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Karyotype and C-banding Pattern of the Domestic Geese *Anser anser* Populations (Aves: Anatidae) in Egypt

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The karyotype and C-banding pattern of domestic Greylag geese *Anser anser anser* populations collected from five localities in El Minia, Egypt, that have either whitish grey or white feather color patterns were described. All populations have a diploid number of 2n= 80 chromosomes. Of the 80 chromosomes, 10 pairs, including ZW chromosomes, were macrochromosomes and the remaining 30 pairs were microchromosomes. Slight variation in the size of macrochromosomes was observed amongst populations. However, obvious variation of C-banding distribution was found and attributed to variation of euchromatin content and its correlation with chromosome size and arrangement of constitutive heterochromatin. Nevertheless, significant variation in the mean number of C-heterochromatin blocks in microchromosomes was attributed to either transformation of heterochromatin into euchromatin and *vice versa* or to involvement of structural chromosomal aberrations during karyotype evolution. The present results show that *A. anser* populations common in Egypt could be distinguished from those of *A. anser* and *A. cygnoides* occurring elsewhere in Europe and Asia via variability in chromosome morphology of pairs nos. 2, 3 and 4.

Key words: C-bands, karyotype, domestic geese, Anatidae, *Anser anser*, Egypt.

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The geese of the genus *Anser* Linnaeus 1758 include all the grey and sometimes the white geese. This genus belongs to the subfamily Anserinae comprising the true geese and swan (LIVEZEY 1986; CARBONERAS 1992) and contains ten living species, which span nearly the whole range of true goose shapes and sizes. Only two of these ten species have been domesticated (CRAWFORD 1990) and one of them is *Anser anser* which is a European species and is represented by many breeds (WÓJCIK & SMALEC 2007). One of these breeds is common in Egypt and is called Greylag goose *Anser anser anser*. These geese measure about 70-89 cm long and the feather color ranges from brownish grey or whitish grey, with pale-edged feather in the upper parts, to totally pure white or grey. The tail is grey with a white tip and the tail coverts are white. The breast and abdomen are brownish grey, with a few small gray spots. The beak, legs and feet color ranges from orange to pinkish. The sexes are very similar in appearance but the male is usually larger than the female (EEAA 1997).

Birds are considered one of the least karyotypically examined animal groups due to their karyotype specificity, i.e. small chromosomes, a large diploid chromosome number and the separation of chromosomes into macro- and microchromosomes (CHRISTIDIS 1989; RODIONOV 1997; STEVENS 1997; SHETTY *et al*. 1999; WÓJCIK & SMALEC 2007). The classical avian karyotype, although it is highly conserved among very divergent lineages of birds from ratites to passerines, has an extremely variable diploid number ranging from 40 to 142 chromosomes (CHRISTIDIS 1990; GRIFFIN *et al*. 2007). The large sized chromosomes range from four to eight microns in length and constitute only a few chromosome pairs. The remaining pairs are described as microchromosomes and are usually smaller than two microns in length and in many cases they appear as points (CHRISTIDIS

1989; SHETTY *et al*. 1999). Microchromosomes in comparison with macrochromosomes are characterised by a higher content of guanine-cytosine pairs (G-C). In addition, half of the identifiable genes are situated on microchromosomes (FILLON *et al*. 1998; GREGORY 2002). Moreover, bird microchromosomes are characterized by a three-fold higher crossing-over frequency than the macrochromosomes and thus the possibility of their correct segregation during meiotic division is considerably increased (RODIONOV 1996).

Cytogenetic research on birds is stimulated by scarce knowledge about waterfowl genomes (VIGNAL *et al*. 1999). With the development of chromosome banding techniques in the 1970s, it became possible to identify precisely the homological pairs as well as to identify and analyze chromosomal aberrations (WÓJCIK & SMALEC 2007). Chromosome banding patterns were performed for man and many farm animal species (ISCNDA 1989; ISCN 1995; ANSARI *et al*. 1999; ISCNDB 2000). However, in poultry the banding pattern, which includes the nine largest pairs of chromosomes, was obtained only for *Gallus domesticus* (LADJALI-MOHAMMEDI *et al*. 1999).

Conventional banding techniques do not always enable differentiation of bird chromosomes even in relation to the centromere location (BITGOOD & SHOFFNER 1990). One of the most frequently applied chromosome banding techniques is the RBG banding method. Another popular chromosome banding method is the CBG banding method (WÓJCIK & SMALEC 2007, 2008). Constitutive heterochromatin, constituting about 20 % of the genome, is a structural part of C-bands and has been proven to differentiate between very similar karyotypes (SHAHIN & ATA 2004). A pair of homologous chromosomes may be heteromorphic when one member has more heterochromatin material than the other, i.e. an addition or deletion making the homologues unequal (SHAHIN & ATA 2004). C-heterochromatin is placed in the centromeric, telomeric and interstitial parts of chromosomes (BURKHOLDER & DUCZEK 1982; SHAHIN & ATA 2004). Moreover sex chromosomes W, Z and B-chromosomes of animals can be entirely or mostly made up of heterochromatin (ŚWITOŃSKI 1998). In birds, C-banding is primarily considered a diagnostic technique for the detection of the W chromosomes (WANG & SHOFFNER 1974). The C-banding staining technique indicated that W is rich in constitutive heterochromatin, which in otherwise only found in high densities at the centromeres of microchromosomes and at one end of the Z chromosome (ELLEGREN *et al*. 2007; SCHMID *et al*. 2005).

Most morphological karyotype and banding pattern studies of various goose species were carried out on individuals occurring outside Egypt (HAMMAR 1966; BHATNAGAR 1968; BEÇAK *et al*. 1975; SHOFFNER *et al*. 1979; BELTERMAN & DE BOER 1984; SILVERSIDES *et al*. 1988; CHRISTIDIS 1989; CRAWFORD 1990; HIDAS 1993; APITZ *et al*. 1995; RABSZTYN *et al*. 1998; ANDRASZEK *et al*. 2007, 2010; WÓJCIK & SMALEC 2007, 2008, 2011, 2012), however, up to date no further studies involved geese common from Egypt.

