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CONSTITUTIVE HETEROCHROMATIN (C-BANDS) AND C-POLYMORPHISM IN THE KARYOTYPE OF THE ESTONIAN BREED OF THE JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*)

Introduction

Constitutive heterochromatin is a quantitatively important component of eukaryotic chromosomes, and it usually contains more repetitive DNA than other chromatin components. Constitutive heterochromatin of eukaryotic chromosomes has sequences which, although they are peculiar to every species, show homology in phylogenetically related ones. On the other hand, constitutive heterochromatin may be quite variable even within the limits of one single species — cases of heterochromatin polymorphism are actually not rare at all (De Lucca, 1983; Christidis, 1986). Constitutive heterochromatin polymorphism has been shown in many domesticated bird species (the Domestic fowl, pheasant, turkey, the Japanese quail). Usually, this type of polymorphism concerns the avian Z- and W-chromosomes (gonosomes) (Родионов et al., 1987).

The function of repetitive DNA or of constitutive heterochromatin is still the subject of much conjecture and discussion. In general, except in a few cases, most repetitive DNA is considered to be inert in terms of RNA transcription, but it is supposed that constitutive heterochromatin may influence the expression of the genes located nearby (location effect) (Britten, Davidson, 1971). In addition, constitutive heterochromatin has some structural functions: it is usually located in the centromeric area of chromosomes, and gives these regions greater mechanical resistance (De Lucca, 1983).

Taking into account the heterogeneity and, possibly, polymorphic character of constitutive heterochromatin, the aim of our research was to reveal constitutive heterochromatin, its amount and character in the karyotype of a new breed of the Japanese quail — the Estonian quail.

Material and methods

Thus, the object of our research was the Estonian breed of the Japanese quail (*Coturnix coturnix japonica*). This breed is quite a new one — it was officially recognized only in 1988. The breed is characterized by high meat production (adult live weight in males 169 g; in females 191 g), average annual egg production is 285. The breed resulted from a long-term selection work carried out by selectionists of the Estonian Academy of Sciences and Kaarepere Experimental Station (Estonia) (Tikk, 1989).

In our experiments we used five-day-old quail embryos. 0.1 ml colchicine (0.1 mg/ml) was injected into the air-chamber of the egg. Incubation lasted for 1—2 h-s. Then the embryos were taken out, chopped up and incubated in hypotonic solution (1.25% Na-citrit) for 30 min, 37 °C. After that the embryonic cells were fixed 4 times in methanol-acetic acid fixative (3 : 1). Preparations were made on ice-cold slides and air-dried.

The slides were stained by the following C-banding method: incubation in saturated $Ba(OH)_2$ for 1 h, 60 °C; incubation in 2×SSC, 1 h, 60 °C; staining in Giemsa solution (4%) in phosphate buffer, pH 6.8, 1 h.

Results and discussion

Avian karyotypes are characterized by some specific traits that make the investigation of these somewhat difficult. This mainly concerns their large number of chromosomes (usually more than 80), and their small dimensions (the largest of them are usually smaller than the middle-sized mouse chromosomes, and their visualization lies on the border of light-microscope capacity) (Родионов et al., 1987). Up to now, the main part of the Japanese quail (and other avian) chromosomes have not yet been identified. Taking into account the mentioned difficulties, it is essentially important and interesting to study chromosomal polymorphism and look for genetic markers on this material.

According to our results, there are 73—78 chromosomes in the mitotic karyotype of the Estonian quail (Fig. 1). From these, the majority are formed by microchromosomes that are identified with difficulties or not identified at all. It is supposed that the varying chromosome number in avian karyotypes is caused by asynchronic spiralization of microchromosomes. This results in their different staining ability. Therefore authors usually talk about the modal number of microchromosomes. It is agreed to be 66 (Яковлев, 1985). All microchromosomes (including the W-chromosome) are of heterochromatic character and on our material we could see prominent C-bands in most microchromosomes.

