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THE KARYOTYPE OF THE ESTONIAN QUAIL. CHROMOSOME NUMBER, SIZE AND MORPHOLOGY

Introduction

At present there are approximately 8900 extant species of birds, and only about 10% of them (521 species) have been investigated cytogenetically (Belterman, De Boer, 1990). Although *Galliformes* belongs to the orders that have been studied comparatively well, the karyotypes of only some 30 from over 250 species are known to date (De Boer, Belterman, 1981).

The karyotype of the Japanese quail (*Coturnix coturnix japonica*) has been studied by several authors (see De Boer, 1984); it must be noted that at present there exist several commercial breeds of the Japanese quail, yet not much is known about their cytogenetics. As a matter of fact, systematic position of laboratory and commercial lines (breeds) of quail belonging to the *Coturnix* genus is still obscure, so their relationships to each other as well as to *Gallus*, and, particularly, to other *Galliformes* species, deserves more detailed study (Родионов et al., 1987; Belterman, De Boer, 1990). One of these new breeds is the Estonian quail, officially recognized only in 1988 (see Tikk, 1989).

The aim of the present paper is to describe the karyotype of the Estonian quail from the standpoint of its chromosome number, chromosome size and morphology, as well as to try and find its place among other avian karyotypes.

Material and methods

Material. Estonian quails were obtained from Kaiavere Quail Farm (Estonia). Short-term bone-marrow cultures (Christidis, 1985) were used for chromosome preparations. 100 metaphases were analyzed.

Methods. The slides were routinely stained with Giemsa stain solution in tap-water (1:50) for 10 min. *Microscopy.* The slides were examined under photomicroscope (Biolar PI, Poland) and photographed. Measurements were made on photos.

Results

Chromosome number. The diploid chromosome number of the Estonian quail ranged between 70—78. The whole chromosome set could be divided into macro- and microchromosomes, though there is no strict border between the two groups. We considered as macrochromosomes the first five largest autosome pairs and the *Z*-chromosome. These chromosomes could

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be easily counted and identified according to their length and morphological type. The number of macrochromosomes was in all metaphases, without exceptions, 12 (six pairs).

The rest of the karyotype consisted of microchromosomes. They were difficult to count, especially the smallest ones, and their number ranged from 58 to 66 in different metaphases. Therefore, as it has been proposed before (Яковлев, 1985), we consider it sensible to talk about the modal number of microchromosomes. In our case it was 62. Microchromosomes were not individually identified, and they were arranged into a karyogram according to their length. A karyotype with 78 chromosomes is shown in Fig. 1.

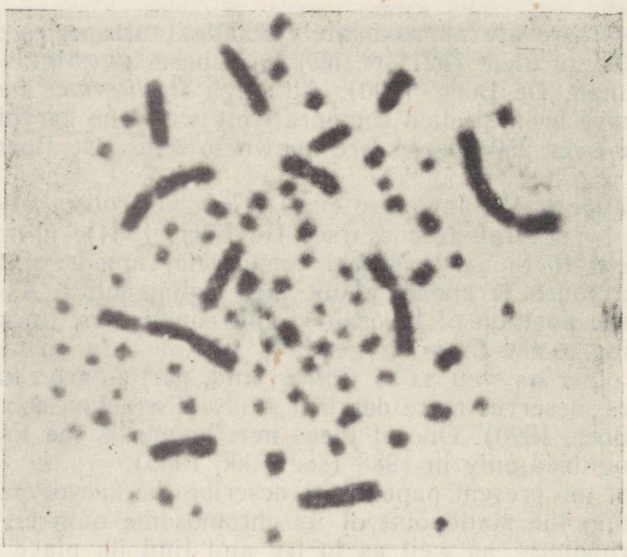

Fig. 1. A routinely stained karyotype of the Estonian quail (100×1.5×12.5).

Chromosome size. In our study only mid-metaphase preparations were used, which means that all the metaphases were of almost the same stage of condensation. In order to choose metaphases with the same spiralization index, we took the absolute length of the first autosome for our basis. Only the mid-metaphase plates where the absolute length of the first autosome was 8.6—9.0 microns were chosen for the analysis. At this stage of condensation the absolute length of the second autosome was approximately 6 microns, the third was about 4 microns, the fourth was about 3.6 microns, and the fifth was about 3 microns. The *Z*-chromosome was of the same length as the fourth autosome — 3.6 microns.