Hence, the present study was undertaken to carry out a detailed survey of the Greylag goose *A. a. anser* populations having either whitish grey or pure white feather color patterns in El Minia (Upper Egypt), with the major aims of: 1) testing whether there are different karyotype forms based upon the existing feather color pattern variation in these populations; 2) identifying and characterizing the karyotype of these predictable forms; 3) assessing karyotype evolution among these forms using the C-banding technique; and 4) comparing the present results with those available on other geese species occurring elsewhere.

Material and Methods

Adult male and female individuals of the domestic Greylag goose *Anser anser anser* Linnaeus 1758 populations having whitish grey and pure white feather color patterns were collected from local markets and houses of five localities (ten samples each) in El Minia province (Upper Egypt). The collecting sites and the corresponding sample sizes, sex and feather color patterns are indicated in Table 1 and Fig. 1. The geese samples were killed and femurs were dissected. Subsequently, the following techniques were undertaken.

Conventional preparation

Mitotic chromosome spreads from the femoral bone marrow cells were prepared by the air drying technique using the method of YOSIDA (1973) and ATA *et al*. (2005), with some modifications. The bone marrow cells were treated in vitro with 0.025% colchicine solution made in EDTA for 15 min at room temperature. About 100 metaphase plates from both males and females of each population were examined at ×100 magnification and good spreads (about 50) from both sexes of each population were scored and photographed using an Olympus BX51 microscope with a C-4040 zoom digital camera. The karyotype was determined on the basis of 50 well-spread metaphase cells from each population. Macrochromosomes were measured under the microscope using the Soft Imaging System (SIS) analysis program (Version 3.0) edited in 1999 by Soft Imaging System

Table 1

The collecting sites and the corresponding sample number based on feather color and sex variation

Location	Number of collected samples based on feather color				Total number of collected samples
	Whitish grey		White		
	Male	Female	Male	Female	
Samalot	5	5	–	–	10
Bani Mazar	4	4	1	1	10
Mallawy	3	3	2	2	10
El Minia	4	4	1	1	10
Abu Qurqas	4	4	1	1	10

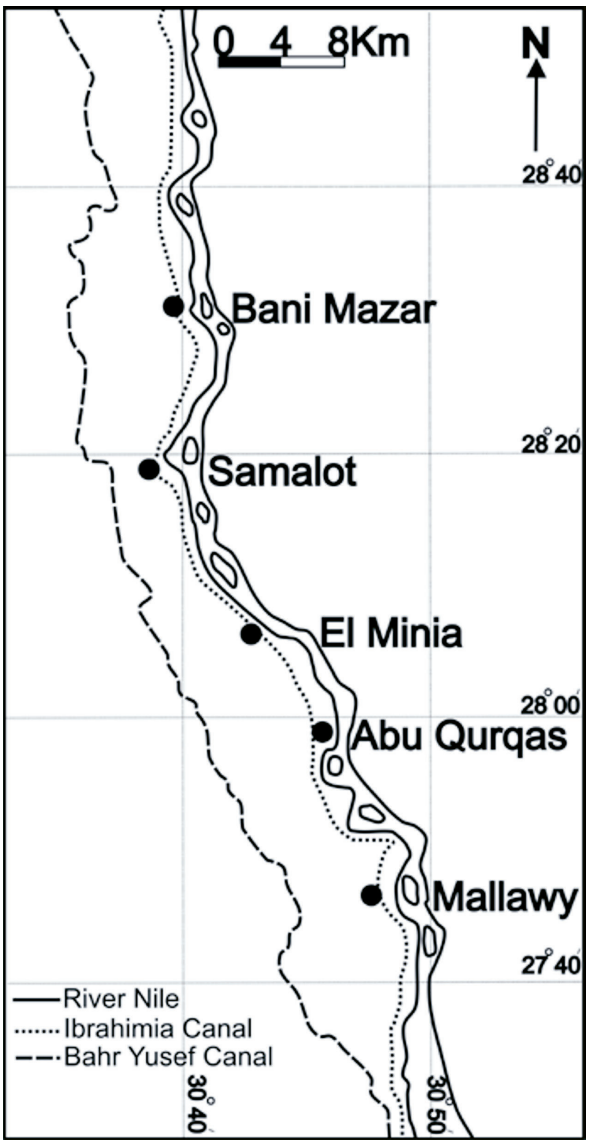

Fig. 1. The localities from which domestic Greylag goose populations were collected from the five localities in El Minia province (Upper Egypt).

GmbH, Germany, and classified according to the system of nomenclature proposed by LEVAN *et al*. (1964).

C-banding technique

C-bands were obtained by using the standard protocol of SUMMNER (1972) with major modifications as described by SHAHIN and ATA (2004) and ATA *et al*. (2005). About 100 metaphase plates from both males and females of each population were examined and photographed using an Olympus BX 51 microscope with a C-4040 zoom digital camera. The C-banding size and distribution on the macrochromosomes of both males and females of the five localities were described. In addition, the number of C-bands in the microchromosomes was counted for both sexes of each population of the five localities and their mean numbers were statistically analyzed by using MSTATC program version 2.10 (GOMEZ & GOMEZ 1984).