It is known that the amount of constitutive heterochromatin in the karyotype of the Japanese quail surpasses that of the Domestic fowl chromosomes (Lance-Jones, Lagenaur, 1987). As to the amount of DNA, 1/3 of the quail genome is formed by microchromosomes (Яковлев, 1985). Fluorochrome staining, carried out by us earlier, showed that microchromosomes of the Estonian quail contain GC-rich DNA (Kummik, Raudsepp, 1987; Родионов et al., 1987). There are data according to which the totally heterochromatinized W-chromosome contains GC-rich DNA in its centromeric area and AT-rich DNA in terminal regions (Stock, Bunch, 1982). In our work we could not show it, as we could not show the W-chromosome either. Being one of the microchromosomes, it turned out to be very difficult and doubtful for us to identify it.

As macrochromosomes, we considered 5 pairs of autosomes and the gonosome Z. Of course, the border beetween macro- and microchromosomes is quite stipulated and depends on one's ability to identify the concrete chromosome. As to their centromere position, the Estonian quail's 1st autosome is submetacentric; the 2nd autosome is metacentric; the 3rd, the 4th and the 5th autosomes are acrocentric, and the Z-chromosome is metacentric. C-banding occurred in the centromeric areas of all macrochromosomes, whereas the Z-chromosome had no centromeric C-band. Telomeric C-bands were seen in 1q and 3q, and on both arms of the Z-chromosome. In some metaphases, C-banded regions were seen in the middle of chromosome arms. The staining ability and the amount of telomeric and interstitial constitutive heterochromatin tended to vary in different cells as well as between homologous chromosomes.

As it has been shown earlier (Kummik, Raudsepp, 1987), it is interesting that the centromeric C-blocks of the 1st and the 2nd autosomes are AT-rich, whereas the analogous regions in the Domestic fowl chromosomes are GC-rich (Родионов et al., 1987). The C-banded macrochromosomes of the Estonian quail are shown on an idiogram (Fig. 2).

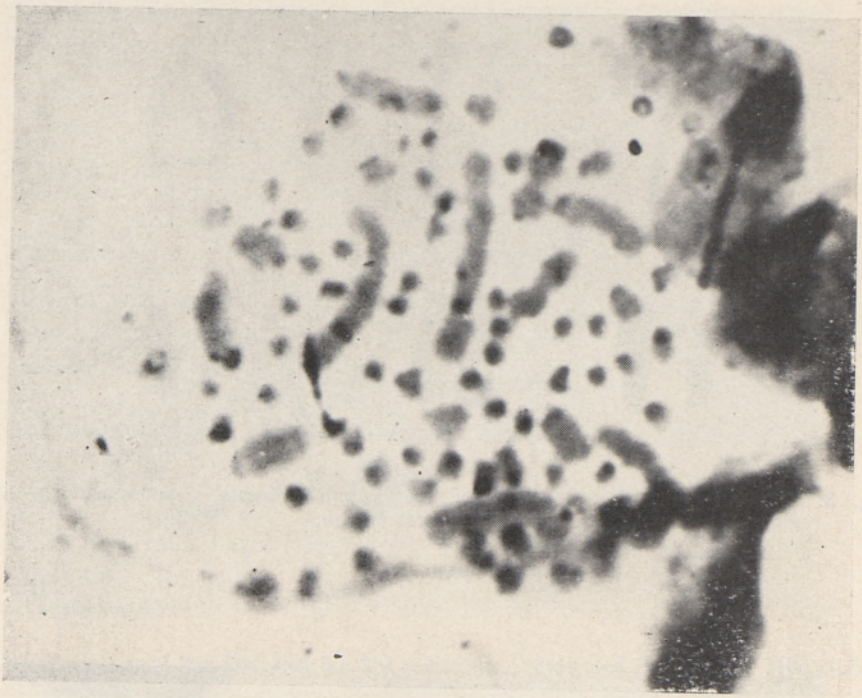

Fig. 1. C-banded karyotype of the Estonian breed of the Japanese quail (100×1.5×
×12.5×10).

