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Genetic Polymorphism of the *DCT* gene in local ducks raised in Iraq

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Abstract

The study was conducted in the field of waterfowls birds breeding / College of Agriculture / University of Basrah during the period from 5/12/2023 to 1/30/2024, to detect the genetic polymorphism of the (*DCT*) gene. In this study (270 chicks from three genetic groups of local ducks were used according to the feather colours (white, gray) and mallard ducks with (90) chicks for each group. (25) blood samples were collected from each group at the age of (6) weeks. *DCT* gene amplification and DNA sequencing revealed four base-change sites (70T>C), (148C>T), (234G>A), and (370A>G) and a group of alleles associated with feather colour. The genotypes showed an equilibrium of different mutation sites, the genetic mutation (70T>C) was equilibrium in all feather colour groups of ducks, while the other genetic mutation (148C>T) is for ducks with gray and mallards, and the two genetic mutations (234G>A) for white and (370A>G) are in ducks with white and gray feathers. Shannon's index values increased for all mutation sites in local ducks. The frequencies of observed heterozygous genotypes were higher than the frequencies of observed homozygous genotypes, and negative values for the stability index were recorded for all mutation sites.

Keywords: Genetic Polymorphism; Plumage Color; DCT gene; local ducks

I. Introduction

Ducks are among the most important local birds species, known globally for their meat and egg production, in addition to their by-products of livers and feathers. Global production of duck meat increased during the period from 2000 to 2011, reaching 1.3 million tons. Asian countries account for 84% of this production, with China contributing 73%, while Southeast Asian countries produce 83% of the total global production. Consumption of duck meat has also increased over the past decade due to its richness in unsaturated fatty acids (Cherry & Morris, 2008; Miffaf, 2013; FAO, 2014). Global interest in conserving ducks as a genetic resource has increased, allowing for the study of the genetic diversity of different breeds using molecular markers (Sharma *et al*., 2015; Al-Kurd et al.,2019). The Dopachrome tautomerase gene (*DCT*) is an important gene in the field of duck phenotyping. It is located on chromosome 1 and consists of 9 exons and 8 introns. It plays a major role in the synthesis of melanin pigment responsible for the colour of feathers, skin, and retina. It is considered an important factor in selecting the phenotypes of desired feather colors in ducks. It has been observed that genetic mutations in this gene are associated with different feather colors, which has helped in determining the origin of ducks and the extent of their environmental adaptation (Sultana *et al*., 2014).

The *DCT* gene produces the genetic code for the *DCT* enzyme, which catalyzes the conversion of dopachrome to DHICA, 5,6-dihydroxyindole-2-carboxilic acid, an intermediate in the melanin biosynthesis pathway. There are two types of melanin: eumelanin, which produces black or dark brown feathers, and pheomelanin, which produces red or

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yellow. The balance between the production of these two types of melanin determines the feather color of ducks (Huang *et al*., 2013; Sultana *et al*., 2015). The relationship of several genetic mutations in this gene to feather color has been studied using DNA sequence analysis. A study by (Hasina *et al*., 2015), revealed the 726C>T mutation in the fourth intron of the *DCT* gene to differentiate Korean ducks from commercial ducks with white feathers. Sultana *et al*. (2018) also revealed a genetic mutation 938(A>G) in the fifth exon of the *DCT* gene, where the allele (G) was observed to be associated with black feather color and the allele A was associated with white feather color in the Asian duck, while the study showed that the other genetic mutation (762C>T) of the *DCT* gene in the fourth exon was not associated with feather color in the Asian duck, and there was no significant association between the two alleles (C, T) and the inheritance of black and white feather color. Some genetic mutations in the *DCT* gene play a role in reducing the activity of the *DCT* enzyme and affecting the ratio of eumelanin to pheomelanin, which leads to the appearance of feathers in different colors (light, white, white and black). Also, increased gene expression of the *DCT* gene may affect the activity of the *DCT* enzyme and thus make the feather color dark, as a result of changing the amino acid sequence of the *DCT* enzyme protein from the normal sequence, which affects its function and makes it ineffective (Li *et al*., 2012; Wang *et al*., 2024). This study aimed to reveal the genotypes of the *DCT* gene and to study the relationship between the gene's polymorphisms and feather colour and genetic diversity in local ducks in Iraq.

II. Materials and Methods

The study was conducted in the Waterfowl Breeding Field/Agricultural Research and Experiment Station/College of Agriculture/University of Basrah from December 5th , 2023 to January 30th , 2024. (270) birds from three groups of local ducks (white, grey, and green) were raised from one day old to (8) weeks old. The birds were raised in a hall with dimensions of (18 x 5 x 2.5) m², divided into six rooms (2 m² each). The birds were distributed with (90) birds per genetic group, with two replicates (45 birds per replicate). Birds were fed *ad libitum* on starter diet (18% protein and 2900 kcal/kg) from 0-2 weeks of age and grower diet (20% protein and 3100 kcal/kg) from 3-8 weeks of age (NRC, 1994).

