Various traits in rabbits were reported to inherit according to a linkage relation among loci. A review study focused on Albinism and Dominant White Spotting loci

[1]Andrei C. Grădinaru, [2,3,4]I. Valentin Petrescu-Mag

[1]University of Agricultural Sciences and Veterinary Medicine Iași, Faculty of Veterinary Medicine, Iași, Romania; [2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture, Cluj-Napoca, Romania; [3]SC Bioflux SRL, Cluj-Napoca, Romania; [4]University of Oradea, Oradea, Romania. Corresponding author: A. C. Grădinaru, andre_gradinaru@yahoo.com; a.c.gradinaru@uaiasi.ro

Abstract. The beginnings of linked inheritance are found in experiments conducted by Thomas Hunt Morgan with vinegar-fly. The tendency of various loci located on the same chromosome to show linkage was studied in different species of mammals, especially in those with a short period of gestation and a large number of descendants in each litter. A graphically relationship of the *r* locus (red-eyed yellow) between the loci *c* (albinism) and *p* (pink-eyed yellow) was firstly concluded in rats. In rabbits, the first linked relationship was reported on assumed chromosome I among loci for albinism, yellow fat, and brown coat. The second reported linked inheritance referred to the loci for Dutch white spotting *(Du),* English white spotting *(En),* and long (angora) hair *(l).* In past decades, various genes were associated with important loci described in Classical Genetic' studies. For example, the *fgf5* gene was found as a molecular candidate for the angora locus and the *KIT* gene for the spotting pattern in various species of animals. Although in mouse and other species the *KIT* gene was associated with deafness, in rabbits such of association was not reported. Instead, mutations of *KIT* gene are associated in rabbits with Congenital Megacolon. The aim of this paper is to review various reports about the linkage phenomenon in rabbits, focused on albinism and white spotting genes.
Key Words: linkage, coat colours, deafness, megacolon, rabbits.

Introduction. Mendel's principle of combination involved an independent segregation during meiosis of two or more allelic pairs due to their localization on different loci and on different chromosomes (Geiringer 1944). This made it possible a segregation ratio of 9/3/3/1 in the second generation of offspring in the investigated Pea garden plants (*Pisum sativum* L.), in which 9/16 probabilities were for both dominant characters, each 3/16 for alternative one character dominant and the other one recessive, and 1/16 for both recessive characters.

A deviation from this Mendel's second law of heredity was confirmed by Thomas Hunt Morgan while studying two autosomal characters related to the color of the eyes and the length of the wings in *Drosophila melanogaster* L. (vinegar-fly). Performing a testcross among double heterozygous F_1 females (with both dominant characters, red eyes and wings of normal length, respectively) and double homozygous males (with all four recessive alleles and both recessive characters, purple eyes and vestigial wings, respectively), the obtained phenotypical classes in the next filial generation matched the four classes established by Mendel in backcross experiments with Pea garden plants, but the segregation ratio was different from 1/1/1/1 reported when genes were located in different chromosomes and their segregation occurred independently. However, the reported segregation ratio in aforementioned experiment with *Drosophila melanogaster* revealed a greater share (each at slightly more than 40%) of the two parental types of individuals (with both dominant and both recessive characters), and a smaller one (each at slightly more than 5%) for the two non-parental types of individuals (with alternative one character dominant and the other one recessive), indicating a coupling of genes

when both dominant and recessive alleles were physically located together (pr^+ - vg^+ and *pr* - *vg*, respectively) on each chromosome of the homologous pair and segregated together in the obtained gametes (the linkage phenomenon). In the same time, recombinant gametes, with alternative one dominant and another one recessive alleles (pr^+ - *vg* and *pr* – vg^+, respectively) are the result of crossing-over, an exchanging process of parts among homologous chromosomes pair in meiosis (Griffiths et al 2000).

The aim of this paper is to review various reports about the linkage phenomenon in rabbits, which contributed over the time on the physical mapping of genes position in the structure of chromosomes. Far from being exhaustive, this review presents the first linkage studies focused on albinism and white spotting genes in rabbits, with additional data from recent reports.