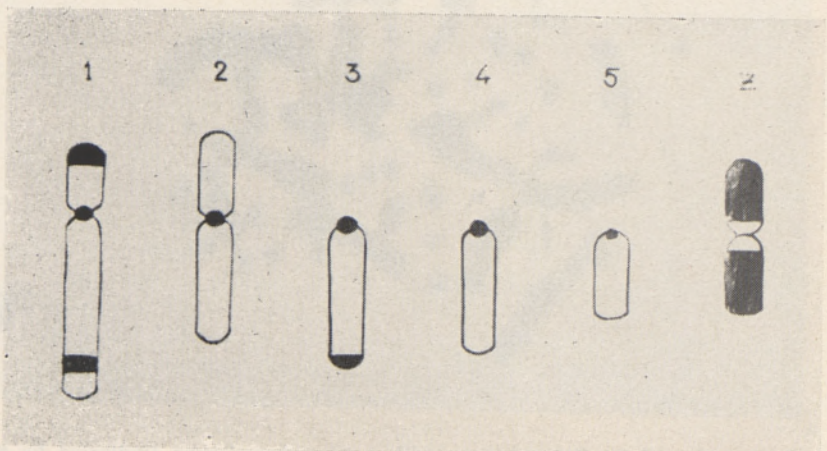

Fig. 2. Idiogram of macrochromosomes of the Estonian quail (C-banding) (100×1.5×
×12.5×10).

Fig. 3. The 1st pair of autosomes of the Estonian quail. C-polymorphism of the centromeric region.

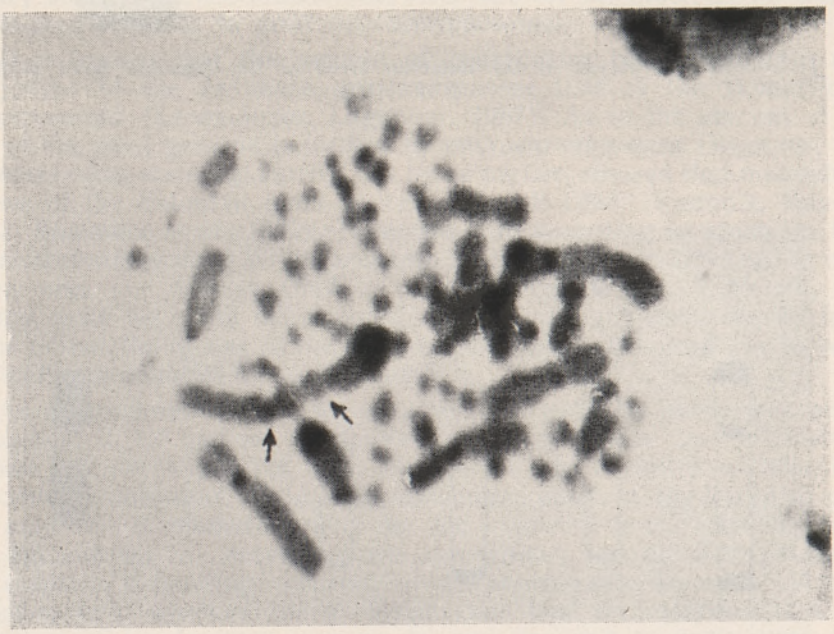

Fig. 4. Conjugation of the 3rd autosome with a microchromosome (indicated by arrows) (100×1.5×12.5×10).