Results

Karyotype

As a rule, the karyotype of the domestic Greylag goose *A. a. anser* populations collected from the five localities and that have either whitish grey or white feather colors was practically similar in morphology and consists of a diploid number of 2n= 80 chromosomes. The chromosome complement consists of 10 pairs, including the sex Z and W chromosomes, which were classified as macrochromosomes, while the remaining 30 pairs were identified as microchromosomes (Table 2). Of the 10 pairs of macrochromosomes, pair no.1 was submetacentric, pairs nos. 2 and 4 were metacentrics, pair no. 3

Table 2

Chromosomal comparisons between Greylag geese populations examined from five localities. Measurements are given in µm and data are presented as means ± standard deviation (SD). Centr. =centromeric, m=metacentric, sm=submetacentric, st=subtelocentric, t=telocentric (acrocentric)

Chromosome No.	Samalot			Bani Mazar			El Minia			Mallawy			Abu Qurqas		
	Centr. Index %	Arm ratio	Type	Centr. Index %	Arm ratio	Type	Centr. Index %	Arm ratio	Type	Centr. Index %	Arm ratio	Type	Centr. Index %	Arm ratio	Type
1	37.18±1.89	1.69±0.14	sm	36.69±1.43	1.74±0.12	sm	34.56±3.83	1.89±0.31	sm	37.27±2.41	1.71±0.15	sm	35.64±2.40	1.75±0.15	sm
2	39.15±3.24	1.57±0.21	m	40.22±2.42	1.50±0.15	m	40.17±4.03	1.514±0.24	m	39.51±1.83	1.54±0.12	m	38.18±3.20	1.66±0.23	m
3	19.19±3.74	4.40±1.14	st	19.76±2.17	4.12±0.56	st	19.98±4.99	4.29±1.42	st	19.00±2.05	4.31±0.59	st	18.28±4.21	4.74±1.11	st
4	44.22±3.68	1.27±0.20	m	45.42±2.25	1.20±0.11	m	45.75±9.91	1.32±0.25	m	46.67±2.32	1.15±0.10	m	44.95±2.79	1.23±0.14	m
5	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t
6	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t
7	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t
8	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t
9	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t	100.0	0.00	t
Z	38.37±3.38	1.62±0.22	sm	35.77±3.18	1.81±0.23	sm	36.05±4.14	1.80±0.31	sm	34.53±5.82	1.83±0.29	sm	35.27±3.37	1.91±0.45	sm
W	47.94±2.07	1.09±0.10	m	48.65±0.86	1.05±0.03	m	44.88±3.06	1.24±0.16	m	46.28±1.94	1.16±0.09	m	46.94±2.99	1.14±0.25	m

Table 3

Distribution of C-blocks into different size categories among macrochromosomes of the Greylag geese populations examined from the five localities. E=entirely heterochromatic, L=large, M= medium, S=small, Sf=small faint, -=no C-blocks

Location	Macrochromosome number										
	1	2	3	4	5	6	7	8	9	Z	W
Samalot	M	M	M	M	Sf	L	L	L	M	M	E
Bani Mazar	–	S	S	S	Sf	S	M	M	M	S	E
Mallawy	S	S	M	S	Sf	L	L	L	M	M	E
El Minia	S	S	S	S	Sf	M	M	M	L	M	E
Abu Qurqas	S	S	M	M	Sf	M	M	M	L	M	E

was subtelocentric, while pairs nos. 5, 6, 7, 8, and 9 were acrocentrics. The sex chromosome Z appeared submetacentric, however, the W chromosome was a medium sized metacentric. For details on the arm ratios and centromeric index values of the chromosome complement in all populations, see Table 2.

C-banding comparison

As a rule, all of the goose populations examined in this study have a relatively small amount of constitutive heterochromatin and their C-banding pattern was characterized by the presence of a centromeric C-band in a variable number of chromosomes of each of these populations. In addition, it is noteworthy to mention here that no variation in the constitutive heterochromatin content was observed amongst geese populations of the five localities that have different feather color patterns. Although the positions of C-bands was frequently similar in the same chromosomes of all populations, obvious variation in the size and occurrence of C-bands was observed between the populations of the five localities (Table 3; Figs 2 & 3).

Macrochromosomes

The C-banding size and occurrence in ten pairs of macrochromosomes were fairly different amongst populations (Table 3; Figs 2 & 3). Chromosome pair no.1 has a small to medium sized centromeric C-band in addition to a telomeric (distal) C-band that appeared in its long arm (q) in the populations of Samalot, Mallawy and Abu Qurqas (Fig. 2), however, it appeared entirely devoid of heterochromatin in Bani Mazar population (Fig. 3). Similarly, pairs nos. 2, 3 and 4 have a small to medium sized centromeric C-band in all populations. However, pair no. 5 has a small sized lightly stained centromeric C-band, while pairs nos. 6, 7, 8 and 9 have a small or medium sized to large darkly stained centromeric band in all populations. In addition, the Z chromosome has a small to medium sized darkly stained centromeric C-band; however, the W chromosome appeared to entirely consist of a large darkly stained C-heterochromatin block in all populations (Figs 2 & 3).

Microchromosomes

Of the 30 pairs of microchromosomes, only two pairs appeared to entirely consist of heterochromatin in all geese populations, while variable sized C-band blocks were recognized in the remaining pairs (Figs 2a & 3a). In addition, although significant variation in the mean number of C-heterochromatin blocks was observed between the geese populations examined ($P \geq 0.05$), no significant variation was scored between the males and females of all populations, except Abu Qurqas population where the mean value was significant. For details on the mean number of C-blocks in males and females of all populations, see Table 4).

