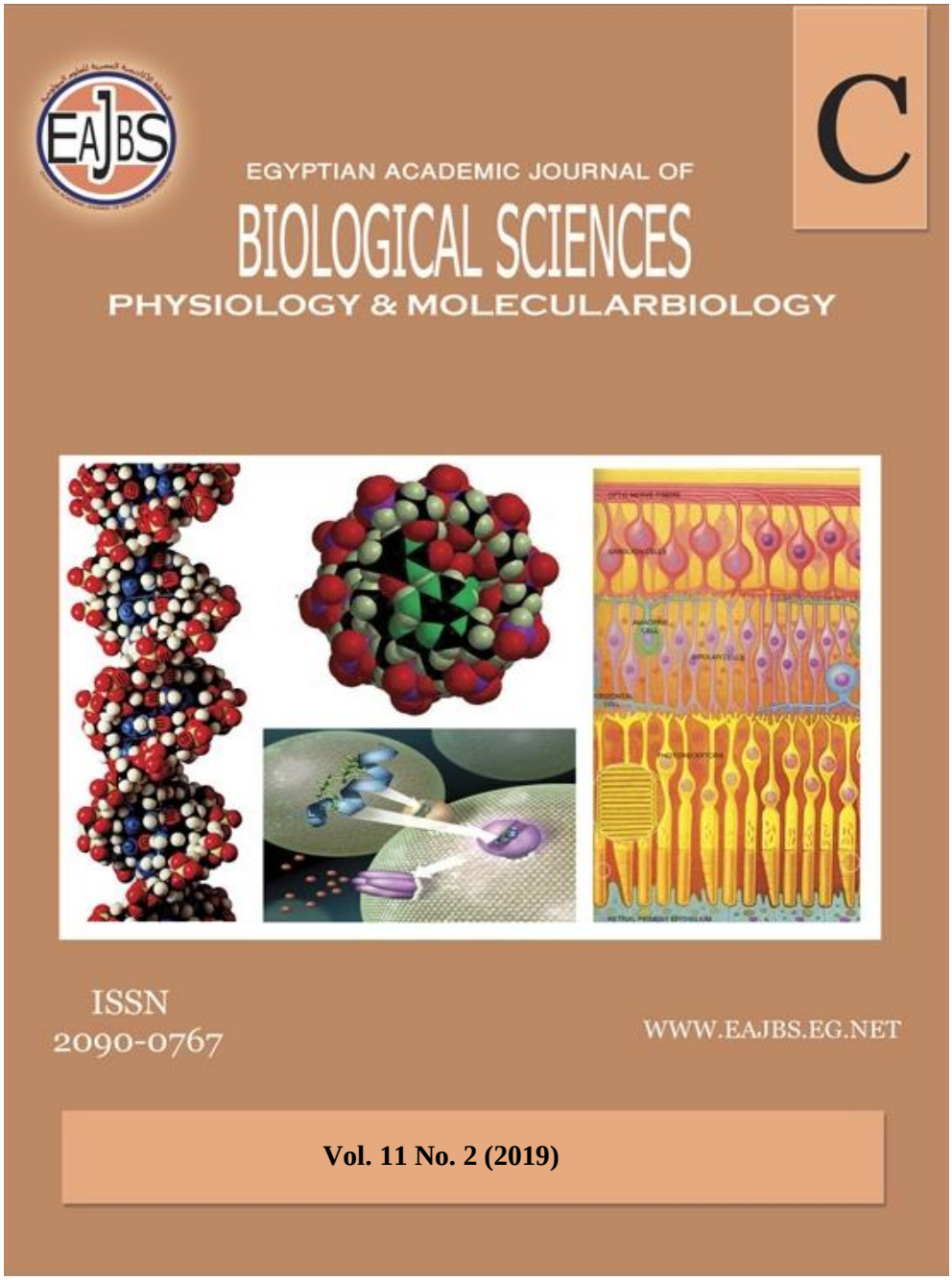

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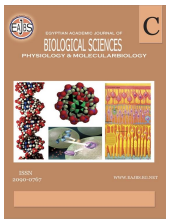

Constitutive Heterochromatin Pattern of Five Domestic Duck Breeds, (Aves: Anatidae) in Egypt

