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COMPARATIVE STUDY OF G-, QFQ- AND QFH-BANDING IN THE MITOTIC KARYOTYPE OF THE ESTONIAN QUAIL

Introduction

From the beginning of the 1970s, when differential banding techniques for chromosomes with quinacrine derivatives (QFQ-banding) and the Giemsa stain (G-banding) were discovered, functional meaning of the produced bands became of continuous interest (Родионов, 1985). According to present knowledge (van Duijn et al., 1985) there exists a correlation between the G- and QFQ-bands, though this coincidence is not just one-to-one. With autoradiographic techniques it has been demonstrated that the G- and QFQ-banding patterns largely correlate with late replicating DNA regions (Родионов, 1985). This initiated the idea that the G- and QFQ-positive bands reflect late replicating, adenine-thymidine (A-T) rich DNA (Shafer et al., 1982). This has been proved by using A-T-specific agents like Hoechst 33258 (Rodionov et al., 1983; Fritschi, Stranzinger, 1985; van Duijn et al., 1985). The resulting chromosome-banding pattern (QFH-banding) is similar to those of G- and QFQ-techniques.

On mammalian material it has been shown (Родионов, 1985) that compared to unstained regions in G-banding and non-fluorescenting regions in QFQ-banding (R-bands) the G- and QFQ-positive regions contain genetically comparatively inert heterochromatin-like material, and fewer genes. It has been proposed that in QFQ- or G-blocks mainly tissue-specific genes and pseudogenes are located and that these regions are rich in "silent" DNA (Родионов, 1985). On the other hand, genes that are actively transcribed in various tissues are located in interband (R-band) regions.

The base pair content does not show the genetic character of the concrete chromosome region one-to-one. Comings (1978) has shown that genetically comparatively inactive facultative heterochromatin located on chromosome arms is A-T rich. Bright QFQ- and G-bands indicate this type of chromatin. Genetically active euchromatin containing most of the important genes (e. g. housekeeping genes) is rich in guanine-cytosine (G-C) base pairs, and corresponds to R-bands. Genetically inactive, highly repetitive constitutive heterochromatin located mainly in pericentromeric regions, may be either G-C or A-T rich.

The chromosome field theory proposed by Lima-de-Faria states that there exists an ordered distribution of specific DNA sequences within the centromere-telomere segment of a chromosome. There is both order and hierarchy within chromosomes, and genes are not located randomly (Lima-de-Faria, 1980; Lima-de-Faria et al., 1986; Lima-de-Faria, Mitelman, 1986).

From the list of karyological references concerning avian cytogenetics (De Boer, 1984) one can see that the G-banded karyotype of the Japanese quail (*Coturnix coturnix japonica*) has been studied in three cases (Ryttman, Tegelström, 1981; Sasaki, 1981; Stock, Bunch, 1982). QFQ-banding

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is discussed in only one short communication by Comings and Wyandt (1976). The A-T and G-C base pair location in the Japanese quail chromosomes is shortly described by Rodionov et al. (Родионов и др., 1987).

At present there exist several commercial breeds of the Japanese quail, and almost nothing is known about their cytogenetics. One of these is the Estonian quail. The breed is quite a new one — officially recognized only in 1988 (see: Tikk, 1989, Raudsepp, 1990). As a matter of fact, systematic position of laboratory and commercial lines (breeds) of quails belonging to the *Coturnix* genus is still obscure (Родионов и др., 1987). There is no common view whether to consider them as subspecies of the Japanese quail or not (Родионов и др., 1987). It is hoped that cytogenetical investigations might help to find some answer to this problem.

The task of the present investigation was to carry out a parallel study of G-, QFQ- and QFH-banding of the Estonian quail's chromosomes in order to 1) compare the results of these three techniques to each other, 2) establish a QFH-banded karyogram of the Estonian quail, 3) detect the localization of A-T-rich DNA regions and find out their optimal position in the chromosome field and 4) compare our results to the data of literature concerning the Japanese quail.

Material and methods

Material. Estonian quails (5 males and 5 females) were received from Kaiavere Quail Farm (Estonia). In order to obtain metaphase chromosome preparations we used short-term bone-marrow cell cultures described by Christidis (1985).

