



THE INFLUENCE OF GENE RECOMBINATION ON FERTILITY AND HATCHABILITY OF FOUR SPECIES OF TORTOISE

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ABSTRACT

Tortoise is characterized by ecological habitats and species distribution genes directly affected productive performance. These are classified species as Marginata (Po), Serrated hinged terrapin (Ps), West African mud (Pc), and Pancake (Pa) study aimed to compare fertility and hatchability among these tortoise species. A total of four (4) sires and forty (40) dams' genetic group were used in this study which was conducted over one year involving both direct and reciprocal crosses. A total number of 62 eggs out of 103 eggs laid were set. Similarly, number of eggs hatched, percentage fertility and hatchability were significantly ($p < 0.01$) affected by both sire and dam species. Percentage fertility were 90.91, 90.00, 88.89 and 86.96 among the Marginata, Pancake, Serrated Hinged Terrapin and West African Mud sired tortoise respectively, value percentage hatchability was 88.96, 87.50, 85.00 and 80.00 respectively. There was also significant ($p < 0.05$) interaction effect between dam and sire species on fertility and hatchability percentages. Marginata sire group produced the highest number of fertile eggs, followed by the Pancake group. The West African Mud (Pc) sire group produced the least fertile eggs. The overall mating between purebred tortoises produced high percentage fertility 88.71 and 87.29 percent hatchability. Hatchability was higher where Pancake and Marginata tortoises were used as dam compared to where they were used as sires. However, the reverse was the case with West African Mud tortoise species. It could therefore be concluded that genes for strain types significantly affects fertility and hatchability among the tortoise species and the marginata genetic group was generally superior to the other groups.

Keywords: Dam, Fertility, Genes, Hatchability, Sire

INTRODUCTION

In tortoises, gene recombination (specially related to sex determination) can significantly impact fertility and hatchability, particularly in the dry season. Temperature-dependent sex determination (TSD) is common in many reptile species, including tortoises, where the incubation temperature of eggs determines the sex of the offspring (Choi *et al.*, 2021). During the dry season, if nests are in areas with fluctuating or extreme temperatures, it can lead to skewed sex ratios (more males or more females) or reduced overall hatchability due to developmental abnormalities.

Many reptile species, including tortoises, lack sex chromosomes and instead rely on the incubation temperature of their eggs to determine the sex of the offspring (Gatto and Reina, 2022). Different species exhibit different TSD patterns, but generally, cooler temperatures produce one sex (often males), while warmer temperatures produce the other (often females). If nests are consistently in hotter or cooler areas, the population might become predominantly male or female, potentially impacting long-term breeding success (Averill-Murray *et al.*, 2021).

Extreme temperatures can negatively affect egg development and lead to lower hatching rates. Abnormal temperature can also cause developmental issues in hatchlings, reducing their survival rates. While some tortoise species prefer drier conditions for nesting, extremely dry conditions can lead to nest desiccation and egg mortality. In the dry season, temperature fluctuations can be more pronounced, especially in areas with less vegetation cover, impacting the nests located in these areas.



Dry conditions can also reduce the availability of food resources for both adult tortoises and hatchlings, potentially affecting their survival and reproductive success (Lovich *et al.*, 2018).

The genetic make-up of an individual is fixed at fertilization and hence, fertility and hatchability are generally considered as a character of the two parents (Mendoza *et al.*, 2021). Fertility is the ability to reproduce; it determines the number of progenies that can be obtained from a given number of eggs. Fertility is expressed as the percentage of eggs fertilized while hatchability refers to the percentage egg hatched or percentage of hatchlings hatched from all eggs placed in the sharp sand as incubator. Hatchability of tortoise's eggs is affected by several factors which include fertility of the egg, the genetic constitution of parents and environmental – temperature and nutrition. Kulshreshitha *et al.*, (2022) stated that it is difficult to determine the relative influence of the sire in transmitting hatchability to offspring.