Early Linkage Studies in Rodent and Non-rodent Species of the Glires Clade. The tendency of genes located on the same chromosome to show linkage (to be transmitted together through meiosis) was studied in various species of mammals, especially in those with a short period of gestation and a large number of descendants in each litter. Among the very first reports on linkage in animals, Dunn (1920) reminded those of Darbishire (1904), Cuénot (1907), Castle (1914, 1916, 1919), Castle & Wright (1915), Haldane et al (1915), Whiting & King (1915). They reported valuable linked traits in mice and rats, such as those between the genes for red eyes and pink eyes in yellow Norway rats, the genes for pink-eyes and albinism in house mice, the genes for albinism, pink-eye and red-eye, and the genes for red-eyed yellow and albinism in rats. In 1920 Dunn concluded an arrangement of three linked genes in rats based on the amount of crossing over between them, in a graphically relationship of the *r* locus (red-eyed yellow) between the loci *c* (albinism) and *p* (pink-eyed yellow). These findings were important both for involved species in the studies but also for other members of the sister orders Rodentia (guinea-pigs) and Lagomorpha (rabbits), various conditions being similar in their expresion among these related species of the Glires clade and not only among them [until 1912 rabbits were classed as rodents but as Gidley proposed, they were considered a part of a separate order, Lagomorpha (Jellison & Parker 1945). However, in 1996, Graur et al showed that *Lagomorpha* is significantly more closely related to *Primates* and Scandentia than it is to rodents].

In different mammalian species, various alleles are known at the *C* locus, the most dominant of them *(C)* being involved in a full production of melanin in the fur, skin, and eyes, while the *c* recessive allele inhibits the melanin synthesis by the absence of tyrosinase, a peroxidase enzyme involved in the first step of the melanine synthesys pathway (Grădinaru 2017; Covrig et al 2013; Cronin et al 2003). A genetic linkage between the albino *(c)* and hemoglobin β-chain *(Hbβ)* loci was firstly demonstrated in mice and rats [in a graphically relationship comprising the genes for pink-eyed dilution *(p)* – albino *(c)* – hemoglobin β-chain *(Hbb)* – Warfarin resistance *(War)*], and afterwards in rabbits, the observed linkage homologies among these related species being confirmed by a close estimated map distance between the two loci (9±6cM in rabbits and 8cM in mice and rats). Moreover, it was concluded that there is a possibility of conservation in various mammalian species, including man, of the albino and hemoglobin β-chain linked loci, their localization in mouse being reported in the structure of chromosome 7, and assumed in chromosome 1 in rabbits and in chromosome 11 in man (Sandberg & Andersson 1987). In all these three species (mice, rats, rabbits), as were reported in various mammals (pigs, cattle, humans), the recombination frequencies caused by crossing over were higher in females than in males (crossing over taking place more frequently in oogenesis than in spermatogenesis) (Sternstein et al 2015; Sandberg & Andersson 1987; Dunn & Bennett 1967; Dunn 1920).

Completions to Well-known Linked Loci and Phenotypes in Rabbits. Various studies on different phenotypes in domestic rabbits *(Oryctolagus cuniculus* L.*)* are of a real concern, since many passionate breeders grow rabbits for their beauty, meat or fur productions. These animals are important medical models in human medicine due to some common hereditary diseases (for example: aortic atherosclerosis, cataracts,

hypertension, hypertrophic cardiomyopathy, epilepsy, spina bifida, osteoporosis), and various studies include them for toxicological trials (Carneiro et al 2011). Cytogenetically, those 22 pairs of chromosomes are the host of a completely sequenced genome (but only 82% mapped), the physical position of the genes being checked by molecular studies in different rabbit populations (Sternstein et al 2015).

One of the most studied linked locus in rabbits is that of albinism. For its phenotypic expression, the individuals must have a homozygous recessive genotype *(cc),* such a condition being also encountered in various animal species (Covrig et al 2013). The Himalayan pattern color in rabbits is possible both in the conditions of $c^h c^h$ and $c^h c$ genotypes, but the genotype including the allele for albinism lead to a fader expression (McNitt et al 2013).

As Castle & Sawin (1941) reviewed, the studies of Castle (1924, 1933, 1936) and Pease (1927) put the bases of the first linked relationship among three loci in rabbits in a physical position of *c-y-b* (genes for albinism – yellow fat – brown coat) on an assumed chromosome 1. Regardless the gender of heterozygous individuals (F_1), their map distances based on the frequencies of crossing over were lower among loci for albinism and yellow fat than those among yellow fat and brown coat. The yellow fat is considered an undesirable trait which inherits as a simple Mendelian recessive to normal white fat. Its occurrence is due to the lack of a hepatic enzyme able to metabolize the fat soluble carotene and xanthophyl pigments found in carrots and in the chlorophyl of green plants, respectively (Wilson & Dudley 1946; Castle 1933). In true albino rabbits, the share of yellow fat individuals was reported to be about 14-fold higher than in other rabbits, males being about two times more affected than females (Wilson & Dudley 1946). Yellow fat sporadically occurred in Flemish Giant rabbits (Castle 1933). Although Himalayan and albino are allelomorphs, Wilson & Dudley (1946) reported no case of yellow fat in Himalayan rabbits. There were also no records of yellow fat in Sable and Chinchilla rabbits, but Chocolate phenotype revealed a share slightly lower than 5% for individuals with yellow fat.