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ABSTRACT

Constitutive heterochromatin patterns of five domestic duck breeds three of *Anas platyrhynchos* (Dumyati, Khample and Pekin) and two of *Cairina moschata* (Muscovy and Sudani) occurring in Egypt have been described. Results indicated that although the constitutive heterochromatin (C-banding) pattern of three *Anas platyrhynchos* breeds shared the same distribution of heterochromatin there was an obvious variation in heterochromatin size. In addition, the C-banding patterns of the two *Cairina moschata* breeds (Muscovy and Sudani) were approximately similar. There was variation in size, number and occurrence of C-band blocks on micro-chromosomes between the examined five duck breeds. This may be attributed to the variation of euchromatin content and its correlation with chromosome size and arrangement of constitutive heterochromatin. Furthermore, it may be due to genome mixing through hybridization between related species and/or genera. The present results have shown that discrimination between *Anas platyrhynchos* (Dumyati, Khample and Pekin) and *Cairina moschata* (Muscovy and Sudani) duck breeds could be realized via cytogenetical markers such as presence of entirely heterochromatic chromosome pair no.8 in breeds belong *Anas platyrhynchos* whereas it was euchromatic in breeds of *Cairina moschata*. In addition to chromosome pair no.8, C-banding pattern of autosome pair no.3 and sex chromosome Z Could discriminate between the duck breeds of *Anas platyrhynchos* and *Cairina moschata*. Moreover, the constitutive heterochromatin amounts have significantly varied between the ducks belonging to the two genera. Thus, about only one-third of the micro-chromosomes in the three *Anas* breeds, exhibited large and visible C-banding blocks in both males and females while up to half of them in the two *Cairina* breeds. Hence, the duck breeds common in Egypt could be distinguished from others occurring elsewhere by C-banding pattern but this will be confirmed in further biochemical and molecular studies (under publication).

INTRODUCTION

Ducks belong to the order Anseriformes, family Anatidae, (Linnaeus, 1975). They have diverged from the chicken (Galliformes) a long time ago. The main radiation of modern duck took place during the Miocene, 5-23 million years ago (Olson, 1985). Ducks together with the ostrich, emsu, peacock, turkey, quail, and other birds play a major role in studies on bird evolution. They are important species in poultry production. Moreover, they are valued for such products as meat, fat, eggs and down-feathers (for making bedding and warm jackets). Domestic ducks have served as a source of food and income for people in many parts of the world. Their meat and eggs are good dietary sources of high-quality protein, energy and several vitamins and minerals. Seo, *et al.* (2016) reported that domestic ducks are believed to have originated from the Mallard (*Anas platyrhynchos*) and spot-billed duck (*Anas poecilorhyncha*). Some of the well-known breeds of common ducks include the Pekin, Aylesbury, Rouen, Call, Indian Runner, Khaki Campbell, Cayuga, Albio, Maya, and Tsaiya. Different breeds and varieties of common ducks can interbreed and produce fertile offspring (William and Tirah, 2014).

In Poland, the most commonly raised species of duck are the Mallard duck and the Muscovy duck. Moreover, The Pekin Duck is a domesticated form of the Mallard (*Anas platyrhynchos*), and the domestic Muscovy duck is derived from the wild Muscovy duck native to Central and South America (*Cairina moschata*). It has capable of adapting to different climates (Su, *et al.*2006).

In Egypt, there are five well-known domesticated Duck breeds. Pekin is the most common type of duck breeds for egg and meat production (FAO, 2014). The other known breed is Khample duck, a domesticated duck that originated in England and is kept for its high level of egg production. Moreover, an indigenous breed of Duck known near the Mediterranean shore in a coastal city in north-east, Egypt is Dumyati and named after Damietta. These three breeds (Pekin, Khample and Dumyati) are taxonomically belonging to *Anas Platyrhynchos*. In addition, Muscovy duck, (also in some contexts, Barbary duck) is a large duck native to Mexico, Central, and South America and Sudani duck is an indigenous breed of duck known all over Egypt and is similar to the Muscovy duck in its features and characteristics, with mixed white and black feathers. These latter two breeds (Muscovy and Sudani) are belonging to *Cairina moschata*.