The length of microchromosomes ranged from 1.6 to 0.3 microns. The smallest microchromosomes were seen only as Giemsa stained dots. In fact, the *W*-chromosome (females are heterogametic, having *ZW* gonosomes) belongs to microchromosomes, and the present staining technique did not enable us to identify it.

Chromosome morphology. Morphological type of chromosomes is usually detected according to their centromere position. We used the method of Naranjo et al. (1983). It was revealed that the first and the second autosomes were submetacentrics; the third, the fourth and the fifth autosomes were acrocentrics; the *Z*-chromosome was metacentric.

Due to their small size it was rather difficult to detect the morphological type of the microchromosomes. As far as it could be seen, they were acrocentrics. The morphological type of the *W*-chromosome remained obscure. The whole set of Estonian quail's karyotype, arranged into a karyogram, can be seen in Fig. 2.

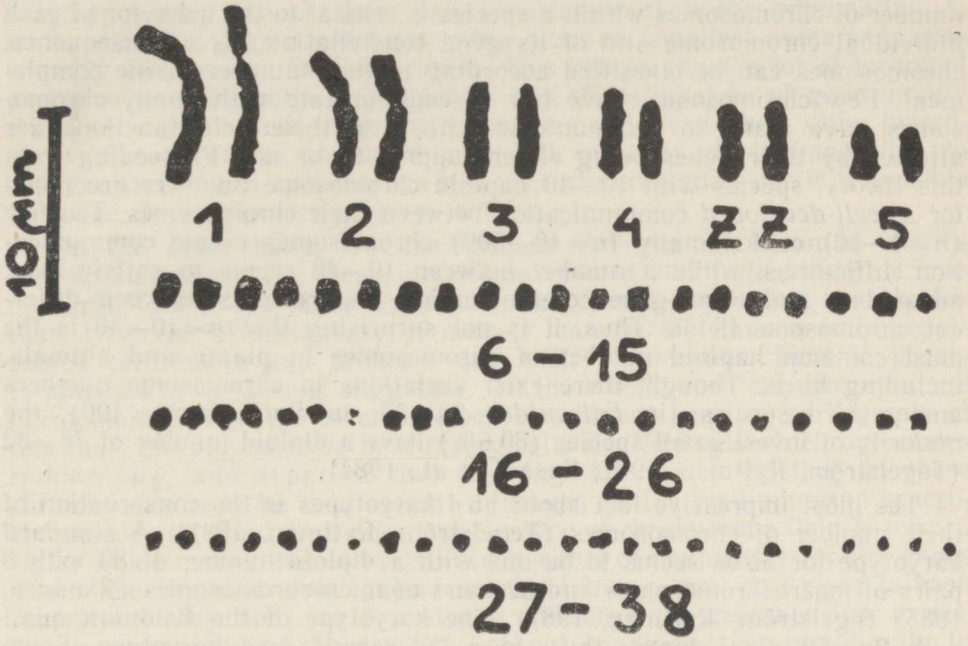

Fig. 2. Karyogram of the Estonian quail, $2n=78$.

Discussion

Chromosome number. An accurate diploid number can only be determined when large numbers of well-dried spreads are obtained, and even then the results tend to vary in some limits. The number of macrochromosomes can be detected quite easily: in our case it was 12. Now, microchromosomes present a problem. We can point out several technical reasons that might cause differences in microchromosome numbers: 1) microchromosomes can escape from the metaphase plates when the cell suspension is dropped on to the slide; or 2) microchromosomes can be hidden near macrochromosomes; or 3) they can lie indistinguishable near each other, and/or 4) small spots of dye or stained dust can be misjudged as microchromosomes. Furthermore, there may be other reasons to cause variations in microchromosome number. One of these is the asynchronous spiralization of microchromosomes that affects their staining ability with Giemsa (Яковлев, 1985). In *Gallus domesticus* it has been shown (see Яковлев, 1985) that there exists a clear correlation between the number of microchromosomes and the stage of mitotic spiralization of chromosomes. According to these data, there appears to be a sharp decrease of the number of microchromosomes when the length of the first autosome lies between 17—16 microns; the number of microchromosomes increases considerably when the length of the first autosome decreases to 15.5—12 microns, and, accompanying further condensation, the number of micro-

chromosomes decreases again. The authors propose to investigate the karyotype of *Gallus domesticus* at the stage where the length of the first autosome lies between 10.7—12.2 microns. At this stage of mitosis there exists a specific plateau of the number of microchromosomes. In the karyotype of the Estonian quail we noticed the same phenomenon at the stage when the length of the first autosome was 8.6—9.0 microns.