(25) blood samples were collected from the jugular vein from each group at the age of (6) weeks and the samples were stored in tubes containing anticoagulant (EDTA) placed inside a refrigerated container at 20°C. The steps for extracting deoxyribonucleic acid (DNA) were used according to the steps recommended in the Geneaid Kit prepared by the American company, Promega. DNA was detected using electrophoresis on agarose gel (Sultana et al., 2015). The forward (GGCTTTTGAACCACATTCAAGGCF) and reverse (GCCTTATGCCATCTGGGATGR) primers for the *DCT* gene were used, the used primers were prepared by the Korean company, Macrogene in the form of a lyophilized powder. The nitrogenous base sequences of the primers were matched with the National Center for Biotechnology Information database (NCBI) (Sultana *et al*., 2015) (Hasina *et al*., 2015). The forward and reverse primers were dissolved by adding 300 µL of deionized water to be the concentration 100 pmol (stock solution). To dilute the primer, 10 µL of the stock solution was taken and 90 µL of distilled water was added to it to be the concentration 10 pmol (Sultana *et al*., 2015). The Polymerase Chain Reaction (PCR) program (Table 1) was used to amplify the target fragment of the *DCT* gene.

Table (1): Steps of PCR reaction

Gene	Steps	Temperatures	Time	Number of cycles
	Initial denaturation	95C°	5	1
	Denaturation	95C°	0.30	

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DCT Gene	Annealing	54 C°	0.30	35
	Elongation	72C°	0.45	
	Final elongation	72C°	10	1

The amplification product was electrophoresed on a 1.5% agarose gel containing 0.45 g agarose in 30 ml of TBE 1x buffer. 5 µL of PCR product was loaded with 2 µL of Diamond™ Nucleic Acid Dye into each pit, with one pit designated as the DNA Ladder. The transformation was run at 70 V, 85 mA for 45 min, and the band images were captured using a UV Gel Documentation device (Sultana et al., 2015; Sultana et al., 2018). Samples were sent to the Korean company (Macrogene) for analysis of the nitrogenous base sequences of the target region of the gene. The forward and reverse strands were then sequenced and aligned using Bio Edit software for comparison with the NCBI database. Genotypes were determined according to the mutation sites of the *DCT* gene using the Pop gene program to calculate the numbers of birds observed and expected, allelic frequencies, genotype frequencies, chi-square, Shannon index, and stability index (Hall, 1999).

III. Results and Discussion

Electrophoresis of the *DCT* gene amplification results and the nitrogenous bases change sites

The electrophoresis results on a 1.5% agarose gel showed that the *DCT* gene amplification results showed a band of (652) base pairs (Photo 1).

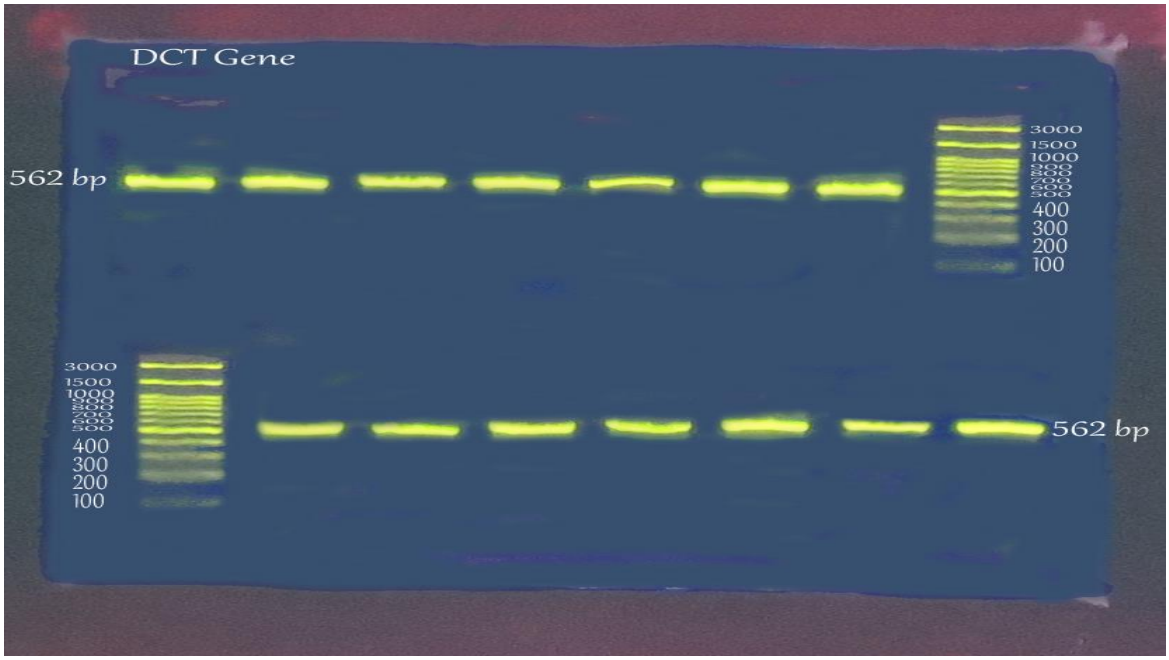

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Photo (1): Amplification product of the *DCT* gene on a 1.5% agarose gel

Four sites of base changes were detected in the first exon of the *DCT* gene using DNA sequence analysis: the site (70T>C) (Fig. 1) which is a silent mutation leading to the change of the nitrogenous base thymine (T) to the nitrogenous base cytosine (C) and causing the change of the nitrogenous base sequences from AGT to AGC which was registered in GenBank according to the genotypes (TT, TC, CC) under the accession numbers (LC822357), (LC822358) and (LC822359) respectively.