Many Cytogenetic studies were aimed to develop a standard for chromosome banding patterns for ducks and geese (Apitz *et al.*, 1995; Denjean *et al.*, 1997; Andraszek and Smalec, 2007; W´ojcik and Smalec, 2007a, b;W´ojcik and Smalec, 2008a,b; W´ojcik and Smalec, 2017; Shahin *et al.*, 2014; Ata *et al*, 2005, 2007, 2012 and 2017). The only band pattern standard thus far approved by the International System for Standardized Avian Karyotypes is for *Gallus domesticus* (Ladjali-Mohammedi *et al.*, 1999). Cytogeneticists have shown little interest in birds due to their karyotype specificity, (i.e. small size chromosomes, and the division into macro and microchromosomes). Although birds have high degree of conservatism in their chromosomes, the number in chromosomes can range from 2n = 40 to 142 (Christidis, 1990; Rodionov, 1997; Griffin *et al.*, 2007). In ducks (*Anas platyrhynchos* and *Cairina moschata*) and geese (*A. anser* and *A. cygnoides*) the diploid number of chromosomes is 80 (Denjean *et al.*, 1997; W´ojcik and Smalec, 2007a, b;

W´ojcik and Smalec 2008a, b; W´ojcik and Smalec, 2017 Shahin *et al.,* 2014; Ata *et al.* 2017). Macrochromosomes are 4 to 15 μm long and they are about ten pairs in the karyotype (W´ojcik and Smalec, 2007a, b; W´ojcik and Smalec, 2008a, b), while the remaining pairs of chromosomes are dot-like microchromosomes and are usually smaller than two microns in length (Christidis, 1989; Shetty *et al.,* 1999). Microchromosomes are rich in G-C pairs and characterized by a high frequency of crossover, which is conducive to proper segregation in cell divisions. A large number of GC bases in the microchromosomes is due to the loss of bases characteristic of repeated sequences, i.e. AT bases. Microchromosomes account for about 25 to 35% of the total length of the genome of birds. It is believed that most of the functional genes about 50% of the genes are located on microchromosomes (Fillon *et al.,* 1998; Gregory, 2002; Masabanda *et al.,* 2004; Griffin *et al.,* 2007, 2015). Conventional chromosome preparation does not always enable differentiation of bird chromosomes even in relation to the centromere location (Bitgood and Shoffner, 1990). Various techniques for staining chromosome structure (Chromosome banding) are used in the cytogenetic analysis. One of the most commonly used techniques is the CBG banding method (W´ojcik and Smalec 2007a, 2008a; 2017; Shahin et al., 2014). Constitutive heterochromatin, representing about 20 % of the genome, is a structural part of C-bands and has been proven to differentiate between very similar karyotypes (Shahin and Ata, 2004). It may locate in the centromeric, telomeric and interstitial parts of chromosomes or entirely heterochromatin as in W chromosome in birds (Wang and Shoffner, 1974; W´ojcik and Smalec 2007a, b 2008a, b, c; 2017; Shahin *et al.,* 2014). Heterochromatin includes DNA sequences that are tandem-repetitive, AT-rich or hypermethylated, mobile genetic elements, and sporadic genes (Henikoff, 2000; Reddy and Jia, 2008). It plays a role in genetic silencing, because genes located in its region or too close to heterochromatin domains can become silenced (Pidoux and Allshire, 2005; Reddy and Jia, 2008; Djupedal and Ekwall, 2008; Cam *et al.,* 2009). Constitutive heterochromatin and other proteins can enhance the initiation of replication process (Murzina et al., 1999; Maison and Almouzni, 2004).

In Egypt, conventional karyotype was made by (Ata *et al.,* 2017) in order to clarify and characterize the chromosome variation between the five domestic duck breeds (Dumyati, Khample, Pekin, Muscovy and Sudani). The results revealed that there was a similarity to some extent in the karyotype of the five duck breeds. Hence, the present study was undertaken to carry out the C-banding technique between the five ducks breeds in Egypt in order to: 1) identifying the five domestic duck breeds occurring in Egypt by using the C-banding technique. 2) Assessing karyotype evolution among these breeds. 3) Comparing the present results with those available on other duck species occurring elsewhere based on the available data on other duck species.

MATERIALS AND METHODS

Animals:

Domestic ducks from the five breeds were obtained from El-Serw Waterfalls Research Station, Dimiatta, Animal Product Research Institute, Agriculture Research Center, Egypt. Birds transported in large bird cages supplied with food to Molecular Genetics Lab., Faculty of Agricultural, Minia University. Samples were taken from 25 birds, 5 (3 males and 2 females) from each breed for mitotic chromosome preparation.

Chromosome Preparations:

The mitotic chromosome preparations were carried out

according to the air-drying method (Yosida, 1973) with some modifications (Ata *et al.,* 2005).