Discussion

Chromosome examination of the domestic Greylag geese *A. a. anser* populations collected from five localities in El Minia province with either whitish grey or white feather color revealed similar karyotypes consisting of 2n= 80 chromosomes. This means that the karyotype of *A. anser* is conserved in these geographically isolated and distinctively colored populations and their karyotype evolution occurs slowly. A conclusion that coincides with the assumption of CHRISTIDS (1990), STEVENS (1997), RODIONOV (1997) and SHETTY *et al*. (1999) that the avian karyotype is

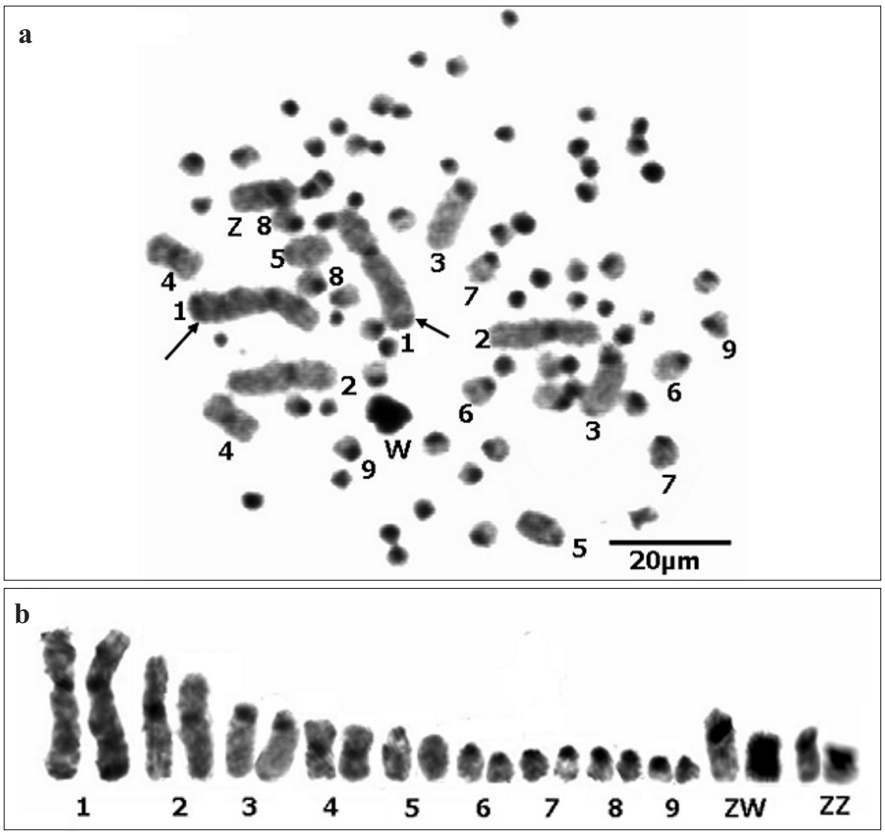

Fig. 2. Image of C-bands of metaphase cell chromosomes (a) and karyotype (b) of a male Greylag goose *Anser anser anser* collected from Samalot locality. Note the female ZZ chromosomes are added in (b). Arrows in (a) refer to telomeric bands on q arms of pair no. 1 and numbers refer to macrochromosomes.

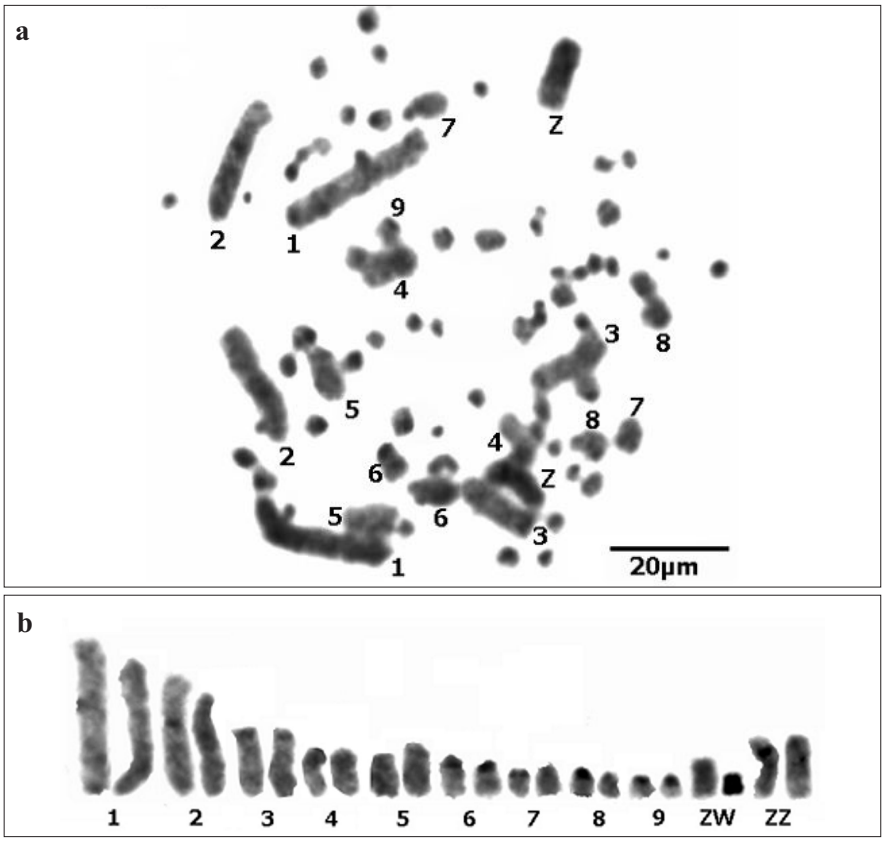

Fig. 3. Picture of C-bands of metaphase cell chromosomes (a) and karyotype (b) of a female Greylag goose *Anser anser anser* collected from Bani Mazar locality showing that the pair no. 1 is entirely devoid of heterochromatin. Note the male ZW chromosomes are added in (b) and numbers refer to macrochromosomes.

Table 4

The mean number and standard deviation (±SD) of C-band blocks on the microchromosomes of the Greylag geese populations examined from the five localities. Means having the same letters vertically are not significant at $P \leq 0.05$ according to DUNCAN´s (1955) multiple range tests

Location	Sex		Overall mean
	Male	Female	
Samalot	45.68^{A}±0.1	46.64^{A}±1.9	46.16^{A}±1.3
Bani Mazar	39.07^{B}±1.9	37.65^{B}±1.8	38.36^{B}±1.8
El Minia	38.60^{B}±2.1	38.40^{B}±3.9	38.50^{B}±2.8
Mallawy	45.10^{A}±9.2	43.66^{A}±1.0	44.38^{A}±5.9
Abu Qurqas	44.04^{A}±4.3	39.84^{B}±3.8	41.94^{AB}±4.3
Least Significant Difference (LSD)	3.611		4.662

highly conserved in many species and its evolution occurs rather slowly.