Methods. *G-banding* procedure was carried out according to Ryttman et al. (1979) with Giemsa stain (Difco, USA).

QFQ-banding was obtained by using quinacrine dihydrochloride (Sigma, USA), and the procedure was carried out according to Dev and Tantravahi (1982).

QFH-banding procedure was a slight modification of that proposed by Rodionov et al. (1984): preparations were dehydrated in 90%, 70% and 30% ethanol solutions; rinsed in Sörensen buffer (SB) (pH 7) for 10 min; stained with Hoechst 33258 (Serva, FRG, 0.5 µg/ml in SB; pH 7); covered with cover slip and kept in the dark for 15 min; rinsed in SB (pH 7) for 10 min, and, following that, rinsed in distilled water. In addition, Hoechst 33258 stained chromosomes were treated with an actinomycin D solution (0.03 mM in 0.01 M Na-phosphate, pH 6.8 for 7—12 min) for counterstain-enhanced chromosome banding. Preparations were mounted in Mowiol (Aldrich, USA), and covered with cover slip.

Microscopy. The G-banded preparations were examined under photomicroscope (Biolar PI, Poland). The QFQ- and QFH-banded slides were examined under Olympus fluorescence photomicroscope AH-2 (Japan).

Arm-frame method. To get information concerning relationships between specific DNA regions (in our study the A-T rich DNA) and the chromosome organization, the arm-frame method (Lima-de-Faria, 1980) was used. This is a simple procedure in which all the arms of the chromosome complement (in our study only macrochromosomes were included) of a species are located according to their length with all the centromeres on a vertical line (at 0 microns), and telomeres on a line that forms a 45° angle with the centromere line. The arms are located according to their length irrespective of the chromosome to which they belong. Each arm is divided irrespective of its length into three equal parts: a proximal third, a median third, and a distal third. These three segments build the centron, medon, and telon regions, respectively. All measurements were made directly on photographs.

Results

As it has been shown before (Raudsepp, 1990), there are approximately 73—78 chromosomes in the mitotic karyotype of the Estonian quail. The majority of them are microchromosomes identifiable with difficulties or not at all. The modal length of microchromosomes (established on photographs) is less than 1 micron. That makes some of these chromosomes almost invisible under light microscope to say nothing of the possibility to identify them according to G-, QFQ- or QFH-banding patterns. As a matter of fact, the W-chromosome (females are heterogametic, having ZW gonosomes) is also one of the microchromosomes and thus difficult to identify. As we could not determine the W-chromosome with full certainty it is left out of discussion.

Five pairs of autosomes and the gonosome Z were considered as macrochromosomes. Their length extends approximately from 9.2 to 3.2 microns, depending on the condensation of chromosomes in different metaphases. Using the method of chromosome classification on the basis of centromere position (Naranjo et al., 1983), it was revealed that the 1st and 2nd autosomes were of submedian type; the 3rd, 4th and 5th autosomes were acrocentrics, and the Z-chromosome was metacentric.

G-banding. A general view of the G-banded karyotype of the Estonian quail showed Giemsa-positive bands on all the macrochromosomes and on all the largest microchromosomes, while the majority of microchromosomes remained weakly stained (Fig. 1). The G-banding pattern of the macrochromosomes was clear enough for identifying every single pair of them (Fig. 6). The G-banding of the centromeric regions of most autosomes and the Z-chromosome was negative. At the same time the centromeric region of the 1st autosome showed a clear G-positive band.

QFQ-banding. On the QFQ-banded karyotype of the Estonian quail (Fig. 2) it could be seen that all chromosomes, considered by us as macrochromosomes, and some chromosomes of the median size showed bright fluorescence. Microchromosomes showed dull fluorescence. Fluorescenting regions on macrochromosomes were located on chromosomes' arms, chromosome tips and centric regions showed dull fluorescence. As no clear QFQ-bands were received, the description of the QFQ-banded karyotype is limited to general view only.

QFH-banding. A-T specific Hoechst 33258 gave a clear banding pattern along chromosome arms (Fig. 3). All macrochromosomes showed bright fluorescence. The microchromosomes remained comparatively dull. The resolution of QFH-banding enabled to arrange macrochromosomes' karyogram of the Estonian quail (Fig. 4) and to describe each macrochromosome according to its QFH-banding pattern. A more schematic approach was seen on the QFH-banded idiogram (Fig. 6).