According to the *chromosome field theory* (Lima-de-Faria, 1980), the number of chromosomes within a species is critical to the behavior of each individual chromosome and of its gene constellation. As a consequence, chromosomes can be classified according to their number in the complement. Few chromosomes have few to communicate with, many chromosomes have many to communicate with, and their field functions are affected by their genes being either suppressed or not. Proceeding from this theory, species with 10—40 haploid chromosome numbers are noted for a *well-developed* communication between their chromosomes. Too few ($n=4$—10) or too many ($n=40$—500) chromosomes create communication difficulties, while a number between 10—40 seems to satisfy most adequately molecular gene communication requirements between different chromosome fields. Thus, it is not surprising that $n=10$—40 is the most common haploid number of chromosomes in plants and animals, including birds. Though, there exist variations in chromosome numbers among bird species (in *Falconidae* $2n=50$; in *Rallidae* $2n=100$), the majority of investigated species (80.6%) have a diploid number of 78—82 (Tegelström, Ryttman, 1981; Sasaki et al., 1984).

The most impressive fact about bird karyotypes is the conservation of their number of chromosomes (Tegelström, Ryttman, 1981). A standard karyotype for birds seems to be one with a diploid number of 80 with 8 pairs of macrochromosomes and 32 pairs of microchromosomes (Яковлев, 1985; Tegelström, Ryttman, 1981). The karyotype of the Estonian quail with $2n=70$—78 belongs, thus, to a "classical" bird karyotype. Some authors (Belterman, De Boer, 1984) propose that the ancestral karyotype of the *Galliformes* most probably included approximately 80 chromosomes. So, the extant bird species share almost the same diploid chromosome number, and few changes in chromosome number seem to have taken place during the evolution. It may be supposed that one reason for this might be the specific and quite conservative body building as well as life style of birds, but this is only a speculation.

The microchromosome problem of bird karyotypes is quite intriguing. As these minute elements are found in such bird groups as *Carinatae* and *Ratitae*, and in nearly all of their orders (Belterman, De Boer, 1990), their origin dates back at least a 100 million years, which indicates that they must have a very significant evolutionary/adaptive significance for otherwise they would have disappeared randomly.

The data of literature (Tegelström, Ryttman, 1981) show a negative correlation between the number of macro- and microchromosomes. So, the number of microchromosomes decreases with an increase in the number of macrochromosomes. This indicates an evolutionary connection between the two groups of chromosomes.

Proceeding from the aforesaid, the karyotype of the Estonian quail belongs to avian karyotypes with a low number of macro- and a high number of microchromosomes and with a well developed chromosome field, so we presume that internal gene organization along the chromosome and gene communication mechanisms favors the given chromosome number.

Chromosome size. Another interesting feature of bird karyotypes is the size of chromosomes. In the case of Estonian quail the largest chromosomes were not longer than 9.2 microns, while the smallest microchromo-

somes resembled minute stained dots. The same is true about many other avian karyotypes (Belterman, De Boer, 1984). It has been shown (Tegelström, Ryttman, 1981; Belterman, De Boer, 1984) that avian nuclear DNA content is only 40% of that of mammals, whereas the average bird karyotype has usually twice the number of chromosomes found in an average mammalian karyotype. Thus, on the average, bird chromosomes are only one fifth the length of mammalian chromosomes. In most avian karyotypes only two or three pairs are larger than the mammalian *X*, while often less than 8 pairs are larger than the smallest human chromosome 22 (Belterman, De Boer, 1984). This brings about a lot of technical problems in cytogenetic investigation. The slides must be of good quality with well-spread and long chromosomes, and even then only macrochromosomes can be identified with full certainty. It is impossible to distinguish between routinely stained microchromosomes, nor is it possible to define the centromere position in them.

On the other hand, we can discuss the size of Estonian quail's chromosomes from the viewpoint of the *chromosome field theory* (Lima-de-Faria, 1980). The latter postulates that chromosomes that are shorter than 1 micron at metaphase of mitosis, can hardly have a fully represented centromere and telomere and their chromosome field is poorly established or is lacking altogether. On the whole, such chromosomes are uncommon, though the avian microchromosomes comprise an example of this case. The *chromosome field theory* calls the field of such chromosomes *rudimentary*, and supposes that the very few genes existing in these chromosomes may be in a special situation. In the medium-sized macrochromosomes (about 3—4 microns) the type of chromosome field is termed *rigid*, which means that there is little freedom of manoeuvre for the genes due to the fact that their centromere and telomere are very close to each other, and they both exert a strong influence on neighbouring genes. Chromosome field working under optimal conditions is present only in the largest macrochromosomes (4—12 microns), and this is termed *balanced* field. So, proceeding from the *chromosome field theory*, the chromosomes of the Estonian quail have three types of chromosome fields: rudimentary, rigid and balanced. Taking into account the fact that *Galliformes* karyotypes have remained very conservative during their evolution (Belterman, De Boer, 1984), it may be supposed that there exists an optimal balance between chromosome number and their size, and also between different chromosome fields in the karyotypes under consideration.