C-banding Technique:

C-bands were obtained by using the standard protocol of (Summner, 1972) with major modifications as described by Ata et al., 2005; 2017 and Shahin *et al.,* 2014. At least 20 metaphase spreads of each bird were photographed and analyzed using Olympus BX51 microscope with a C-4040 zoom digital camera. The C-banding size and distribution on the macrochromosomes (nine somatic pairs and ZW chromosomes) of both males and females in the five duck breeds were described. In addition, the location of C-band and their size in each macro-chromosome, the number of heterochromatin blocks per micro-chromosomes in the examined cells of the five duck breeds were calculated. Analysis of variance (ANOVA) for numbers of heterochromatin blocks in microchromosomes in each breed was statistically analyzed by using MSTAT C program version 2.10 (Gomez and Gomez, 1984). Moreover, the ideograms of band patterns on the analyzed macro-chromosomes were drawn for each breed using Microsoft Excel 2010 program.

RESULTS

In the present study, description and comparison of c-banding patterns of three duck breeds (Dumyati, Khample and Pekin) belonging to *Anas platyrhynchos* and two belonging to *Cairina moschata* (Muscovy and Sudani) were carried out. Generally, by investigating chromosomes stained by C-banding in the five domestic duck breeds, it was possible to compare and describe the karyotype of these breeds. Moreover, the results of C- banding revealed variation between duck breeds of *Anas platyrhynchos* and breeds of *Cairina moschata.* There was an obvious variation in size, occurrence of C-bands and number of C-bands blocks on micro-chromosomes between the examined duck breeds as illustrated in (Table 1; Figures 1,2,3&4). The ideogram (Figures 2,& 4) for duck breeds was constructed according to karyological data of (Ata *et al.,* 2017) on these five duck breeds.

1. ***Anas Platyrhynchos* Breeds*:***

1.1. Macrochromosomes:

C-banded metaphase chromosomes of males and females of the three duck breeds (Dumyati, Khample and Pekin) belonging to *Anas platyrhynchos* are shown in Figure (1). The C- banding size and occurrence in ten macrochromosome pairs were different in the same complement. No considerable differences of C-bands are found among chromosome complements of the three breeds. There was no obvious constitutive heterochromatin on chromosome pairs no. 1 and 2, while chromosome no. 3 exhibited a small weak C-band. Faint C-bands were observed on the centromeric regions of chromosome pair nos. 4 and 5. On the other side, chromosome pairs nos. 6, 7, and 9 showed clear C-banding blocks. In almost all examined cells, macro-chromosome pair no. 8 appeared as entirely heterochromatic (Figs. 1a, b, c and f). In addition, the subacrocentric Z chromosome has a small sized centromeric C-band. However, the W chromosome is entirely heterochromatic in all three *Anas* breeds (figures 1a, b, and c).

1.2. Microchromosomes

Blocks of constitutive heterochromatin were observed and counted in microchromosomes for each metaphase set in all the examined three duck breeds (Dumyati, Khample and Pekin) belonging to *Anas platyrhynchos*. Results revealed that the average numbers of C-band blocks per cell ranged from 20.13 in Dumyati male cells to 21.67 in Khample female cells. In addition, analysis of variance showed, there was no significant variation in the mean number of C-heterochromatin blocks between the three *Anas platyrhynchos* breeds

(Dumyati, Khample and Pekin). Moreover, statistical analysis revealed that mean number of C-heterochromatin blocks per cell was significantly different between males and females of the three *Anas platyrhynchos* breeds (Table 1).

2. ***Cairina Moschata* Breeds:**

2.1. Macrochromosomes

C-banding patterns of ten pairs macro-chromosome of two duck breeds (Muscovy and Sudani) belonging to *Cairina moschata* and their corresponding ideogram are shown in Figures (3a, b, c, d and 4). There was variation in the C- banding size and its occurrence in ten macrochromosome pairs. There were no visible C-bands on chromosome pairs nos. 1, 2 and 3 in almost all examined cells of the two *Cairina moschata* breeds (Muscovy and Sudani). In contrast, macrochromosome pairs nos. 4-9 showed visible and clear centromeric C-bands which ranged from small to large blocks. In addition, the acrocentric Z chromosome showed an obvious small terminal C-band on the centromeric region in both Muscovy and Sudani ducks. As expected, W chromosome was entirely heterochromatic (Figs. 3a and b).