In general, three types of QFH-bands could be discerned on macrochromosomes: especially bright bands, more or less bright bands, and almost dull, non-fluorescenting regions or interbands. As Hoechst 33258 is an A-T base pairs specific agent, the bright and especially bright bands indicate the prevalence of A-T-rich DNA in these regions.

The centromeric regions of macrochromosomes usually remained dull. The only exception here was the first autosome, where a bright pericentromeric QFH-band could be seen. Out of 50 examined metaphases the centromeric regions of this autosome showed some kind of variability in fluorescence intensity — in some metaphases fluorescence was bright, whereas, in some particularly bright.

Comparison between the G-, QFQ- and QFH-banding. Comparing the results of the three used staining methods, a general tendency could be

brought out — macrochromosomes were stained or banded while most of the microchromosomes remained dull.

Comparing the G- and QFH-banded karyograms more precisely, one can see that the Giemsa-positive bands coincided with bright QFH-bands (Fig. 5). Especially good accordance was between the darkest G-bands and the brightest QFH-bands. It is important to note that the centromeric region of the first autosome was stained (or fluorescenting) with both techniques.

There can be only a very general comparison of the QFQ-banding with the two other techniques. So, due to poor band resolution, no specific band relationships could be revealed.

Arranging the chromosome field. The chromosome field theory, worked out by A. Lima-de-Faria (1980) gave us an idea that there may exist some kind of ordered distribution of the A-T-rich clusters within the centromere-telomere field. In order to check up on this, we arranged chromosome field for all 5 pairs of macrochromosomes and the gonosome Z (Fig. 7). On the centromere-telomere field only the brightest QFH-bands were placed, as in these the prevalence of A-T base pairs was evident. It could be seen that the A-T-rich clusters in all the macrochromosomes were located in an area that covered the central part or the beginning of the third third of a chromosome arm. Within the arm-frame they started far away from centromere and ended in the longer arm, away or slightly away from telomere. In shorter arms they ended in some cases at telomere. The QFH-bands tended to avoid proximal and distal regions (Fig. 7). Looking at the idiogram (Fig. 5), one can see that the just bright QFH-bands tend to occupy the same territory in the chromosome field. The only exception is the bright centromeric QFH-band in the first autosome, which is discussed below.

Discussion

So, as Hoechst 33258 is an A-T-base-pair specific agent, it may be concluded that the bright and particularly bright QFH-bands correspond to A-T-rich chromatin. On the other hand, as there exists a certain correspondence between the G-, QFQ- and QFH-banding patterns, it is reasonable to suppose that G- and QFQ-banding techniques reveal chromosome regions of the same character. Of course, the QFQ-technique did not show a too good banding pattern, but the general view of the karyotype was the same as with the other two methods. These results are in full accordance with the data of literature (Comings, 1978; Shafer et al., 1982; Fritschi, Stranzinger, 1985; van Duijn et al., 1985; Родионов, 1985). On the basis of this, it may be concluded that the Giemsa-positive bands, bright QFQ-regions, and bright QFH-bands on chromosome arms correspond to facultatively heterochromatic regions. These regions are A-T rich, they contain comparatively few genes, and are genetically relatively inactive (in the sense of transcriptional activity) (Comings, 1978). Moreover, using these three techniques it may be said that A-T-rich DNA regions generally tend to be located on the arms of the largest chromosomes only. Most of the microchromosomes do not contain this type of DNA. Some authors (Comings, Wyandt, 1976; Родионов и др., 1987) have shown that the microchromosomes of the Japanese quail are G-C rich. It is likely that the same is true about the Estonian quail, although further investigations should be made.