The interaction between genes involved in TSD and environmental factors like temperature can affect gene expression and ultimately, the development of the offspring. Changes in gene expression due to environmental factors can drive evolutionary changes in populations, potentially leading to adaptation to specific environmental conditions (Kperegbeyi *et al.*, 2025).

Understanding the impact of gene segregation and environmental factors on tortoise fertility and hatchability is crucial for conservation efforts. Protecting suitable nesting habitats, managing temperature fluctuations, and ensuring the availability of food resources are essential for maintaining healthy tortoise populations. In conclusion, gene segregation, particularly in the context of TSD, plays a significant role in tortoise reproduction. Dry season conditions can exacerbate the effects of TSD, impacting fertility and hatchability (Hunter *et al.*, 2021). Understanding these interactions is vital for effective tortoise conservation.

Nevertheless, there is paucity of information on the influence of sire and the interaction between sire and dam genotypes on fertility and hatchability of tortoise's eggs in the Niger Delta region of Nigeria. Therefore, this was initiated to fill this gap and examine the segregation effect of major genes on the fertility and hatchability of tortoise genotypes.

MATERIALS AND METHODS

Experimental Site

The research was conducted at the Department of Animal Production Research Farm (DAPRF), Southern Delta University (SDU), Ozoro. Latitude 5° 32' N and Longitude 6° 15' E of Greenwich meridian place the center in mid-western Nigeria's rainforest. Humidity averages 2500-3000 mm per year and 27.4°C and 85 % are the mean temperature and RH (SDU, 2025).

Experimental Animal and Management Procedures

Forty (40) dams and four (4) sires' tortoise were used for the experiment, and are allocated into 4 groups Marginata tortoise (*Testudo marginata*); Serrated hinged terrapin (*Pelusios sinuatus*); West African mud tortoise (*Pelusios castaneus*) and Pancake tortoise (*Malacochersus torrieni*). These groups are maintained for adaptive breeding purpose in the University Departmental Farm. Intensive housing system was used for this experiment. The dimension of the area is 12ft x 12ft. The house was ant proof to prevent ants not to invade the unit. A pen (ditched) was constructed measuring 8ft x 2ft. The tortoise was fed with concentrate diet (Top Omega feed), cooked meat, fermented cassava (Akpu/Fufu), and Palm fruits. Feed and water were given *ad libitum*. The animal was grouped into four (4) treatments e.g TRT 1, TRT 2, TRT 3, and TRT 4 respectively. The tortoises were given the same treatment throughout the experimental period under the same managerial conditions. The ditch was filled with enough water to reach the bridge where top shell (carapace) meets the bottom shell (plastron). Some routine preventive hygiene was done frequently such as providing fresh water, feeding, changing of water regularly at all times, observing the behaviour of the tortoises, signs or symptoms of any disease condition should be observed by physical examination. The tortoises were properly identified with an indelible mark on their back labeling T1, T2, T3 and so on.

Data Collection

All tortoises were weighed individually with an electronic scale measured in grams (g) to obtain the body weight (BWT). The morphometric was also gauged with a tape calibrated in

centimeters (cm). The BWT was taken with a digital scale on a monthly basis. The experiment lasted for a period of 52 weeks.

Egg collection

The experiment lasted for 52 weeks between January and November in 2023–2024. The female's tortoise lay their eggs on a heap sharp sand between October and September. Eggs were carefully examined to records the number of eggs produce per clutch, Number of eggs laid, number of fertile eggs, and number of eggs hatched. The fertility and hatchability percentage were computed

Statistical Analysis

The response variables were analyzed by fitting a fixed effect model using the least squares procedures of the generalized linear model (SAS, 2018). This was also used to pre-adjust the data for the effects of sire age and egg batch. The fixed effects included in the final analysis were sire specie, dam species and their interactive effects. Linear contrast of least squares class means was computed using New Duncan Multiple Range Test (Steel and Torrie, 2010) to determine the significance of specific classes.