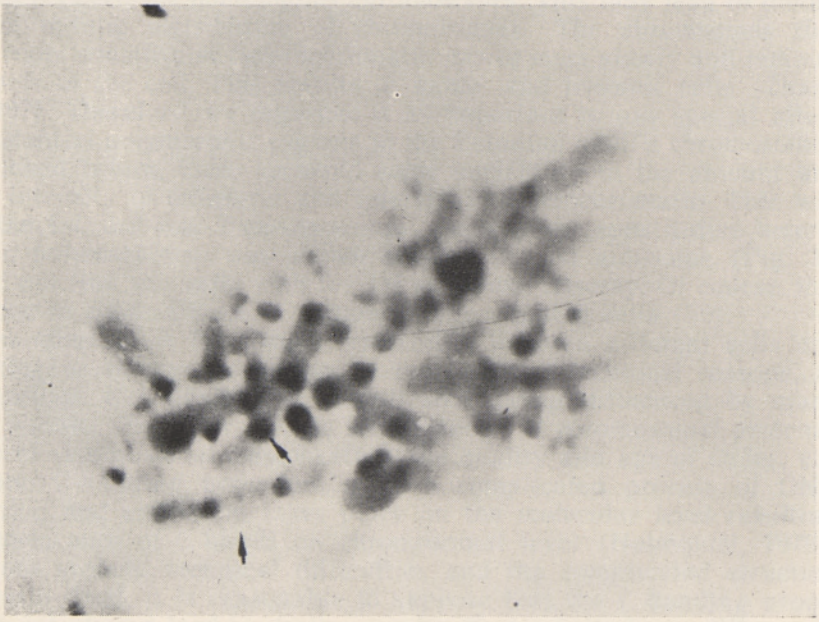

Fig. 5. Conjugation of the 2nd and the 3rd autosomes (indicated by arrows) (100×1.5×
×12.5×10).

According to the data of literature, one of the possible causes for trisomy in man is the large differences of the amount of constitutive heterochromatin between the homologous chromosomes. This may lead to disturbances in mitotic distribution of chromosomes. The chromosome that contains more constitutive heterochromatin moves more slowly and remains on one pole of the cell. This results in mono- and trisomic cells (Zhang et al., 1987). Therefore, we regarded it as important to pay special attention to potential polymorphic regions in the Estonian quail's chromosomes. As mentioned before, there occurred some variability in the amount and staining intensity of telomeric and interstitial heterochromatin. This was seen in the 1st, 3rd and Z-chromosomes. Centromeric constitutive heterochromatin polymorphism was shown only in the 1st autosome (Fig. 3). We could not find any cells with the 1st autosome trisomy. Of course, this may be due to relatively small number of investigated cells (50).

On the other hand, the C-band variability between different cells and the homologous chromosomes may be the result of technical reasons, as the mechanism of C-banding is complicated, being influenced by many different factors. The latter include the character of chromosomal proteins, the stage of chromosomal condensation, the quality and age of slides, etc. During the C-banding procedure quite a complicated process of DNA denaturation and extraction takes place on the molecular level resulting in the appearance of C-bands on chromosomal level (Holmquist, 1979). Taking into account technical difficulties and the complicated structure of chromatin itself, it is reasonable to suppose that the C-banding procedure does not always lead to similar results. Particularly large differences in banding pattern must take place in regions with a low or heterogenic content of constitutive heterochromatin. Respectively, such chromosome regions which contain especially large amounts of constitutive heterochromatin must stain in more or less all the investigated cells. On the whole it must be mentioned that the sensitiveness of the existing C-banding methods is not high enough to reveal very small chromatin blocks (Pollock, Fechheimer, 1981).

Taking into consideration the above-mentioned facts, we may assume that the stained regions of macrochromosomes and microchromosomes of the Estonian quail contain comparatively large amounts of constitutive heterochromatin, as those regions were stained in most of the investigated cells. Especially bright C-bands were seen in the 1st autosome, but in order to prove the existence of chromosomal polymorphism in this chromosome, further investigations are needed.

As the telomeric and interstitial regions tended to show variable C-banding, it is supposed that the content of constitutive heterochromatin in these regions is low or heterogenic.

In the investigated material (50 cells) we could find 3 triploid cells and some macro- and mircochromosome translocations. On the whole, the C-banding method is a good tool to reveal chromosomal abberrations, especially pericentric translocations. According to the data of literature, there occur more chromosomal abnormalities in avian karyotypes as compared to the karyotypes of mammals (Tegelström et al., 1983; Яковлев, 1985).