Of the 80 chromosomes, ten pairs including the Z and W chromosomes were identified as macrochromosomes, while the remaining 30 pairs were microchromosomes. According to results of parallel studies on *A. anser*, WÓJCIK and SMALEC (2007) described only 14 pairs (13 pairs of autosomes and ZW chromosomes) out of the 40 pairs of chromosomes. However, in addition to the ZW chromosomes, ANDRASZEK *et al*. (2007) recognized only eight pairs, compared to only five pairs by ANDRASZEK *et al*. (2010) and SILVERSIDES *et al*. (1988). In addition, the karyotype morphology was frequently similar in all populations. Of the ten pairs of macrochromosomes, pair no. 1 was submetacentric in all populations. This finding is consistent with that found in both *A. anser* and *A. cygnoides* (SILVERSIDES *et al*. 1988; HIDAS 1993, 1999; APITZ *et al*. 1995; ANDRASZEK *et al*. 2007; WÓJCIK & SMALEC 2007, 2008). Pair no. 2 was metacentric; however, on the contrary, it is identified by the previous authors as submetacentric. Similarly, pair no. 3 was recognized herein as subtelocentric, while it is acrocentric in ANDRASZEK *et al*. (2007) and WÓJCIK and SMALEC (2007, 2008). Nevertheless, pair no. 4 was metacentric; this is in agreement with the description of BELTERMAN and DE BOER (1984) and WÓJCIK and SMALEC (2008) but it contradicts the findings of SILVERSIDES *et al*. (1988), HIDAS (1993, 1999), APITZ *et al*. (1995), ANDRASZEK *et al*. (2007) and WÓJCIK and SMALEC (2007) who described it as submetacentric.

Frequent accounts have mentioned the differences in size and morphology of the fourth chromosome in birds. CHOWDCHARY and RAUDSEPP (2000) pointed out that the fourth chromosome has been considered the greatest enigma in the evolution of birds. BURT *et al*. (1999) mentioned that these differences are due to the structural chromosomal aberrations that occurred during bird evolution. Amongst goose populations, however, this morphological variation in the structure of the fourth pair of autosomes is attributed to the pericentric inversions that have occurred during karyotype evolution (SHOFFNER *et al*. 1979; SILVERSIDES *et al*. 1988; APITZ *et al*. 1995; RABSZTYN *et al*. 1998**)**. Similarly, the morphological variation in pairs nos. 2 and 3 between the present species and *A. anser* and *A. cygnoides* domesticated elsewhere could be explained also in view of pericentric inversions during karyotype evolution.

Moreover, the autosome pairs nos. 5 to 9 were classified as acrocentrics in the present study, a result that is in agreement with that reported by ANDRASZEK *et al*. (2007) and WÓJCIK and SMALEC (2007, 2008). Furthermore, the Z chromosome was submetacentric in all populations. This agrees with the findings of GOLDSCHMIDT *et al*. (2000), ANDRASZEK *et al*. (2007) and WÓJCIK and SMALEC (2007, 2008). Conversely, the W chromosome was metacentric in all populations examined; this result is consistent with the data presented by GOLDSCHMIDT *et al*. (2000), however, it contradicts SILVERSIDES *et al*. (1988), APITZ *et al*. (1995) and WÓJCIK and SMALEC (2007, 2008) who assigned it as submetacentric. This difference in chromosome nomenclature may be due to some intrachromosomal rearrangements such as addition or deletion of chromosomal segments caused by pericentric inversion (GRIFFIN *et al*. 2007; SHIBUSAWA *et al*. 2004; NANDA *et al*. 2008).

All of the Greylag geese populations examined have a common pattern of constitutive heterochromatin represented by the occurrence of a variable sized centromeric C-band in all chromosomes except the Bani Mazar population in which pair no. 1 appeared entirely devoid of heterochromatin. Absence of heterochromatin blocks in *A. anser* chromosomes has also been described by WÓJCIK and SMALEC (2007) in pair no. 4. In addition, a telomeric (distal) C-band was detected only on the long arm (q) of this pair in the populations of Samalot, Mallawy and Abu Qurqas. Occurrence of telomeric C-band either on the long arms or on both arms of some chromosomes of *Anser* species has also been recorded by HAMMAR (1966), MAYR *et al*. (1990), APITZ *et al*. (1995) and WÓJCIK and SMALEC (2007, 2008) as a result of the presence of guanine-cytosine regions (CMA-2 positive). However, the lack of these telomeric C-bands in the distal parts of chromosomes is attributed to short digestion times of the chromosomes with barium hydroxide (WÓJCIK & SMALEC 2007).

Many explanations have been put forward to account for variation in C-bands between the individual homologue pair chromosomes or among chromosomes of the same karyotype or even among karyotypes of closely related species. For example, it has been attributed by many authors to transformation of heterochromatin into euchromatin or *vice versa* (KING 1980, 1991; KING & JOHN 1980; CABRERO *et al*. 1985; CUEVAS & FORMAS 2003; SHAHIN & ATA 2004) or to deletion or duplication of heterochromatic segments among karyotypes of related species (WHITE 1973; CABRERO *et al*. 1985). In the present study, it is evident that the heterogeneity of C-banding distribution in morphologically similar chromosomes could be attributed to variation of euchromatin content and its correlation with chromosome size and arrangement of constitutive heterochromatin.