As to centromeric regions of macrochromosomes, it has been shown (Raudsepp, 1990) that all macrochromosomes (except the Z-chromosome) and microchromosomes contain more or less pericentromeric constitutive heterochromatin. Our present results indicate that only the first autosome

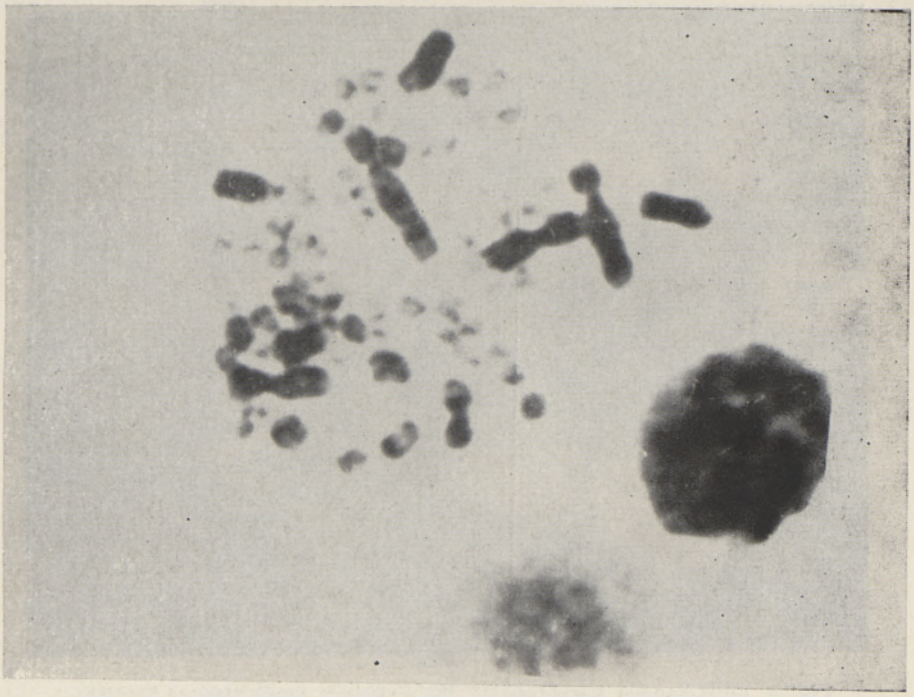

Fig. 1. Giemsa banded (G-banded) karyotype of the Estonian quail (100×1,5×12,5).

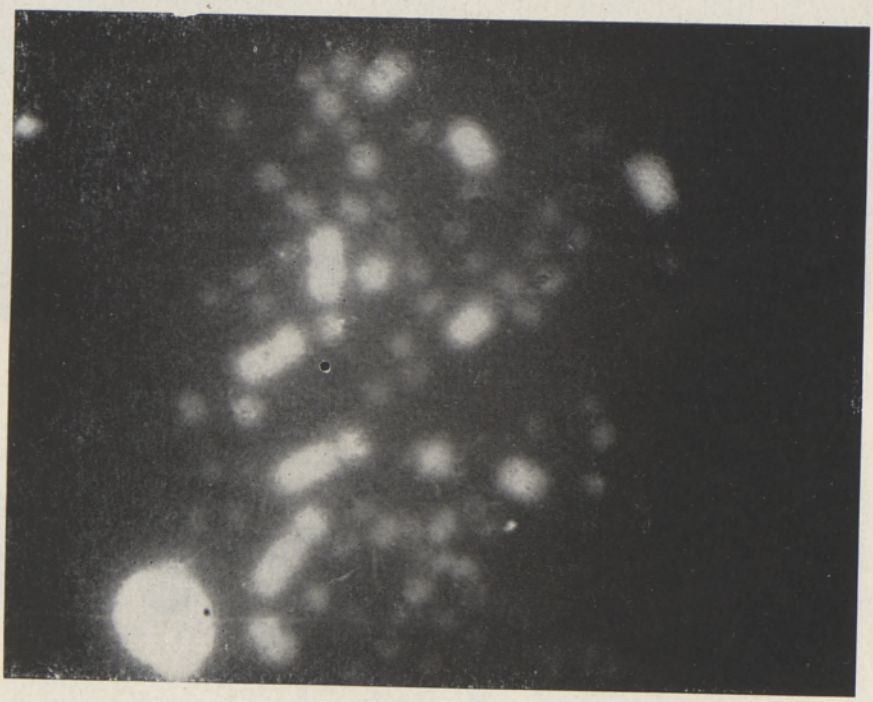

Fig. 2. QFQ-banded karyotype of the Estonian quail (100×1,5×12,5).