2.2. Microchromosomes

The mean numbers of C-heterochromatin blocks on microchromosomes ranged from 30.47 in Sudani breed to 32.0 in Muscovy breed (Table 1). Results of analysis of variance revealed that there was no significant variation between the two *Cairina* breeds (Muscovy and Sudani). Moreover, the mean value of C-heterochromatin blocks was significantly different between males and females of the two *Cairina moschata* breeds.

Table 1. Mean Number ± Standard Error of C-banding blocks on microchromosomes of the five studied duck breeds

	Anas platyrhynchos			***Cairina moschata***		L.S.D
	Dumyati	Khample	Pekin	Muscovy	Sudani	
Females	21.00 ± 0.42	21.67 ± 0.37	21.47 ± 0.27	32.00 ± 0.46	31.33 ± 0.48	1.17
Males	20.13 ± 0.41	20.80 ± 0.12	20.27 ± 0.47	31.33 ± 0.48	30.47 ± 0.33	1.23
Both Sexes	20.57 ± 0.38	21.23 ± 0.24	20.87 ± 0.37	31.67 ± 0.47	30.90 ± 0.40	1.14

L. S. D least significant difference value

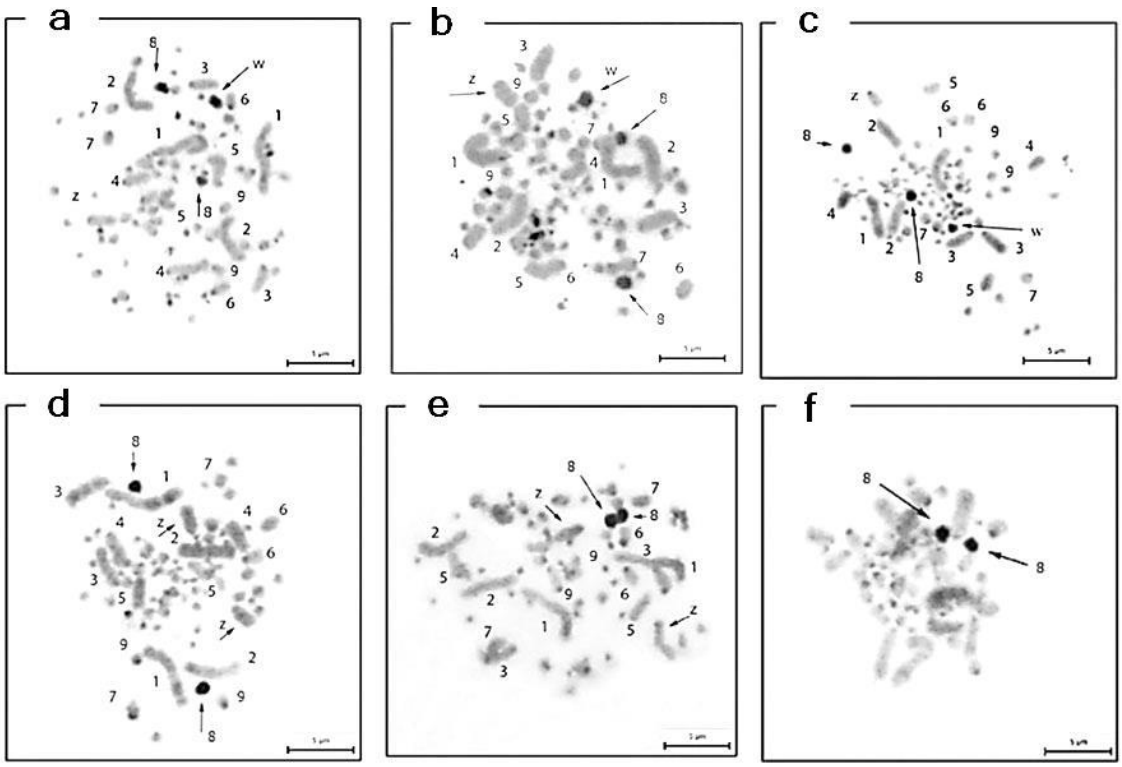

Fig. 1. Metaphase chromosome of Dumyati, Khample and Pekin breeds belonging to *Anas platyrhynchos* is showing C-bands of females (a, b and c) and Males (d, e and f), respectively. Arrows indicate to chromosome numbers according to their size, Chromosome pair no.8, Z chromosome and W chromosome. Scale bars = 5 microns.