Moreover, chromosome pair no. 4 has a small to medium sized centromeric C-band in the *A. a. anser* populations examined. CBG banding techniques explained to a certain extent the differences in the morphology, size and heterochromatin content of the fourth chromosome in the karyotype of *A. anser* and *A. cygnoides* (APITZ *et al*. 1995; ANDRASZEK *et al*. 2007; WÓJCIK & SMALEC 2007, 2008). In *A. anser* the fourth chromosome appeared totally devoid of constitutive heterochromatin (ANDRASZEK *et al*. 2007; WÓJCIK & SMALEC 2007). However, on the contrary, it has centromeric C-bands in the interstitial part of the p arm and in the subcentromeric region in *A. cygnoides* (APITZ *et al*. 1995; WÓJCIK & SMALEC 2008). Additionally, the previous authors attributed the difference of the fourth chromosome morphology between metacentric and submetacentric to the genetic heterozygosity that resulted from cross breeding between wild European *A. anser* and Asian *A. cygnoides*. Therefore, chromosome pair no.4 could be used as a marker to distinguish between the Greylag geese *A. a. anser* domesticated in Egypt and other species of *Anser* occurring elsewhere in Europe and Asia.

Furthermore, the present results revealed that the Z chromosome has a small to medium sized darkly stained centromeric C-band in the populations examined. Nevertheless, WÓJCIK and SMALEC (2007) detected a centromeric band only on the short arm (p) of the Z chromosome of *A. anser* in addition to the interstitial blocks that amounted to 41% of its total length, while ATA *et al*. (2005) scored a terminal large C-band block on only one arm of the Z chromosome in three galliform species (turkey, quail and chicken). This change in the position of heterochromatin content could be explained in terms of ancestral pericentric inversion and deletion of the centromeric heterochromatin.

On the other hand, the W chromosome was metacentric and entirely heterochromatic in all populations. However, WÓJCIK and SMALEC (2007) mentioned that the heterochromatin blocks are found on the interstitial parts of the submetacentric W chromosome and measure about 75% of its total length. Nonetheless, ATA *et al*. (2005) found that the W chromosome is submetacentric in chicken and turkey and subacrocentric in quail and is entirely heterochromatic in the three galliform species.

Regarding the C-banding pattern of microchromosomes, it was shown that the mean number of C-heterochromatin blocks were significantly different between the geese populations examined (Table 4). However, no significant variation was found between the males and females of all populations, except the Abu Qurqas population where the mean value was significant. This difference could be attributed to either transformation of heterochromatin into euchromatin and *vice versa* (KING 1991) or to involvement of structural chromosomal aberrations during karyotype evolution (WHITE 1973). In addition, it was found that the mean number of C-heterochromatin blocks in both sexes of the Samalot population was significantly higher than in other populations (Table 4). This increase in the constitutive heterochromatin content could be explained in terms of multiple chromosomal aberrations that might have occurred during evolution (BURT *et al*. 1999).

In conclusion, the present cytological data indicated that the domestic Greylag geese *A. a. anser* populations collected from the five localities in El Minia province with feather colors either whitish grey or pure white have the same karyotype of 2n= 80 chromosomes. However, slight variation in

the size of macrochromosomes was observed amongst populations. In addition, all of the populations examined have a common pattern of constitutive heterochromatin represented by the occurrence of a variable sized centromeric C-band in all chromosomes except the Bani Mazar population in which pair no. 1 appeared entirely devoid of heterochromatin. This variation of C-banding distribution in morphologically similar chromosomes could be attributed to variation of euchromatin content and its correlation with chromosome size and arrangement of constitutive heterochromatin. Moreover, significant variation in the mean number of C-heterochromatin blocks in microchromosomes could be attributed to either transformation of heterochromatin into euchromatin and *vice versa* or to involvement of structural chromosomal aberrations during karyotype evolution. However, the significantly higher increase in the mean number of C-heterochromatin blocks in microchromosomes of males and females of the Samalot population than other populations could be explained in terms of multiple chromosomal aberrations that might have occurred during evolution. Additionally, the Greylag goose *A. a. anser* common in Egypt could be distinguished from those of *A. anser* and *A. cygnoides* occurring in Europe and Asia via variability in the morphology of chromosome pairs nos. 2, 3 and 4.

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References

ANDRASZEK K., SMALEC E., WRZASZCZ L. 2007. Description of G bands on the chromosomes of the European domestic goose (*Anser anser*). Arch. Geflügelk. **71**: 272-277.

ANDRASZEK K., SZYMAŃSKA J., SMALEC E. 2010. Identification of T-bands and subtelomeric areas in chromosomes of the European domestic goose (*Anser anser*). Roczn. Nauk. Pol. Tow. Zoot. **6**: 9-16.

ANSARI H. A., BOSMA A. A., BROAD T. E., BUNCH T. D., LONG S. E., MAHER D. W., PEARCE P. D., POPESCU C. P. 1999. Standard G-, Q-, and R-banded ideograms of the domestic sheep (*Ovis aries*): homology with cattle (*Bos taurus*). Report of the committee for the standardization of the sheep karyotype. Cytogenet. Cell Genet. **87**: 134-142.

APITZ M., WAGNER K. U., SAAR W. 1995. Karyotype characteristics in domestic ducks and geese. Proc. 10th Europ. Symp. on Waterfowl, Halle, Germany: 465-472.

ATA M. A., FAHMY A. M., AHMED E. A., WALID M. A. 2005. Cytogenetic studies on three species of domestic birds (Galliformes, Aves): I- Chromosomal morphology and C-banding analysis. Minia J. Agric. Res. Develop. **25**: 977-1000.

BEÇAK M. L., BEÇAK W., CHEN T. R., SHOFFNER R. N. 1975. Chromosome atlas: Fish, amphibians, reptiles, and birds. Springer-Verlag, New York. Pp. 228.

BELTERMAN R. H. R., DE BOER L. E. M. 1984. A kariological study of 55 species of birds, including karyotypes of 39 species new to cytology. Genetica **65**: 39-82.

BHATNAGAR M. K. 1968. Mitotic chromosomes of white Chinese Geese. J. Hered. **59**: 191-195.