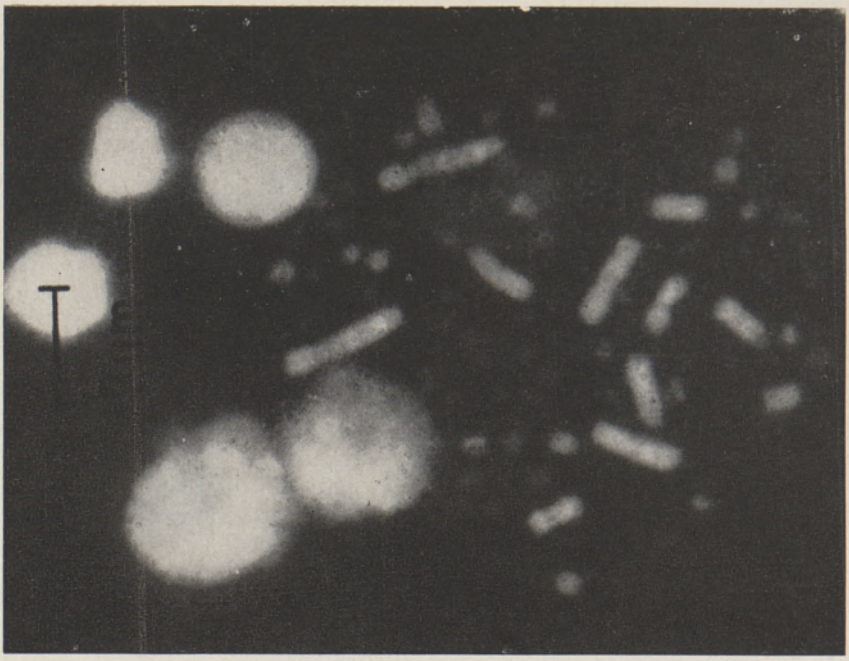

Fig. 3. QFH-banded karyotype of the Estonian quail (100×1,5×12,5).

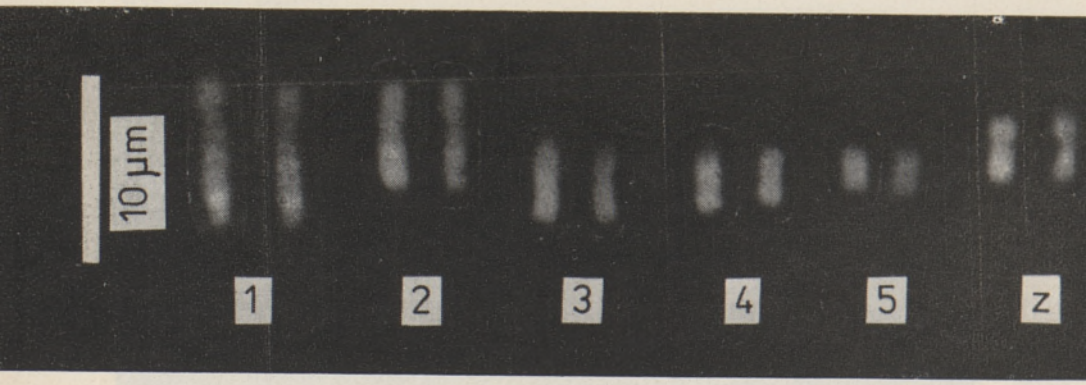

Fig. 4. QFH-banded karyogram of the Estonian quail's macrochromosomes.

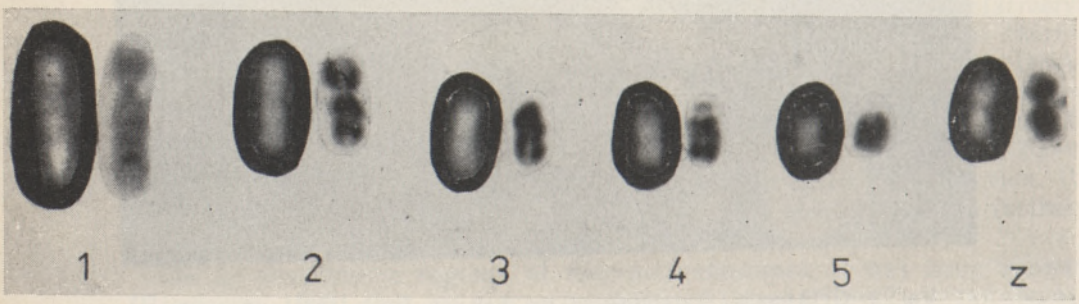

Fig. 5. Comparison between the G- and QFH-banded karyograms of the Estonian quail. Right — G-banding; left — QFH-banding.