RESULTS AND DISCUSSION

Fertility and hatchability are the most vital determinants of producing more hatchlings from a given number of breeding stocks within a stipulated period of time to boost the profitability of the tortoise farming. Sire and dam genotype significantly ($p < 0.01$) affected the number of eggs laid, number of fertile eggs, percentage fertility, and percentage hatchability ($p < 0.05$) in this present study. The least square means and the significant test influence sire genetic group, dam genetic group and their interactive effect on fertility and hatchability of eggs of the tortoise species are presented in Table 1.

Table 1: Least Squares Means of Egg Parameters as Influenced by SIRE and Dam Genotype

Variable	No. clutch size	No. of egg laid	No. fertile eggs	No. Eggs hatched	% of fertile eggs	% of eggs hatch
Overall	34	62	55	48	88.71	87.27
Sire genotype						
Marginata (Po)	6 ^b	11 ^c	10 ^c	8 ^d	90.91±5.23 ^a	80.00±5.32 ^d
Serrated hinged terrapin (Ps)	10 ^a	18 ^b	16 ^b	14 ^b	88.89±6.10 ^b	87.50±6.15 ^b
West African Mud (Pc)	12 ^a	23 ^a	20 ^a	17 ^a	86.96±5.25 ^c	85.00±4.53 ^c
Pancake (Pa)	6 ^b	10 ^d	9 ^c	9 ^c	90.00±4.53 ^a	88.96±6.43 ^a
Dam genotype						
Marginata (Po)	8 ^b	16 ^b	14 ^b	13 ^b	87.10±6.31 ^b	92.86±6.41 ^b
Serrated hinged terrapin (Ps)	6 ^d	10 ^d	9 ^d	9 ^c	90.00±7.24 ^a	100.00±7.30 ^a
West African Mud (Pc)	13 ^a	24 ^a	22 ^a	17 ^a	91.67±6.62 ^a	77.27±5.13 ^d
Pancake (Pa)	7 ^c	12 ^c	10 ^c	9 ^c	83.33±5.74 ^c	90.00±6.55 ^c

Values within the same column with different letters are significantly ($p < 0.05$) different

The significant effect of sire genotype on fertility and hatchability in this study is consistent with the reports of Kperegbeyi *et al.*, (2025) who stated that the duration of sperm fertilizing ability varied widely among individuals within specific parent male lines and within the lines used for their study. In the present study, the marginata sire group produced the highest number of fertile eggs, followed by the pancake group. The West African Mud (Pc) sire group produced the least fertile eggs. The corresponding percentage values observed were 90.91%, 90.00%, 88.89% and 86.96%, respectively. The better performance of the marginata (Po) tortoises in this paper could be associated with positive adaptive genes influence of the marginata traits significantly affecting thermoregulatory ability (Kubisch and Ibarguengoytia, 2021). Nevertheless, the pancake (Pa) group produced eggs with the highest hatchability percentage. Corresponding percentage hatchability values for Pa, Pc, and Po was 88.96%, 87.50%, 85.00% and 80.00%, respectively. Percentage fertility and hatchability was consistently lower among eggs sired by the Pc genetic group. This result is consistent with the reports of Lovich *et al.* (2018); Averill-Murray *et al.* (2021); Segura *et al.* (2021) and Cordero *et al.* (2022), who reported that increase in embryonic mortality (up to 10% in pure species) found among Pa/Pa



and to a lesser extent, among their Pa/Po counterpart put them at a slight disadvantage to the Pc control tortoises. There was a 6.1% reduction in embryonic survival as compared to Pc genotype in this investigation. This value is less than the 13% reduction in embryonic survival reported by Segura *et al.* (2020). According to their report, embryonic mortality normally occurs during the last stage of incubation (67-70 days).