Chromosome morphology can also be discussed on the basis of the *chromosome field theory* (Lima-de-Faria, 1980). In asymmetric chromosomes (submetacentrics) the field is discordant, and in one-armed chromosomes (acrocentrics) the field is extremely discordant. This is due to asymmetry in chromosome arms that have fields of different types, also in one-armed chromosomes all field relationships are concentrated on one side of the centromere.

To complement this, Lima-de-Faria (1980) has collected evidence as to the relationship between karyotype symmetry and the degree of specialization of the organism. A low degree of specialization is found in organisms with symmetric chromosomes. Again, increased specialization is connected with the presence of asymmetric chromosomes. Chromosomes with a single arm are more common in animals than in plants, while animals are much more specialized organisms than plants. So, the Estonian quail, as a single case, and avian karyotypes on the whole, with a high number of one-armed chromosomes, must present another evidence of high specialization.

Studies where karyotypes of many different bird species were analyzed

(Tegelström, Ryttman, 1981), reveal that there seems to be a correlation between the number of microchromosomes and the morphological type of macrochromosomes. One-armed (acro- or telocentric) macrochromosomes are more common (40.2%) in species with many microchromosomes. The opposite is true for bi-armed (meta- or submetacentric) macrochromosomes. The authors propose that Robertsonian translocations of microchromosomes giving rise to small, bi-armed macrochromosomes, are responsible for these chromosomal changes. The karyotype of the Estonian quail belongs, thus, to that more common group of bird karyotypes with a large number of microchromosomes and with no bi-armed smaller macrochromosomes, for bi-armed are only the first two of its macrochromosomes and the 'Z-chromosome.

Conclusions

Proceeding from our results and from the data of literature it may be concluded that the karyotype of the Estonian quail is quite a typical avian karyotype: its chromosomes are small, there is a high number of microchromosomes and a low number of macrochromosomes, and in chromosome morphology asymmetry is prevailing. We can point out the following relationships that describe the studied karyotype:

1) relationship between the number of macro- and microchromosomes;
2) relationship between chromosome number and size;
3) relationship between the number of microchromosomes and the morphology of macrochromosomes;
4) relationship between karyotype asymmetry and high specialization of the organism.

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EESTI VUTI KARÜOTÜÜP. KROMOSOOMIDE ARV, SUURUS JA MORFOLOOGIA

On uuritud eesti vuti rutiinsel meetodil värvitud karüotüüpi ja näidatud, et kromosoomide arv kõigub 70—78 piires. Nendest kuus kromosoomipaari kuulub makrokromosoomide ja ülejäänud mikrokromosoomide hulka. Kromosoomide suurus ulatub 9,2 mikronist 0,3 mikronini. Esimene ja teine kromosoom on morfoloogiliselt tüübilt submetatsentrilised, kolmas, neljas ja viies kromosoom akrotsentrilised, *Z*-kromosoom submetatsentriline ja mikrokromosoomid akrotsentrilised. Kromosoomide arvu, suurust ja kuju on analüüsitud kromosoomiväljateooria alusel. Uurimuses on esile toodud lindude karüotüübile iseloomulikud tendentsid.

Терье РАУДСЕПП

КАРИОТИП ЭСТОНСКОГО ПЕРЕПЕЛА. ЧИСЛО, РАЗМЕРЫ И МОРФОЛОГИЯ ХРОМОСОМ

Кариотип эстонского перепела был изучен с помощью рутинного окрашивания. Показано, что число хромосом колеблется в пределах 70—78. Из них 6 пар являются макрохромосомами, остальные микрохромосомами. Размеры митотических хромосом колеблются в пределах 9,2—0,3 микрона. Первая и вторая аутосомные пары являются субметацентрическими; третья, четвертая и пятая пары — акроцентрическими; *Z*-хромосома — метацентрическая и все микрохромосомы — акроцентрические. Число, размеры и морфология хромосом анализируются по теории хромосомного поля. В работе представлены типичные тенденции для кариотипов птиц.