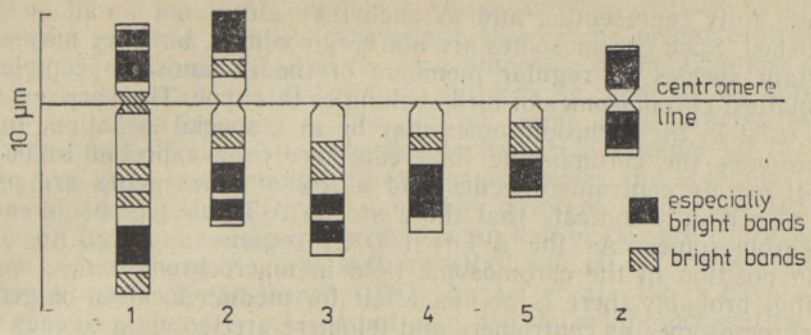

Fig. 6. QFH-banded idiogram of the Estonian quail's macrochromosomes.

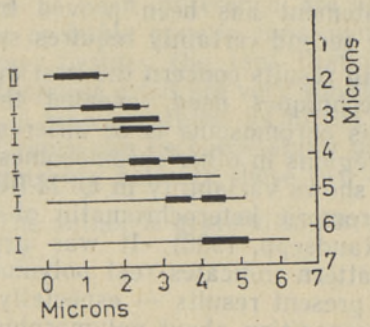

Fig. 7. Location of especially bright QFH-bands (A-T-rich DNA regions) in Estonian quail's macrochromosomes. Distributed as medons in the chromosome field. (Roman numerals to the right of chromosome arms indicate on the number of arms of the same length.)

contains A-T-rich constitutive heterochromatin in its pericentromeric region. Comings (1978) has proposed that the constitutive heterochromatin may be either A-T- or G-C-rich. So we can speculate that the pericentromeric regions of other chromosomes of the Estonian quail contain G-C-rich constitutive heterochromatin. This has been shown in the Japanese quail (Родионов и др., 1987). Still, the supposition concerning the Estonian quail needs further verification.

Our results do not only indicate coincidence of G-, QFQ- and QFH-banding but there also exists visible regularity in the location of A-T-rich DNA clusters over the whole karyotype as well as in individual chromosomes. The *arm-frame* method enabled to show that the A-T-rich clusters located on chromosome arms are not situated randomly. The brightest QFH-bands can be classified as medons, as they cover the territory that is usually avoided by typical centrons and telons. According to our results, the optimal territory for A-T-rich DNA is in the second third or the third third of a chromosome arm irrespective of the chromosome. This is in full accordance with Lima-de-Faria's findings on several species (1980) where Giemsa bands and facultatively heterochromatic regions are classified as medons. Our results show that the mentioned regions contain A-T-rich DNA.

The absence of G-, QFQ- and QFH-bands in microchromosomes is understandable. The chromosome field theory (Lima-de-Faria, 1980; Lima-de-Faria et al., 1986; Lima-de-Faria, Mitelman, 1986) classifies not only specific genes but also whole chromosomes. A chromosome that is shorter than 1 micron at metaphase of mitosis, can hardly have a centromere and

telomere fully represented, and as such the field is not at all or poorly established. Such chromosomes are not very common, but they may appear in certain species as regular members of the chromosome complement. The smallest chromosomes of birds belong to this type. The very few genes which exist in these chromosomes may be in a special situation. In such chromosomes the chromosome field can hardly be expected to be fully formed: merely centromere genes and a few of other genes are present there. Our results indicate that there are no A-T-rich regions in most of microchromosomes. As the A-T-rich DNA regions appeared to occupy median position in the chromosome field in macrochromosomes, we suppose that probably there is no space left for median location of genes in microchromosomes as centromere and telomere are too close to each other. Moreover, the chromosome field theory enables us to predict that the smallest chromosomes contain mainly genes referred to as centrons (e. g. constitutive heterochromatin) and telons (e.g. nucleolus organizing regions). The first statement has been proved by C-banding technique (Raudsepp, 1990), the second certainly requires special investigation.