BITGOOD J., SHOFFNER R. N. 1990. Cytology and cytogenetics. (In: Poultry Breeding and Genetics. B. D. Crowford ed. Amsterdam-Oxford-New York-Tokyo, Elsevier): 401-427.

BURKHOLDER G. D., DUCZEK L. L. 1982. The effect of chromosome banding techniques on the histone and nonhistone proteins of isolated chromatin. Can. J. Biochem. **60**: 328-337.

BURT D., BRULEY Ch., DUNN I. C., JONES Ch. T., RAMAGE A., LAW A. S., MORRICE D. R., PATON I. R., SMITH J., WINDSOR D., SAZANOV A., FRIES R., WADDINGTON D. 1999. The dynamics of chromosome evolution in birds and mammals. Nature **402**: 411-413.

CABRERO J., CAMACHO J. P. M., PASCUAL F. 1985. Cytotaxonomic studies on Pamphaagids genus *Eumigus* detection of two chromosomal races in *E. monticola* (Rambur) (Insecta, Orthoptera). Caryologia **38**: 1-12.

CARBONERAS C. 1992. Family Anatidae (Ducks, Geese and Swans). (In: Handbook of Birds of the World (Vol. 1: Ostrich to Ducks). J. del Hoyo, A. Elliott, J. Sargatal eds. Lynx Edicions, Barcelona): 536-629.

CHOWDCHARY B. P., RAUDSEPP T. 2000. HSA4 and GGA4: Remarkable conservation despite 300-Myr divergence. Genomics **64**: 102-105.

CHRISTIDIS L. 1989. Karyotypic analyses in birds. (In: Cytogenetics of Animals. C. Halmaun ed.): 125-132.

CHRISTIDS L. 1990. Animal Cytogenetics: Chordata 3b: Aves. Gebrüder Borntraeger ed. Stuttgart, Germany.

CRAMP S. 1977. The birds of the Western Palearctic. Oxford University Press, Oxford. Pp. 722.

CRAWFORD R. D. 1990. Origin and history of poultry species. (In: Poultry Breeding and Genetics. Elsevier, Amsterdam-Oxford-New York-Tokyo ed.): 1-41.

CUEVAS C. C., FORMAS J. R. 2003. Cytogenetic analysis of four species of the genus *Alsodes* (Anura, Leptodactylidae) with comments about the karyological evolution of the genus. Hereditas **138**: 138-147.

DUNCAN B. D. 1955. Multiple ranges and multiple F test. Biometric **11**: 1-42.

EEAA 1997. Egyptian Environmental Affairs Agency: Birds known to occur in Egypt. Publication of National Biodiversity Unit, Cabinet of Ministers, Dept. Nature Protection, Arab republic of Egypt.

ELLEGREN H., HULTIN-ROSENBERG L., BRUNSTROM B., DENCKER L., KULTIMA K., SCHOLZ B. 2007. Faced with inequality: Chicken do not have a general dosage compensation of sex-linked genes. BMC Biol. **5**: 40, doi:10.1186/1741-7007-5-40. Published online, September 20.

FILLON V., MORISSON M., ZOOROB R., AUFFRAY C., DOUAIRE M., GELLIN J., VIGNAL A. 1998. Identification of sixteen chicken microchromosomes by molecular markers using two colour fluorescent in situ hybridization (FISH). Chrom. Res. **6**: 307-313.

GOLDSCHMIDT B., NOGUEIRA M. D., SILVA K. P. A., SOUZA L. M. D. 2000. Study of the karyotype of *Oryzoborus maximiliani* (Passeriformes- Aves) using young feather pulp cultures. Genet. Mol. Biol. **23**: 371-373.

GOMEZ K. A., GOMEZ A. A. 1984. Statistical Procedures of Agricultural Research. 2nd edition, John Willy & Sons Inc. New York. Pp. 680.

GREGORY T. R. 2002. A bird's-eye view of the C-value enigma: Genome size, cell size, and metabolic rate in the Class Aves. Int. J. Org. Evolution **56**: 121-130.

GRIFFIN D. K., ROBERTSON L. B. W., TEMPEST H. G., SKINNER B. M. 2007. The evolution of the avian genome as revealed by comparative molecular cytogenetics. Cytogenet. Genome Res. **117**: 64-77.

HAMMAR B. 1966. The karyotypes of nine birds. Hereditas **55**: 367-585.

HIDAS A. 1993. Cytogenetic studies on a species hybrid goose breed. Proc. 8th North American Colloquium on Domestic Animal Cytogenetic and Gene Mapping, Guelph, Canada: 153-155.

HIDAS A. 1999. Molecular cytogenetic studies in domestic goose. Proc. 13th European Colloquium on Cytogenetics of Domestic Animals, Budapest, Hungary. Állattenyésztés és Takarmányozás **48**: 78-80.

ISCN 1995. An international system for human cytogenetic nomenclature. Mitelman F. ed. S. Karger, Basel.

ISCNDA 1989. International System for Cytogenetic Nomenclature of Domestic Animals (1989). The Second Intern. Conf. on Standardization of Domestic Animal Karyotypes. Di Berardino D., Hayes H., Fries R., Long S. 1990. Cytogenet. Cell Genet. **53**: 65-79.

ISCNDB 2000. International System for Chromosome Nomenclature of Domestic Bovids (2000). Di Berardino D., Di Meo G.P., Gallagher D. S., Hayes H., Iannuzzi L. 2001. Cytogenet. Cell Genet. **92**: 283-299.

KING M. 1980. C-banding studies on Australian hylid frogs: Secondary constriction structure and the concept of euchromatin transformation. Chromosoma **80**: 191-217.

KING M. 1991. The evolution of heterochromatin in amphibian genome. (In: Amphipian Cytogenetics and Evolution. D. M. Green and S. K. Sessions eds. Academic Press): 359-381.

KING M., JOHN B. 1980. Regularities and restrictions governing C-band variation in acridoid grasshoppers. Chromosoma **76**: 123-150.