Identified by Castle since 1926, the second group of loci which respects the linked inheritance refers to those for Dutch White Spotting *(Du)* – English White Spotting *(En)* – long (angora) hair *(l).* The Dutch and English White Spotting are additional loci to the major A-E coat color series in rabbits. They are tightly linked, considering the distance of about 1.2 map units among them. The physical position among English White Spotting and long (angora) hair was reported to be about 11-fold higher than that between Dutch White Spotting and English White Spotting loci, their order in the structure of assumed chromosome 2 being *Du-En-l* (McNitt et al 2013; Castle & Sawin 1941). The affiliation of this group genes at the linkage group II in rabbits was also reported in 1970 by Searle & Truslove. Using a microsatellite linkage map, these loci belonging to the linkage group II were subsequently mapped to chromosome 15 (Fontanesi et al 2014). In 2004, Mulsant et al confirmed the fibroblast growth factor 5 *(fgf5) gene* in rabbits as a molecular candidate for the angora locus. The recessive mutation related to abnormally long hair growing (the angora type, also found in mice, goats, dogs, and cats) is associated with a defect of *fgf5* gene, whose product normally regulates the growth of hair follicles (Mulsant et al 2004).

An interesting association in several species is that between the hereditary deafness and loci for white pigmentation. This condition was well-studied in dogs and cats, the pigment-association of cochleo-saccular pathology involving the lack of melanocytes (besides their pigment-producing role in the structures of the skin, melanocytes are also a part of cohlear duct' stria vascularis, whose function is to maintain the ionic composition of endolymph). However, albinos seem to do not exhibit hereditary deafness, because their melanocytes are present in the stria vascularis and in the structures of the skin, where do not produce melanin due to the *TYR* (tyrosinase) gene mutation (Strain 2015, 2010). Considering the aforediscussed *Du* and *En* linked loci in rabbits, they are involved in white pigmentation in different ways of expression. For example, at *Du* locus are found three alleles, the most dominant of them *(Du)* codifying little or no white pigmentation, and the others two, a minimal *(du^d)* and a substantial white spotting *(du^w).* In contrast, the dominant homozygous individuals *(EnEn)* are mostly white (Charlies) and

the recessive ones *(enen)* are with no white spotting on the body (the heterozygous individuals *Enen* are normally spotted) (McNitt et al 2013). In 2014, Fontanesi et al reported an association between the *KIT* (Tyrosine-protein Kinase) gene and the Dominant White Spotting locus *(English Spotting locus).* The *KIT* gene is involved in the migration of melanocytes in the target structures of the developing organism. Although mutations of *KIT* gene produce deafness in the mouse and other species, in rabbits was not reported as a cause of hereditary deafness (Strain 2015). Instead, mutations affecting *KIT* gene associated to its expression decreasing in the cecum of *EnEn* rabbits led to the megacolon condition due to the gut musculature affected motility and the lack of nerve cell function in the gut wall (Fontanesi et al 2014; McNitt et al 2013).

Conclusions. The linkage phenomenon is a non-Mendelian inheritance pattern of genes located on the same chromosome. It was reported in various species of animals, including rabbits. Considering the albino and Dominant White Spotting loci, these are linked with hemoglobin β-chain, yellow fat and brown coat on assumed chromosome 1, and with Dutch White Spotting and long angora hair, respectively, on the assumed chromosome 2 but subsequently mapped chromosome 15. Although hereditary deafness in various species was associated with loci for white pigmentation, in rabbits associations among these genes and deafness were not reported in reviewed literature.

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Author:
Andrei Cristian Grădinaru, Department of Preclinics, Faculty of Veterinary Medicine, „Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iaşi, 8 Sadoveanu Alley, 700489, Iaşi, Romania, e-mail: andre_gradinaru@yahoo.com, a.c.gradinaru@uaiasi.ro
Ioan Valentin Petrescu-Mag, SC Bioflux SRL, Romania, Cluj, Cluj-Napoca 400488, 54 Ceahlău Street; Department of Environment and Plant Protection, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine, Romania, Cluj-Napoca, 400372, 3-5 Calea Mănăştur Street; University of Oradea, Romania, Oradea, 410087, 1 Universitatii Street, e-mail: zoobiomag2004@yahoo.com

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