The most interesting results concern the first autosome of the Estonian quail. The banding techniques used revealed that the pericentromeric heterochromatin of this chromosome is of different base-pair consistence than pericentromeric regions in other chromosomes are. It is A-T rich and, in addition to this, it shows variability in QFH-fluorescence. Polymorphic behaviour of pericentromeric heterochromatin of the first autosome has been shown before (Raudsepp, 1990). It was argued then whether the variable C-banding pattern indicates real polymorphism or it represents only an artefact. The present results — especially the QFH-banding pattern — support the supposition about polymorphism.

In order to specify the systematic position of the Estonian quail we compared our G-banding results with the data of literature concerning the Japanese quail and the Domestic chicken (*Gallus domesticus*) (Ryttman, Tegelström, 1981; Sasaki, 1981; Stock, Bunch, 1982; et al., 1984). Actually, it is most appropriate to use information concerning the Domestic chicken. It has been shown (Ryttman, Tegelström, 1981) that the largest chromosomes of the Japanese quail and the Domestic chicken have almost analogous G-banding patterns; G-banding pattern of the first autosome of these two species is identical, the only difference is a pericentric inversion that has taken place in the first autosome of the Japanese quail, but the centromeric G-band has remained the same as in *Gallus domesticus*.

Comparison indicates that the chromosome number and morphology of the Japanese quail and the Estonian quail are the same. In our previous paper (Raudsepp, 1990) we described the second autosome of the Estonian quail as being metacentric. Now it is apparent that this conclusion was erroneous. The second autosome of the Estonian quail the same as that of the Japanese quail belongs to a submedian type.

Comparison of G-banding patterns was somewhat difficult: in the data of literature (Tegelström, Ryttman, 1981; Sasaki, 1981; Stock, Bunch, 1982) elongated prometaphase or early metaphase chromosomes with high-resolution G-banding pattern are used. In our results the chromosomes were obtained from middle metaphase, where chromosomes are more condensed and the resolution of G-bands is not so clear. Nevertheless, correspondence between the G-positive bands in these two quails is evident, though in our results several smaller bands appeared as one larger band. The only difference between these two quails lies in the G-positive centromeric band in the Estonian quail's first autosome. Analogous band is not indicated either in the Japanese quail or in the Domestic chicken. As to comparing the QFQ- and QFH-banded karyotypes, there are only two short communications concerning the Japanese quail (Comings, Wyandt,

1976; Родионов и др., 1987), neither is the description of these two banding patterns given in detail. Nevertheless, there is no bright centromeric QFQ- or QFH-band described in the first autosome of the Japanese quail.

Correspondence of the G-banding patterns in general in both the Estonian and the Japanese quails did not come as a surprise. Mainly because the Estonian quail is regarded to be just a commercial breed of the Japanese quail thus belonging to the same species and secondly, if the Estonian quail happens to have somewhat different systematic position, it must be taken into consideration that avian karyotypes are characterized by their conservatism. Several authors (Ryttman et al., 1979; Ryttman, Tegelström, 1981; Stock, Bunch, 1982; Ansari et al., 1986) have shown that it is not only closely related but also distant avian species that have surprising identity in G-banding pattern for macrochromosomes. Keeping this fact in mind, it is of particular interest that the centromeric region of the Estonian quail's first autosome shows different G-, QFQ- and QFH-banding pattern. Causes of such difference are not known, nor are we going to speculate about probable genetic consequences that this change may bring about. Anyway, besides the fact that the karyotypes of the Estonian quail and the Japanese quail are very similar, there exists a clear and intriguing difference between them as to the centric region of the first autosome. This difference can be successfully used as a true chromosomal marker in distinguishing between these two quails.