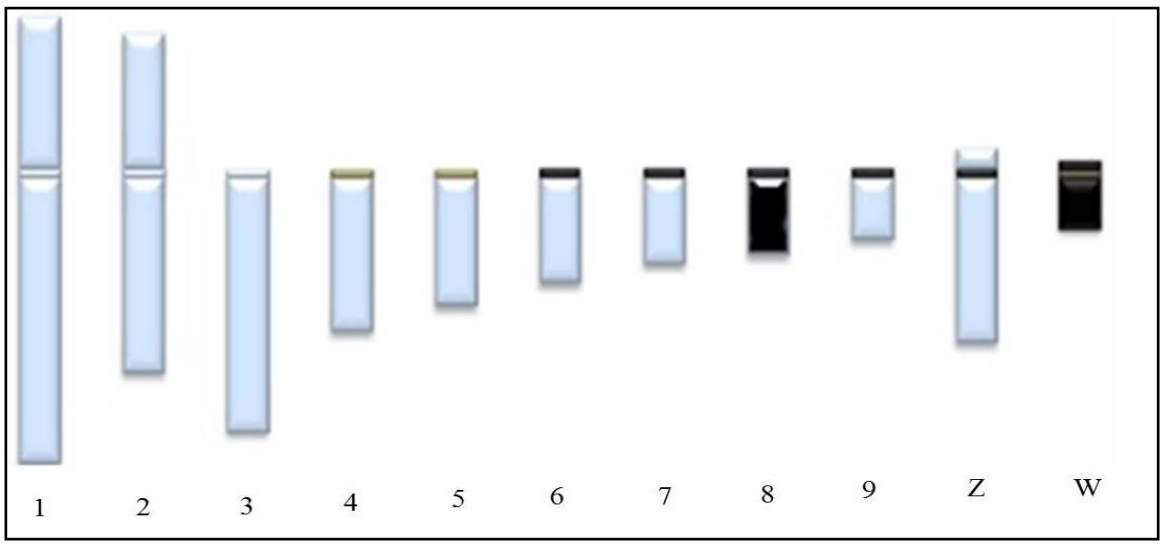

Fig.2. An Ideogram of macro-chromosomes illustrates the position and size of C-heterochromatin in the examined Dumyati, Khample and Pekin breeds belonging to *Anas platyrhynchos***.**

