in the Ngangao group only three had testes scrotum and the other seven had testes abdomen (TA). Five males in the Yale group were testes scrotum while three were testes abdomen. Six males from the Chawia group were testes scrotum and two were testes abdomen (Table 2).

Microscopic observations of the male testes TA and TS differed with respect to lumen diameter of the seminiferous tubules and markedly with the number of interstitial cells between the tubules. In both testes TA and TS, 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.

Table 2: Distribution of testes abdomen(TA) and testes scrotum (TS) among the males from different forest patches

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

TA- Testes Abdomen TS- Testes Scrotum

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 twelve females from Chawia, eight were vagina open and four were vagina closed (VC). Five females from Ngangao were vagina open (VO) while one was vagina closed (Table 3).

Table 3: Vaginal Status of females from different forest patches

Forest patch	VC	VO
Kyulu	2	9
Ngangao	1	5
Yale	1	9
Chawia	4	8

VO- Vagina Open VC- Vagina Closed

Ovarian follicles at different stages of development were observed in the ovaries of both vagina open and vagina closed females.Corpora lutea and pre-ovulatory Graafian follicles were only observed in the ovary of the females with vagina open.

The ovary was chiefly made up of follicles at all stages of development plus corpora lutea.In the ovary of the female with vagina closed (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.

Discussion

Change of Food Habits and Litter Size

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 *Musmusculus* 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). Other studies on *P. delectorum* (Gitonga *et al.*, 2015) indicated that variation in intestine length could not be associated ith compensatory gut changes due to reproduction energy demand.

Intraspecific variation in relative intestine length in *Aethomys chrysophilus* was associated mainly to the presence of reproductive active females in the sample (Korn, 1991). In the European Woodland rodents *Clethrionomys glareolus* and *Apodemus flavicollis* reproductive 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 *Praomysdelectorum* females and male intestine length within the population and mean difference between sexes among the forests was carried out to elucidate that variation does not occur as a result of the breeding females (Gitonga *et al*., 2015).

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 P*raomys delectorum*. Intraspecific differences in litter size could reflect variation in ecological conditions like food availability and quality. Studies by Ghobrial and Hodieb (1982) suggest that the food quality is 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 nutrient enhances growth and reproduction. The most important nutrients like the essential amino acids, certain polyunsaturated fatty acids, a variety of minerals are more abundant in seeds and animal food. Based on the availability of such key nutrients, a partitioning process must dictate among reproductive and non reproductive needs. The acquisitions of these nutrients may not vary in an omnivorous rodent like *P. delectorum* asit has 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.

Histological Observation

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).

Testes abdomen (TA) in males is an indicator that they are either juvenile or are not in the productive active stage. Males with testes scrotum (TS) imply that they are mature and could be reproductive active. Microscopic observations showed that the lumen diameter of seminiferous tubules of males TA were smaller than those of males TS and there were more interstitial cells in the testes of the male with TS. Reproductive 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 males TS may have been preparing for breeding while the males TA were maturing or recovering from a regressive phase (Plate I and II). Even in those rodents that breed throughout the year some reproductive mature males lack spermatids and spermatozoa when they are in the reproductive regressed state (Ghobrial and Hodieb, 1982). Hence the males TA were most likely reproductive inactive or 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 testes TS compared to TA 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 testes scrotum (TS) while in Ngangao the ratio of testes scrotum male to testes abdomen was 3:7, the ratio in Yale of TS to TA was 5:3 and 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 males TS 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.

Ovaries

In this study, the reproductive status of vagina open (VO) was used as an indicator of those females that were reproductive active while those with vagina closed (VC) could have been juvenile or reproductive inactive. Examination of the ovary from the VO females revealed several corpora lutea and this is consistent with the facts that each ovary can release more that one ovum (Pocock and Richard, 2001). From the sample of the 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.

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 from the female VC and VO there were several secondary follicles which seem to have been in the proliferation phase. Based on the fact that rodents like *Praomys* 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 Richard, 2001).

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

In the rodents, the litter size is determined primarily by the number of Graafian follicle that mature. 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 Richard, 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. Particularly high litter size of *Praomys delectorum* coupled with other reproductive aspects of rodents is without doubt, why, like other murid rodents, can be a major agricultural pest (Eisenberg and Isaac, 1970).

Conclusion

- 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 *Praomys* did not affect the litter size. This may suggest that the *P.delectorum*uses other physiological designs to comply with nutritional differences that appears to exist among the different groups.
- Vaginal condition is a good indicator of the reproductive condition in the *P. delectorum*. Those females that had VC were reproductive inactive while those with VO were reproductive active.

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