LADJALI-MOHAMMEDI K., BITGOOD J. J., TIXIER--BOICHARDM, PONCE DE LEON F.A. 1999. International System for Standardized Avian Karyotypes (ISSAK): Standardized banded karyotypes of the domestic fowl (*Gallus domesticus*). Cytogenet. Cell Genet. **86**: 271-276.

LEVAN A., FREDGA K., SANDBERG A. A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201-220.

LIVEZEY B. C. 1986. A phylogenetic analysis of recent anseriform genera using morphological characters. Auk **103**: 737-754.

MADGE S., BURN H. 1988. Wildfowl: an identification guide to the ducks, geese and swans of the world. Christopher Helm, Bromley, Kent. Pp. 352.

MAYR B., LAMBROU M., KALAT M., SCHLEGER W., BIGELBACH A. 1990. Characterization of heterochromatin by sequential counterstain-enhanced fluorescence in three domestic bird species. Hereditas **8**: 468-475.

NANDA I., SCHLEGELMILCH K., HAAF T., SCHARTL M., SCHMID M. 2008. Synteny conversion of the Z chromosome in 14 avian species (11 families) supports a role for Z dosage in avian sex determination. Cytogenet. Genome Res. **122**: 150-156.

RABSZTYN A., JASZCZAK K., JASZCZAK J., KAPKOWSKA E. 1998. Inheritance of two morphological forms of chromosome 4 in Zatorska geese. Proc. 13th Europ. Colloquium on Cytogenetics of Domestic Animals, Budapest, Hungary. Allatenyesztes es Takarmanyozisenetics of Domestic Animals **48**: 72-74.

RODIONOV A.V. 1996. Micro Versus Macro: A review of structure and functions of avian Micro- and Macrochromosomes. Genetika **32**: 597-608.

RODIONOV A.V. 1997. Evolution of avian chromosome and linkage group. Russ. J. Genet. **6**: 342-347.

SCHMID M., NANDA I., HOEHN H., SCHARTL M., HAAF T., BUERSTEDDE J. M., ARAKAWA H., CALDWELL R. B., SWEIGEND S., BURT D.W., SMITH J., GRIFFIN D. K., MASABANDA J .S., GROENEN M. A. M., CROOIJMANS R. P. M. A., VIGNAL A., FILLON V., MORISSON M., PITEL F., VIGNOLES M., GARRIGUES A., GELLIN J., RODIONOV A.V., GALKINA S. A., LUKINA N. A., BEN-ARI G., BLUM S., HILLEL J., TWITO T., LAVI U., DAVID L., FELDMAN M. W., DELANY M. E., CONLEY C. A., FOWLER V. M., HEDGES S. B., GODBOUT R., KATYAL S., SMITH C., HUDSON Q., SINCLAIR A., MIZUNO S. 2005. Second report on chicken genes and chromosomes. Cytogenet. Genome Res. **109**: 415-479.

SHAHIN A. A. B., ATA A. M. 2004. C-banding karyotype and relationship of the dipodids *Allactaga* and *Jaculus* (Mammalia, Rodentia) in Egypt. Folia Biol. (Kraków) **52**: 25-31.

SHETTY S., GRIFFIN D. K., GRANES M. J. A. 1999 Comparative painting reveals strong chromosome homology over 80 million years of bird evolution. Chromosome Res. **7**: 289-295.

SHIBUSAWA M., NISHIBORI M., NISHIDA-UMEHARA C., TSUDZUKI M., MASABANDA J., GRIFFIN D. K., MATSUDA Y. 2004. Karyotypic evolution in the Galliformes: An examination of the process of karyotypic evolution by comparison of the molecular cytogenetic findings with the molecular phylogeny. Cytogenet. Genome Res. **106**: 111-119.

SHOFFNER R. N., WANG N., LEE F., KING R., OTIS J. S. 1979. Chromosome homology between the Ross's and the Emperor goose. J. Hered. **70**: 395-400.

SILVERSIDES F. G., CRAWFORD R. D., WANG H. C. 1988. The cytogenetics of domestic geese. J. Hered. **79**: 6-8.

STEVENS L. 1997. Sex chromosomes and sex determining mechanism in birds. Sci. Prog. **80**: 197-216.

SUMNER A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. **75**: 304- 306.

ŚWITOŃSKI M. 1998. B chromosomes in the common fox (*Vulpes vulpes*), their structure, distribution, inheritance and significance. Rocz. APh.D. Thesis **174**: 1-65. (In Polish).

VIGNAL A., FILLON V., VIGNOLES M., MORISSON M., CROOIJOMANS R. P. M. A., GROENEN M. A. M. 1999. The development of comparative molecular genome analysis in duck. Proc. 1st World Waterfowl Conf., Taichung, Taiwan: 603-606.

WANG N., SHOFFNER J. M. 1974. Trypsine G- band and C-banding for interchange analysis and sex identification in chicken. Chromosoma **47**: 61-69.

WHITE M. J. D. 1973. Animal Cytology and Evolution. 3 ed. Cambridge University Press.

WÓJCIK E., SMALEC E. 2007. Description of the *Anser anser* goose karyotype. Folia Biol. (Kraków) **55**: 35-40.

WÓJCIK E., SMALEC E. 2008. Description of the *Anser cygnoides* goose karyotype. Folia Biol. (Kraków) **56**: 37-42.

WÓJCIK E., SMALEC E. 2011. Comparing the karyotype of the European domestic goose and the Asian Goose on the basis of the karyotype of their interspecific cross-breed, using the RBG chromosome staining technique. Folia Biol. (Kraków) **59**: 107-113.

WÓJCIK E., SMALEC E. 2012. Assessment of chromosome in stability in geese (*Anser anser*). Can. J. Anim. Sci. **92**: 49-57.

YOSIDA T. H. 1973. Evolution of karyotype and differenation in 13 *Rattus* species. Chromosoma **40**: 285-297.