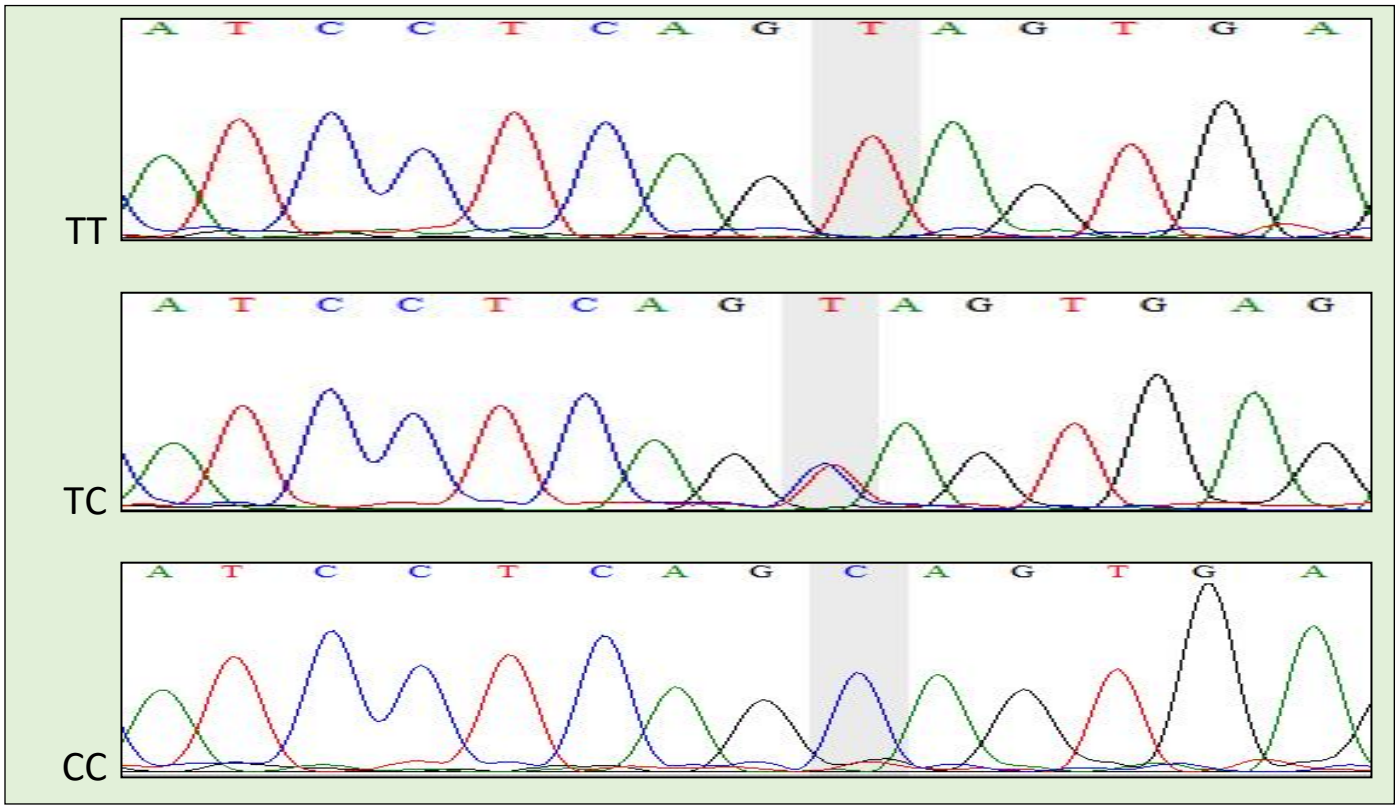

Figure (1) The site of the nitrogenous base change for the genetic mutation (70T>C) of the *DCT* gene.

Figure (2) also shows the site (148C>T *DCT*) which resulted from the occurrence of a silent genetic mutation leading to the change of the nitrogenous base cytosine (C) to the nitrogenous base thymine ((T) which resulted in a change in the sequences of nitrogenous bases from CTC to CTT which was recorded in the gene bank for each genetic combination (CC, CT, TT) under the accession numbers (LC822360), (LC822361) and (LC822362) respectively.