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EESTI VUTI KARÜOTÜÜBI G-, QFQ- JA QFH-VÖÖDISTUSE KÕRVUTAV ANALÜÜS

Eesti vuti mitootilise karüotüübi uurimiseks kasutati kolme erinevat diferentsiaalvärvimismeetodit: värvimine Giemsa värviga, mis toob esile G-vöödistuse; värvimine kinakriindehüdrokloriidiga, mis tekitab QFQ-vöödistuse, ja värvimine Hoechst 33258-ga, mis tekitab QFH-vöödistuse ning näitab ühtlasi ära adeniin-tümidiin (A-T) aluspaaridest rikkad DNA piirkonnad.

Selgus, et diferentsiaalse värvumise üldpilt on kõigil kolmel juhul sarnane. Et Hoechst 33258 seostub A-T aluspaaridega, võib arvata, et ka ülejäänud kaks meetodit toovad esile A-T rikkaid DNA piirkondi. Täheldati tendentsi, mille kohaselt A-T rikkad DNA piirkonnad paiknevad valdavalt makrokromosoomides. Mikrokromosoomid jäävad nimetatud meetodite kasutamisel nõrgalt värvunuks või ei fluorestseeru.

Kromosoomivälja analüüs näitas, et A-T rikkad piirkonnad ei paikne kromosoomides juhuslikult, vaid eelistavad kromosoomiõlgade keskmisi piirkondi, seega kuuluvad nn. medonite hulka.

Eesti vuti karüotüübi G-vöödistust võrreldi kirjanduse andmetest saadud Jaapani põldvuti karüotüübiga. Ilmnes, et karüotüübi üldpilt, kromosoomide arv ja morfoloogia, samuti makrokromosoomide G-vöödistus on neil kahel vutil ühesugused. See on ootuspärane, sest Eesti vutti peetakse üheks Jaapani põldvuti tõuks. Üllatav on aga asjaolu, et Eesti vuti 1. kromosoomi tsentromeeripiirkonnas on tugev G-vööt ja fluorestseeruv QFH-vööt. Viimane puudub Jaapani põldvutil. Muutuse geneetilised põhjused ja tagajärjed on veel selguseta. Igal juhul on nimetatud nähtus tähelepanuväärne ning seda saab kasutada kromosomaalse markerina.

Терье РАУДСЕПП

СРАВНИТЕЛЬНЫЙ АНАЛИЗ G-, QFQ- И QFH-ИСЧЕРЧЕННОСТИ КАРИОТИПА ЭСТОНСКОГО ПЕРЕПЕЛА

Для исследования митотического кариотипа Эстонского перепела были использованы три разных метода дифференциального окрашивания: окрашивание Гимзой (G-исчерченность), окрашивание кинакриндегидрохлоридом (QFQ-исчерченность) и окрашивание Hoechst 33258 (QFH-исчерченность). Следует отметить, что Hoechst 33258 является специфическим агентом для А-Т-нуклеотидных пар ДНК — следовательно, QFH-исчерченность показывает локализацию А-Т-богатых районов ДНК.

Выяснилось, что общая картина результатов всех трех методов похожая. Так как Hoechst 33258 специфически соединяется с А-Т-парами, можно полагать, что и остальные два метода выделяют те же самые районы ДНК. Замечена тенденция, что А-Т-богатые районы находятся в большинстве в макрохромосомах. При использовании указанных методов микрохромосомы остаются слабо окрашенными или слабо флуоресцирующими.

При исследовании хромосомного поля макрохромосом оказалось, что участки, богатые А-Т-парами, не находятся в хромосомах случайно. Скорее наоборот, они предпочитают средние участки хромосомных плечей — следовательно, ведут себя как «медоны».

Кариотип Эстонского перепела сравнивался по литературным данным с кариотипом Японского перепела. Оказалось, что общий вид кариотипа, число и морфология хромосом, также как и G-исчерченность макрохромосом у этих двух перепелов одинаковые. Это и естественно, так как Эстонский перепел считается породой Японского перепела. Тем не менее удивительно, что центромерный район первого аутосома Эстонского перепела окрашивается иначе по сравнению с таковым у Японского перепела. В центромерном районе первого аутосома Эстонского перепела имеется тёмная G- и флуоресцирующая QFH-полоса, у Японского перепела такой полосы нет. Генетические причины и результаты такой разницы ещё не ясны. По всей вероятности, выявленный нами феномен может успешно использоваться в качестве хромосомного маркера.