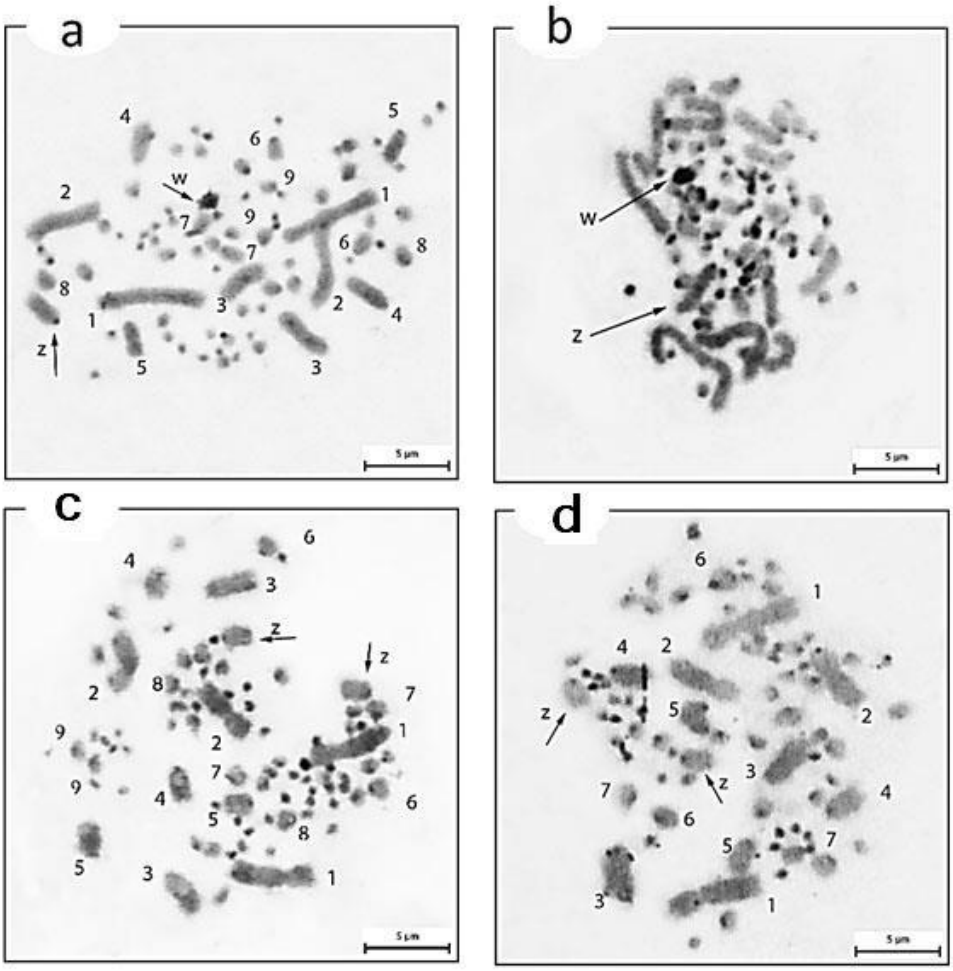

Fig. 3. Metaphase chromosome of Muscovy and Sudani breeds belonging to *Cairina moschata* is showing C-bands of females (a and b) and Males (c and d), respectively. Arrows indicate to chromosome numbers according to their size, Z chromosome and W chromosome. Scale bars = 5 microns

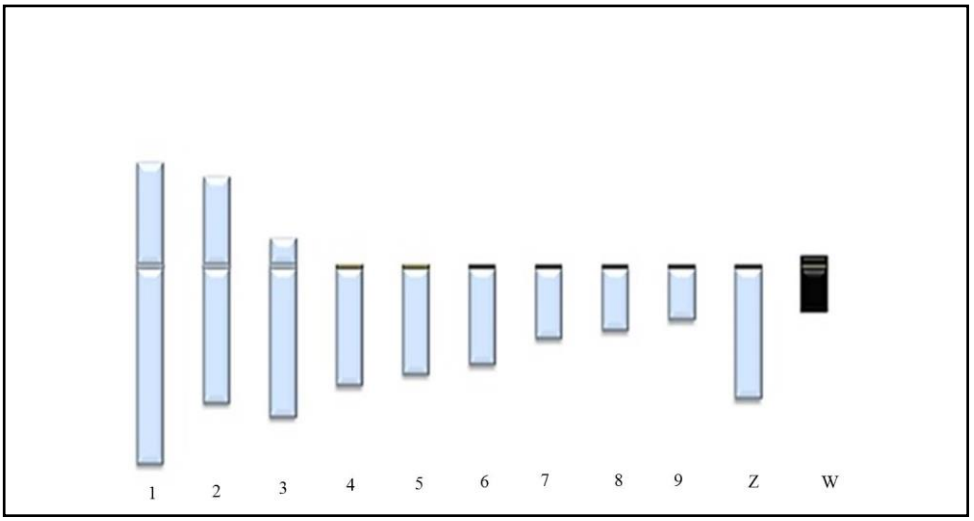

Fig.4. An Ideogram of macro-chromosomes illustrates the position and size of C-heterochromatin in the examined Muscovy and Sudani breeds belonging to *Cairina moschata*.

DISCUSSION

Constitutive heterochromatin patterns investigation of the three duck breeds (Dumyati, Khample and Pekin) belonging to *Anas platyrhynchos* and *Cairina moschata* occurring in Egypt revealed an obvious similarity of C-banding patterns among these three breeds which belonging to *Anas platyrhynchos***.** Similarly, the C-heterochromatin patterns are approximately the same on chromosomes of the two breeds (Muscovy and Sudani) of *Cairina moschata*. These results coincide with the assumption that avian karyotype is conserved and its evolution occurs slowly (Christids, 1990; Stevens, 1997; Shetty, et al. 1999; Shahin et al., 2014; Ata et al., 2017).

Results of C-banding patterns reported herein showed that Pattern of constitutive heterochromatin of the three duck breeds (Dumyati, Khample and Pekin) represented by the presence of variable sized centromeric C- bands in all macrochromosomes except pairs nos.1 and 2 that have no heterochromatin. On the other hand, chromosome pair no.8 appeared entirely heterochromatin as well as W sex chromosome. Moreover, the subacrocentric Z chromosome has a small sized centromeric C-band.