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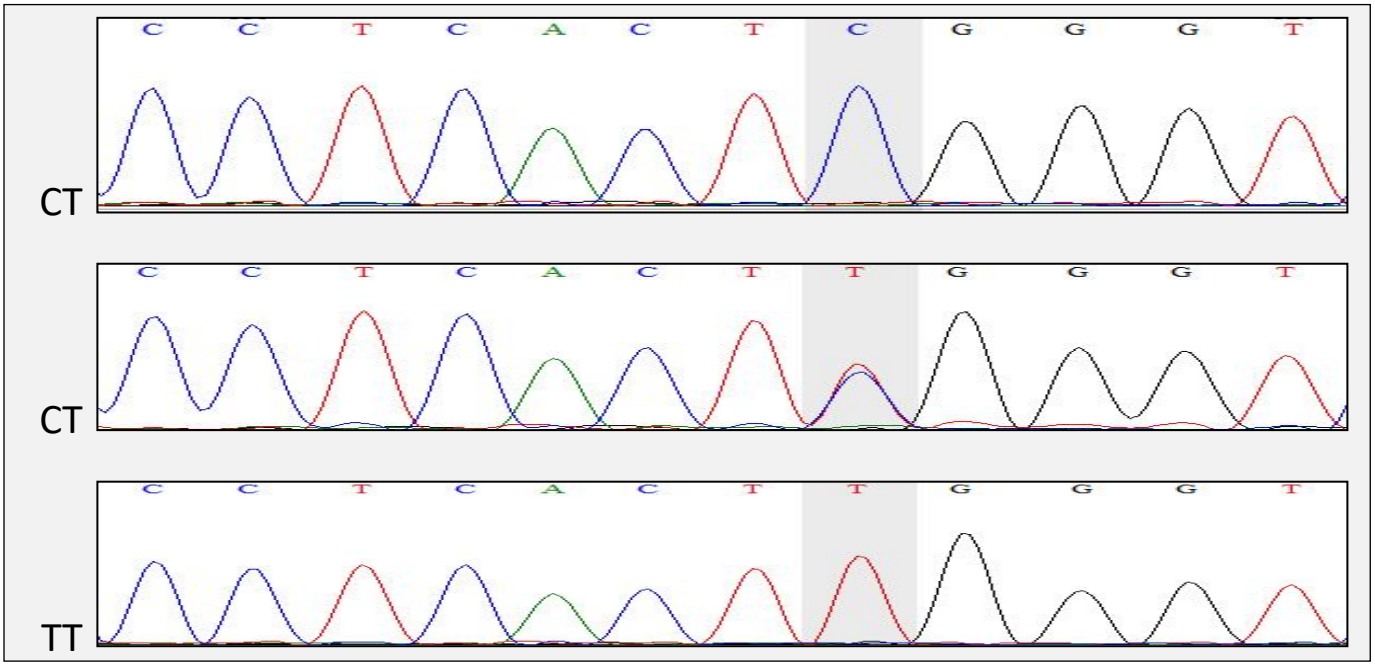

Figure (2) The site of the nitrogenous base change for the genetic mutation (148C>T) of the *DCT* gene

Figure (3) shows the site ((234G>A) of the missense genetic mutation resulting from changing the nitrogenous base guanine (G) to the nitrogenous base adenine (A) and changing the sequence CGC to CAC. It was registered in the gene bank according to the genetic structures (GG, GA, AA) under the accession numbers (LC822363), (LC822364), and (LC822365), respectively

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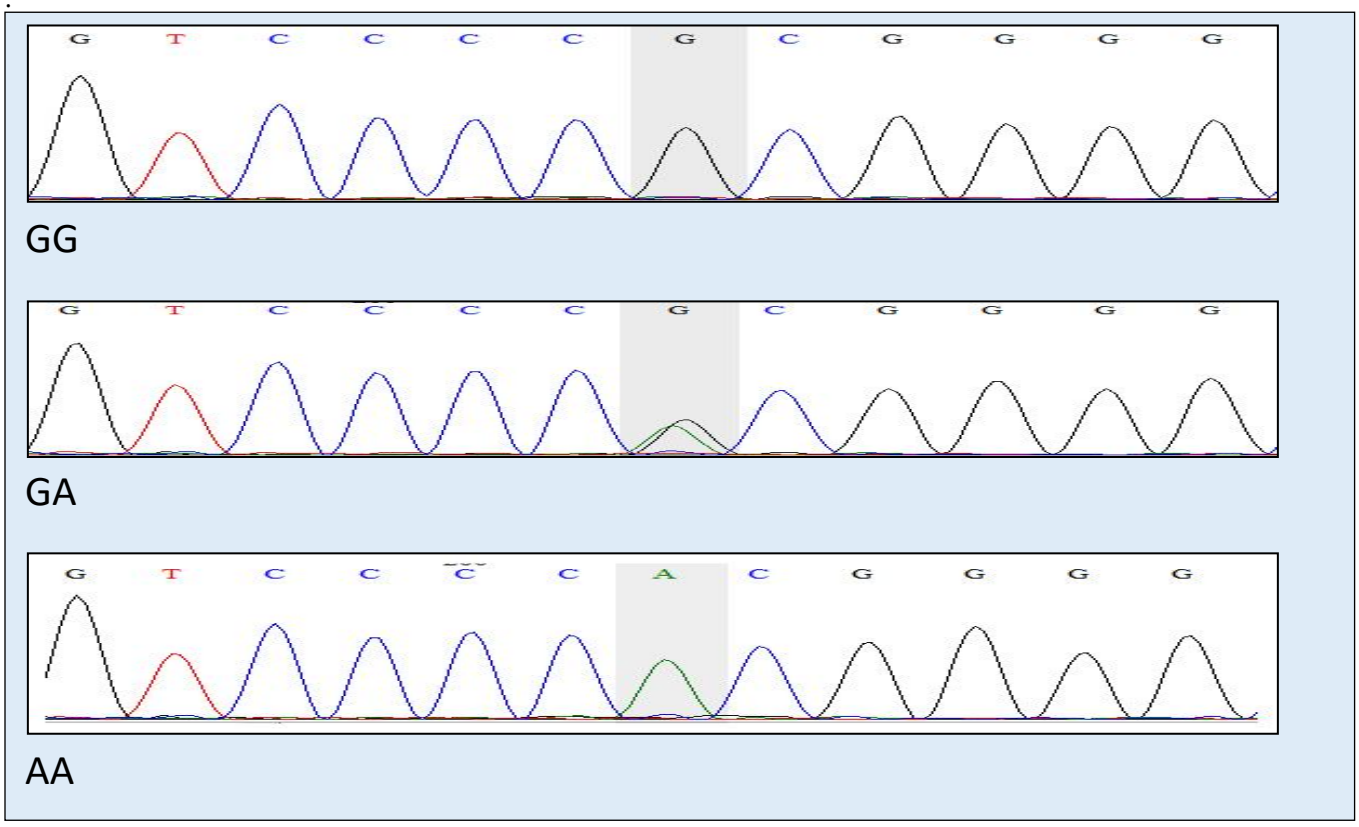