In our experimental material we discovered conjugation of the 3rd autosome to a microchromosome. This "new" chromosome could be detected according to an unusual localization of chromosome arms (Fig. 4). Figure 5 shows the conjugation of the 2nd and the 3rd autosomes with each other. The 3rd autosome appeared to be the most capable in forming pericentric conjugation.

As it has been pointed out, the C-banded karyotype of the Estonian quail needs further investigation with a larger quantity of experimental

material. And certainly, statistical analysis is needed. On the other hand, it would be interesting to compare the C-banded karyotypes of different *Galliformes* species and of different breeds of the Japanese quail. This may provide new information about evolutionary trends within the family.

Usually, the type of constitutive heterochromatin polymorphism shown above is not connected to phenotypic traits, yet it may be useful for hybridization analysis — polymorphic heterochromatic blocks may serve as good genetic markers.

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KONSTITUTIIVNE HETEROKROMATIIN (C-VÖÖDID) JA C-POLÜMORFISM JAAPANI PÕLDVUTI (*COTURNIX COTURNIX JAPONICA*) EESTI TÕU KARÜOTÜÜBIS

On käsitletud konstitutiivse heterokromatiini paiknemist ja iseloomu jaapani põldvuti (*Coturnix coturnix japonica*) uue tõu — eesti vuti — karüotüübis. Kasutades heterokromatiinile omast värvimismeetodit, on näidatud, et konstitutiivset heterokromatiini sisaldavad suurel hulgal kõik mikrokromosoomid ning makrokromosoomide peritsentromeersed piirkonnad. Esimeses autosoomis võib oletada heterokromatiinse polümorfismi olemasolu. Värvunud alasid leidus ka makrokromosoomide õlgadel ja telomeerses piirkonnas (1q; 3q). Makrokromosoomide hulka kuuluvas sugukromosoomis Z ei ole peritsentromeerset heterokromatiini täheldatud, seevastu esinesid C-vöödid Z-kromosoomi kummalgi õlal.

C-värvimismeetod on küllaltki komplitseeritud ning osa mikroskoobis nähtud C-vöötidest tuleb tõenäoliselt artefaktideks arvata. Sellegipoolest on konstitutiivse heterokromatiini uurimine perspektiivikas, sest C-vööte saab kasutada geneetiliste markeritena hübridoloogilistes katsetes. Katsematerjali vähesuse tõttu tuleb siinset uurimust pidada vaid sissejuhatuseks eesti vuti karüotüübi tundmaõppimisel.

Терье РАУДСЕПП

КОНСТИТУТИВНЫЙ ГЕТЕРОХРОМАТИН (*C*-ПОЛОСКИ) И *C*-ПОЛИМОРФИЗМ В КАРИОТИПЕ ЭСТОНСКОЙ ПОРОДЫ ЯПОНСКОГО ПЕРЕПЕЛА

Распределение и характер конститутивного гетерохроматина в кариотипе новой эстонской породы японского перепела (*Coturnix coturnix japonica*) изучались с использованием специфической окраски гетрохроматина. Показано, что конститутивный гетерохроматин содержится в большом количестве во всех микрохромосомах и перицентрических областях макрохромосом. В первой аутосоме предполагается наличие гетерохроматинового полиморфизма. Окрашенные области наблюдались и на плечах, и в теломерной области макрохромосом (1*q*, 3*q*). В половой хромосоме *Z*, которая также относится к макрохромосомам, перицентромерного гетерохроматина не обнаружено. В то же время *C*-полоски наблюдались на обоих плечах *Z*-хромосомы.

Метод *C*-окраски довольно сложен и некоторые *C*-полоски, наблюдаемые на светооптическом уровне, следует считать, по-видимому, артефактами. Тем не менее исследование конститутивного гетерохроматина перспективно, поскольку *C*-полоски могут быть использованы как генетические маркеры в гибридологическом анализе. В связи с недостатком опытного материала данное исследование следует считать первым шагом при изучении кариотипа эстонского перепела.