Furthermore, there was variation in the C- banding size and its occurrence in the two *Cairina moschata* breeds (Muscovy and Sudani). All chromosomes have clear centromeric C-bands which ranged from small to large blocks except chromosomes pairs no.1, 2, 3 that are devoid from heterochromatin. In addition, acrocentric Z chromosome showed an obvious small terminal C-band on the centromeric region. While W chromosome was entirely heterochromatic. From the present results, the entirely hetrochromatic pair no.8, autosome pair no.3 and Z chromosome can be used to differentiate between duck breeds of *Anas platyrhynchos* and *Cairina moschata* occurring in Egypt. In the present study, the obvious variation of C-banding distribution among duck breeds was attributed to variation of euchromatin content and its correlation with chromosome size and arrangement of constitutive heterochromatin as previously reported on avian taxa occurring in Egypt (Ata et al., 2005, 2007, 2012; Shahin et al., 2014). Variation of C-heterochromatin visualization in different duck breeds may be due to genome mixing through hybridization between related species and/or genera.

According to the results of parallel studies on Duck breeds, there were variations in the occurrence and size of heterochromatin in the chromosomes of different duck breeds. For instance, (Wojcik and Smalec, 2017) observed greater amounts of heterochromatin in the homologous chromosomes of *Anas platyrhynchos* than those of the *Cairina moschata* and more heterochromatin was noted in the chromosomes from the Pekin duck than those of the Muscovy duck. Moreover, chromosome no. 10 of the Pekin duck had substantially more heterochromatin than the chromosome from the Muscovy duck (Migliore et al., 1986; Ruixian et al., 1988; Apitz et al., 1995). In addition, variation in the position of heterochromatin on the Z chromosome between Muscovy duck and the Pekin.

Regarding the number of C-banding blocks of microchromosomes, the constitutive heterochromatin amounts have significantly varied between the ducks belonging to the two genera. Thus, about only one-third of the microchromosomes (≈21) in the three *Anas* breeds, while up to half of the microchromosomes (≈31) in the two *Cairina* breeds. Moreover, C-heterochromatin blocks exhibited as large and visible blocks in both males and females. These findings are in agreements with those reported by

Wojcik and Smalec (2017), in which they demonstrated that C-banding karyotype was similar in almost all studied duck breeds belonging to the same species (except those of chromosome pair no. 3 and Z chromosome) while differences in c-heterochromatin blocks were significantly recorded between macro- and micro-chromosomes. Comparison of chromosomes from the duck hybrid with chromosomes of parental genomes of *A. platyrhynchos* and *C. moschata* revealed nearly twice as much constitutive heterochromatin in the chromosomes of the hybrid. Nevertheless, significant variation in the mean number of C-heterochromatin blocks in micro-chromosomes was attributed to either transformation of heterochromatin into euchromatin and vice versa (King, 1991) or to the involvement of structural chromosomal aberrations during karyotype evolution (Burt et al., 1999).

As reported in the present study and in other avian taxa (Ata et al., 2005; Shahin et al., 2014), W chromosome was entirely C-heterochromatic. It could be used as a cytological marker for sex determination at pre and post-embryonic stages (Ata et al., 2007, 2012).

In addition, somatic macro-chromosome pair no. 8 was clearly visible in the present study as entirely heterochromatic and found in almost all examined cells of *Anas platyrhynchos* breeds (Dumyati, Khample and Pekin). Unfortunately, except for the report of (Ruixian et al., 1988), the description of this entirely heterochromatic macro-chromosome no. 8 in *Anas platyrhynchos* in the available literature was very rare.

Hence, it is very important finding out reasons for the remarkable occurrance of entirely hetrerochromatic macro-chromosome pair no.8 which appeared in cells of different breeds belonging to *Anas platyrhynchos*. It may also arise via process like that of recently reported by (Damas et al., 2016). They used chromosome breakpoint data and established that avian inter-chromosomal breakpoints appear in the regions of low density of conserved non-coding elements (CNEs) and that the chromosomal fission sites are further limited to long CNE “deserts”. This corresponds with fission being the rarest type of rearrangement in avian genome evolution. In addition, the appearance of entirely heterochromatic autosome may be related to the origin of Z and W sex chromosomes which was deeply explained by (Bergero and Charlesworth, 2009; Mank, 2013). They thought that sex chromosomes might arise from the somatic chromosomes throughout recombination and/or chromosomal exchanges during evolutionary processes.

Conclusion

In conclusion, the present C-banding patterns indicated that the hertochromatin characterization of duck breeds belonging to *Anas platyrhynchos* was different from those of *Cairina moschata* particularly in chromosome pair no.8. In addition, the duck breeds common in Egypt could be distinguished from those present elsewhere, via the distribution and variability of C-banding patterns. Hence, some molecular and biochemical studies (under publication) will clarify the genetic-make-up of duck breeds occurring in Egypt.

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