Figure (3) The site of the nitrogenous base change for the genetic mutation (234G>A) for the *DCT* gene

Figure (4) also shows a base change in the nitrogenous base sequences from GTA to GTG, which resulted from a silent genetic mutation at the site ((370A>G) and led to the change of the nitrogenous base adenine (A) to the nitrogenous base guanine (G), which was recorded in the gene bank for each genetic combination (GG, AG, AA) under accession numbers (LC822366), (LC822367), and (LC822368), respectively.

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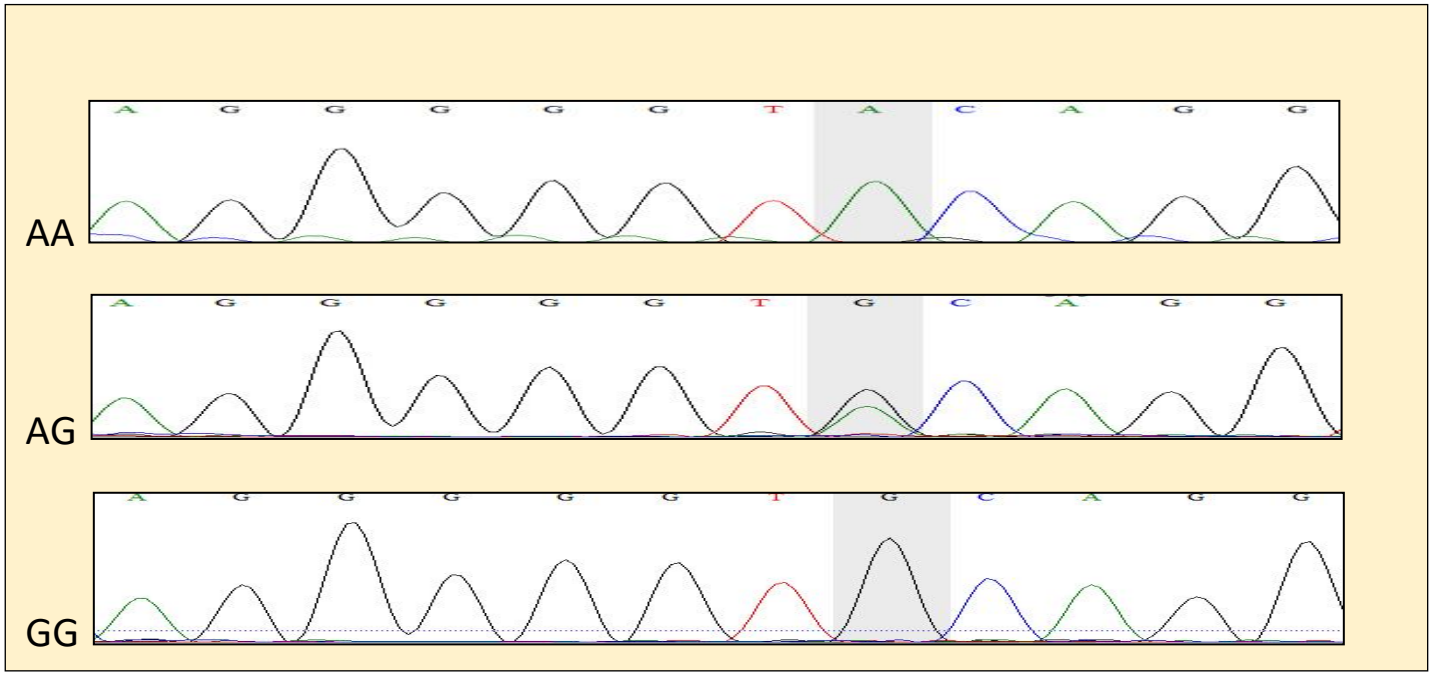