Dam genotype effect was significant ($p < 0.05$), affecting percentage fertility and hatchability. This result also is consistent with the reports of Garviria-Hernandez *et al.* (2021). The West African Mud (Pc) dam produced the highest number of fertile eggs and pancake (Pa) dam the least with corresponding percentage values of 91.67% and 83.33%, respectively. Dam genotype effects on fertility have frequently been attributed to differences in egg production (Kuchling and Hofmeyr 2022) or body composition (Deeming, 2018; Loehr *et al.*, 2019; Gibbs *et al.*, 2020; and Vlachos, 2020). The differences observed in fertility that resulted in the West African Mud dams having the highest fertile eggs and the pancake dam the lowest might be due to possible group/genotype differences in sperm storage and release (Mwaya *et al.*, 2018; Epperson *et al.*, 2020; Pike *et al.*, 2021 and Mendoza *et al.*, 2022). The mean values of hatchability as affected by dam genotype in this study suggest that hatchability may not entirely be a function of fertility, because of some intrinsic factors associated with the eggs (Rhodin *et al.*, 2021; Stemle *et al.*, 2022).

A significant interaction between sire and dam genetic group influenced percentage fertility and hatchability as presented in Table 2.

The least square means of the first order interaction between sire and dam genotypes showed that Marginata tortoises as dams produced a high percentage of fertile eggs in contrast to other genetic groups. Similarly, the percentage hatchability of eggs laid by this group was significantly lower than the values observed for the other groups.

Table 2: Least Squares Means of Egg Parameters as Influenced by the Interaction between Sire and Dam Genotype

Variable	No. of egg laid	No. fertile	No. hatched	% fertile	% hatch
Overall	62	55	48	88.71	87.27
Sire and Dam Genotype					
Po x Po	5 ^b	5	4	100.00±7.32 ^a	80.00±5.63 ^c
Po x Ps	8 ^a	7	6	87.50±4.48 ^b	85.71±5.38 ^b
Po x Pc	3	2	2	66.67±3.75 ^d	100.00±7.23 ^a
Po x Pa	4 ^c	4	3	100.00±7.20 ^a	75.00±5.61 ^d
Ps x Ps	3 ^d	3	3	100.00±6.84 ^a	100.00±7.08 ^a
Ps x Po	3 ^d	3	3	100.00±7.05 ^a	100.00±6.36 ^a
Ps x Pc	5 ^b	4	4	80.00±4.85 ^c	100.00±7.51 ^a
Ps x Po	3 ^d	2	2	66.67±3.61 ^d	100.00±7.40 ^a
Pc x Pc	5 ^b	3	2	60.00±4.72 ^e	66.67±4.93 ^e
Pc x Pa	4 ^c	4	3	100.00±7.53 ^a	75.00±5.21 ^d
Pc x Ps	2 ^e	2	2	100.00±6.85 ^a	100.00±6.45 ^a
Pc x Pa	3 ^d	3	2	100.00±7.51 ^a	66.67±4.62 ^e
Pa x Pa	2 ^e	2	2	100.00±7.38 ^a	100.00±8.12 ^a
Pa x Po	4 ^c	4	3	100.00±6.89 ^a	75.00±4.85 ^d
Pa x Ps	5 ^b	4	4	80.00±5.41 ^c	100.00±7.54 ^a
Pa x Pc	3 ^d	3	3	100.00±6.94 ^a	100.00±6.80 ^a

Values within the same column with different letters are significantly ($p < 0.05$) different

Purebred mating involving pancake tortoises produced about 11.29 per cent infertility corroborating the report of Tiar-Saadi *et al.*, (2022) that the marginata genes is likely to be lethal when present in homozygote form. Mating between West African Mud (Pc) and West African Mud (Pc) tortoises resulted in relatively lower fertility and hatchability contrasted with others, indirectly indicating that, there is a possibility of low combining ability between the genes controlling these traits. This indicates that inbreeding should be avoided in the tortoise breeding farm.



CONCLUSION AND RECOMMENDATION

The results showed that the variations in the genetic groups of the tortoise significantly affected fertility and hatchability of eggs from this species of tortoise. Fertility and hatchability reduced when sires and dams carrying the West African Mud gene were involved in mating to produce fertile eggs. Similarly, purebred mating of pancake tortoise resulted in about 11.29% infertile confirming earlier suggestion of lethal effect of the gene controlling this trait. The marginata genetic group was generally superior to the other groups.

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