Figure (4) The site of the change in the nitrogenous base of the genetic mutation (370G>A) of the *DCT* gene

Genotypes, genotypic and allelic frequencies of the *DCT* gene's nitrogenous base-change sites

The results showed that the number of birds studied for genetic mutation sites in the DCT gene amounted to 69 local ducks. The results of Table (2) reveal the detection of three genotypes for the genetic mutation site (70T>C): which are (TT, TC, TT). It is noted that the number of birds observed with the homozygous genotype (TT) is greater than the number of birds observed with the heterozygous genotype (TC), with the number of birds reaching (15, 9) in the group of the white-feathered ducks, respectively. No bird carrying the homozygous genotype (CC) was observed. Regarding the local duck groups with grey feathers and mallard duck, it is noted that the number of birds with the heterozygous genotype (TC) was increased compared to the number of birds with the two homozygous genotypes (TT, CC), reaching (12, 14) respectively. It is also noted that the frequency of the (T) allele increased compared to the frequency ratio of the (C) allele in all local duck groups, as it reached (0.81, 0.57, 0.67) respectively in the local ducks with white, grey feathers and mallard duck, while the frequency of the (C) allele reached (0.19, 0.43, 0.33) respectively. The chi-square (X^2) values were less than the tabular F values for the local duck groups, reaching (1.11, 0.42, 2.05) respectively.

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Table (2) Genotype, numbers of observed and expected birds, genotypic frequency (%), allelic frequency (%), and chi-square values (X^2) for the genetic mutation site (70T>C) of the DCT gene for local duck groups according to the feather colours.

genetic group	Genotype	numbers of observed birds	numbers of expected birds	Genotypic frequency (%)	Alleles	Allelic frequency(%)	chi-square (X^2)
White Feather Group	TT	15	15.76	0.63	T	0.81	1.11
	TC	9	7.46	0.37	C	0.19	
	CC	---	0.46	0			
Total Number	24						
Gray Feather Group	TT	6	6.73	0.29	T	0.57	0.42
	TC	12	10.53	0.57	C	0.43	
	CC	3	3.73	0.14			
Total Number	21						
Mallard duck Group	TT	9	10.55	0.38	T	0.67	2.05
	TC	14	10.89	0.58	C	0.33	
	CC	1	2.55	0.04			
Total Number	24						

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The results of Table (3) indicate the detection of three genetic genotypes for the genetic mutation site (148C>T) of the *DCT* gene, these genotypes are (TT, CT, TT) in the local duck groups according to the feather colours, it is noted that the number of birds with the heterozygous genetic genotypes (CT) for the white, gray feathers, and mallard ducks increased compared to the number of birds observed with the two homozygous genetic genotypes (TT, CC), the number of birds reached (18, 10, 13) respectively, these results were accompanied by an increase in the genotypic frequency of (CT) genotype in these groups compared to the frequency of the two pure genetic genotypes (TT, CC). The results also indicates an increase in the frequency of the (C) allele compared to the frequency of the (T) allele in the groups of the local white feathers and mallard ducks, reaching (0.58, 0.56) respectively and (0.42, 0.44) respectively, while the frequency of the (T) allele and the (C) allele in the gray duck group reached (0.52, 0.48) respectively. The chi-square (X^2) value of the genetic mutation site (148(C>T) for the local white duck was higher than the grey feathers and mallard ducks groups, reaching (6.54).

Table (3) Genotype, numbers of observed and expected birds, genotypic frequency (%), allelic frequency (%), and chi-square values (X^2) for the genetic mutation site 148(C>T) of the DCT gene for local duck groups according to the feather colors.

genetic group	Genotype	numbers of observed birds	numbers of expected birds	Genotypic frequency (%)	Alleles	Allelic frequency(%)	chi-square (X^2)
White Feather Group	TT	1	4.04	0.04	T	0.42	6.54
	CT	18	11.91	0.75	C	0.58	
	CC	5	8.04	0.21			
Total Number	24						
Gray Feather Group	TT	6	5.63	0.29	T	0.52	0.10
	CT	10	10.73	0.48	C	0.48	
	CC	5	4.63	0.23			
Total Number	21						

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mallard Group	TT	4	4.46	0.17	T	0.44	0.15
	CT	13	12.06	0.54	C	0.56	
	CC	7	7.46	0.29			
Total Number	24						

Three genotype of the genetic mutation site (234G>A) were also detected, which are (GG, GA, AA) for the local duck groups (Table 4). It is noted that the number of birds observed with the heterozygous genotype (GA) increased compared to the number of birds carrying the two homozygous genotypes (GG, AA), reaching (15, 13, 17) birds, respectively. These results also indicate an increase in the genotypic frequency of heterozygous genotype (GA) compared to the genotypic frequencies of the two homozygous genotypes (GG, AA), reaching (0.63, 0.62, 0.71), respectively. It is also noted that the frequency of the (G) allele increased compared to the frequency of the (A) allele in all local duck groups. It reached (0.65, 0.55, 0.60) in the local ducks with white, gray feathers, and mallard ducks, respectively, while the frequency of the (A) allele reached (0.35, 0.45, 0.40) respectively. It is noted from the results that the chi-square value (X^2) for the genetic mutation site (234G>A) for mallard ducks was higher, reaching (5.09) compared to ducks with white and grey feathers.

Table (4) Genotype, numbers of observed and expected birds, genotypic frequency (%), allelic frequency (%), and chi-square values (X^2) for the genetic mutation site 234(G>A) of the DCT gene for local duck groups according to the feather colors.

genetic group	Genotype	numbers of observed birds	numbers of expected birds	Genotypic frequency (%)	Alleles	Allelic frequency(%)	chi-square (X^2)
White Feather Group	GG	8	9.89	0.33	G	0.65	2.88
	GA	15	11.21	0.63	A	0.35	
	AA	1	2.89	0.04			
Total Number	24						
Gray Feather Group	GG	5	6.17	0.24	G	0.55	

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	GA	13	10.65	0.62	A	0.45	1.06
	AA	3	4.17	0.14			
Total Number	21						
Mallard ducks Group	GG	6	8.63	0.25	G	0.60	5.09
	GA	17	11.72	0.71	A	0.40	
	AA	1	3.63	0.04			
Total Number	24						

The results of Table (5) show the detection of three genotypes for the genetic mutation site (370A>G), which are (GG, AG, AA) in local ducks. It is noted that the number of observed birds with heterozygous genotype (AG) increased compared to the number of observed birds with the two homozygous genotypes (GG, AA), reaching (15, 14, and 17) birds, respectively. The results also indicate an increase in the frequency of the heterozygous genotype AG) compared to the genotypic frequencies of the homozygous genotypes (GG, AA). The results also indicate an increase in the frequency of the (A) allele compared to the frequency rates of the (G) allele in all groups of local ducks, reaching (0.65, 0.57, and 0.60), respectively, while the frequency of the (G) allele reached (0.35, 0.43, and 0.40), respectively. The results also indicate a high chi-square value (X^2) for the genotype site (370A>G) in mallard-feathered ducks, reaching (5.09) compared to white and gray feathered ducks.

Table (5) Genotype, numbers of observed and expected birds, genotypic frequency (%), allelic frequency (%), and chi-square values (X^2) for the genetic mutation site 370(A>G) of the DCT gene for local duck groups according to the feather colors.

genetic group	Genotype	numbers of observed birds	numbers of expected birds	Genotypic frequency (%)	Alleles	Allelic frequency(%)	chi-square (X^2)
White Feather Group	GG	1	2.89	0.04	G	0.35	2.88
	AG	15	11.21	0.63	A	0.65	
	AA	8	9.89	0.33			

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Total Number	24						
Gray Feather Group	GG	2	3.73	0.10	G	0.43	2.38
	AG	14	10.53	0.67	A	0.57	
	AA	5	6.73	0.24			
Total Number	21						
Mallard Feather Group	GG	1	3.63	0.04	G	0.40	5.09
	AG	17	11.72	0.71	A	0.60	
	AA	6	8.63	0.25			
Total Number	24						

Number of observed alleles (na), number of effective alleles (ne), Shannon index values, and stability index among local duck groups

The results of Table (6) indicate a high value of Shannon's index, which ranged between (0.68-0.69), and the value of the likelihood square ranged between (0.12-0.74) for the grey-feathered ducks group for all genetic mutation sites compared to the white-feathered and mallard ducks. The results also indicate a high stability index for the white ducks, which ranged between (-0.23) to (-0.54) for all genetic mutation sites compared to the grey-feathered and green-feathered ducks, and that the stability index values are all negative for the local duck groups.

Table (6) Numbers of observed alleles, numbers of effective alleles, Shannon index values, likelihood square (G^2) values, and stability index values for the genetic mutation site of the *DCT* gene for local duck groups according to feather colors.

Genetic group	Mutation sites	Sample size	Alleles	Shannon index	Likelihood square (G^2)	Stability index
White Feather Group	70 T>C	48	C,T	0.48	0.29	0.23-

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	148 C>T	48	C,T	0.67	0.01	0.54-
	234G>A	48	G,A	0.65	0.08	0.36-
	370A>G	48	A,G	0.65	0.08	0.36-
gray Feather Group	70 T>C	42	C,T	0.68	0.51	0.16-
	148 C>T	42	C,T	0.69	0.74	0.04-
	234G>A	42	G,A	0.68	0.30	0.24-
	370A>G	42	A,G	0.68	0.12	0.36-
Mallard Group	70 T>C	48	C,T	0.63	0.15	0.31-
	148 C>T	48	C,T	0.68	0.69	0.10-
	234G>A	48	G,A	0.67	0.02	0.48-
	370A>G	48	A,G	0.67	0.02	0.48-

Percentages of observed and expected genotypes and average of heterozygous ratio of the *DCT* gene among local duck's groups

The results of Table (7) indicate that the observed genotypes ratios in the white-feathered ducks for all genetic mutation sites are high, ranging between (0.25-0.63) compared to the gray-feathered and mallard ducks. It is also noted that the heterozygous genotypes ratios for mallard ducks are high, ranging between (0.54-0.71) for all genetic mutation sites compared to the genotypes ratios for the local white-feathered and gray-feathered ducks. In general, the results show that the observed heterozygous genotypes ratios for the local duck groups are high, ranging between (0.38-0.71) compared to the observed homozygous genotypes ratios, which ranged between (0.25-0.63) for the local duck groups.

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Table (7) The percentages of observed and expected homozygous genotypes (%), the percentages of observed and expected mixed genotypes (%), and the average heterozygous ratio for the genetic mutation sites of the *DCT* gene for local duck groups according to feather colors.

Genotype	Mutation sites	Sample size	Observed homozygous genotypes(%)	Expected homozygous genotypes(%)	Observed mixed genotypes(%)	Expected mixed genotypes(%)
White Feather Group	70 T>C	48	0.63	0.68	0.38	0.31
	148 C>T	48	0.25	0.50	0.75	0.49
	234G>A	48	0.38	0.53	0.63	0.46
	370A>G	48	0.38	0.53	0.63	0.46
gray Feather Group	70 T>C	42	0.43	0.49	0.57	0.50
	148 C>T	42	0.52	0.48	0.48	0.51
	234G>A	42	0.38	0.49	0.61	0.50
	370A>G	42	0.33	0.49	0.66	0.50
Mallard Group	70 T>C	48	0.42	0.54	0.58	0.45
	148 C>T	48	0.45	0.49	0.54	0.50
	234G>A	48	0.29	0.51	0.71	0.48
	370A>G	48	0.29	0.51	0.71	0.48

The variation in the distribution of allelic frequencies and genetic frequencies of the DCT gene across mutation sites and among duck groups in the present results may be attributed to the fact that higher-frequency alleles are considered more suitable for environmental conditions compared to other less frequent alleles. Individuals of heterozygous genotypes may have a higher ability to adapt and survive compared to individuals with homozygous genotypes. Non-

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random mating such as inbreeding, migration, and gene flow may have an effect on changing allelic frequencies and genotypes distribution, and consequently affect the equilibrium state according to the genetic mutation sites in small populations (Sultana *et al*., 2018; Ahmed & Ameen., 2024). In this regard, the results of the study by Sultana et al. (2018) revealed the different allelic frequencies of the *DCT* gene according to the mutation sites in the Asian black and white feathered duck. The frequencies of the two alleles (T, C) reached (0.63, 0.37), (0.66, 0.34) respectively for the genetic mutation site 726(C>T) of the DCT gene in the fourth exon, while the allelic frequencies of the genetic mutation site 348(A>G) of the DCT gene in the second exon reached (0.50, 0.50) for the ((G) allele and (0.49, 0.51) for the (A) allele, while the allelic frequencies of the genetic mutation sites 468(A>G) reached (0.74, 0.26), (0.85, 0.15) respectively.

The moderate Shannon index values for the *DCT* mutation sites in our results indicate that local duck groups are characterized by genetic diversity, enabling them to adapt to environmental conditions. This may be due to the effect of natural selection on achieving a balance between the alleles of this gene, which maintains the proportion of heterozygous genotypes in local duck groups and positively impacts their genetic diversity (Wu *et al*., 2008; Tubelyte *et al*., 2011; Ahmadi *et al*., 2007). In this regard, a study by Ahmed & Ameen (2024) on local ducks in the Kurdistan Region of Iraq confirmed the diversity of local ducks, with an average Shannon index value of 0.147.

The results of the study by Tunca *et al*., (2015) showed that the Shannon index value for some duck groups in Anatolia was (0.198). The results of the study by Padhi & Sahoo, (2022) conducted on Kuzi ducks with white, grey, and blue feathers showed that the percentages of phenotypic were (0.57, 0.57, and 0.34), respectively. The study by Padhi *et al*., (2022) conducted on Kuzi ducks with multi-colored, white, black, white and black, and grey) feathers observed that the percentages of phenotypic according to feather color were (80.76, 2.21, 1.41, 6.89 and 0.55), respectively.

IV. Conclusion

The results of a study examining the effect of the *DCT* gene on melanocyte expression, the melanin synthesis pathway, and feather colour expression, as well as the detection of four mutation sites and the frequencies of their genotype frequencies, showed that Shannon's index values increased while the stability index (negative) values decreased, indicating the instability of the local duck genetic population. Furthermore, the proportions of mixed genotypes were higher than those observed in local duck groups, due to their adaptability, survival, and high genetic diversity.

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