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## **TOBIANO AND LEOPARD ALLELES IN FELIN PONY POPULATION**

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### **ABSTRACT**

The phenotype in 19 tobiano–leopard ponies has been described. The tobiano pattern has been identified in them according to the arrangement of white and dark areas, sharp and smooth edges of the areas, rather big white markings on heads, white legs or large markings on the legs and sometimes wall eyes. The leopard–spotted pattern has been recognised thanks to the dark leopard spots of the base colour or darker, the roaned or frosted areas emerging from white patches or occupying the whole dark patches, jagged patch edges, fragments of mottled skin, eyes with visible sclera and striped hooves. Sometimes, only the visible sclera or a blurred part of the patch edge has indicated the presence of the leopard–spotted pattern.

The pattern ratios in foals produced from 55 matings in Waron sire's progeniture have agreed with the dominant mode of inheritance of the tobiano and leopard–spotted patterns. The results indicate independent inheritance of these patterns.

**Key words:** horse, coat colour, tobiano pattern, leopard–spotted pattern, inheritance

## INTRODUCTION

The attractive tobiano and leopard-spotted patterns are nowadays in demand. They are particularly desired in ponies, which are mainly used by children and leisure riders. In the view of differentiating the Felin Pony colours, two half sibs sired by bay-tobiano pony, Waron (Waluś, Shetland, chestnut-tobiano – Tośka, leopard-spotted), were used in the breeding: Wampir sire, 1991, black-tobiano-leopard (from Sroka, Hucul [ˈhutzuw], blue dun-tobiano) and Wacat sire, 1992, bay-tobiano-leopard (from Wilga, half Shetland, chestnut with grey hairs). Wacat sire was the result of mating full sibs. Sires: Apacz and Polaris, Anglo-Arabes, and Fason, Hucul, also brought to the population Tobiano allele. In 1997, Tobiano allele frequency equalled 0.0857 [10]. Moreover, Lubicz, Anglo-Arabe, and Amoretto, pony, sires, brought Leopard allele which frequency amounted 0.0121.

*Tobiano pattern.* This is one of the pied patterns and may occur with any colour. The tobianos have alternately dark and white areas, which are mainly vertically arranged. The white usually extends over the back, whereas the pigmented areas extend under the belly. The edges are smooth. The legs are white and head dark with usual, rather big markings. The eyes are rarely in a part or whole blue [5] and that is mainly the case when white markings extend over the eye. The dark patches in tobianos develop in embryos from pigmentation centres, successively grow and join together [4]. Hence, their edges are usually convex or straight. The centres of the patches are usually situated between the eye and cheek, on the ear, neck, chest, arm or elbow, back, belly, groin and at the base of the tail.

It should be added, that the edges of white in some places might have almost square angles. In white areas, the skin is unpigmented, though sometimes a halo effect is seen at the edges, where the dark skin is covered with white or mixed hair, as it occurs also in other patterns of white [11]. Groups of small so-called ink spots of the base colour may be present on white areas [27]. Hooves are generally yellowish, as it is in the case of a white marking on a leg.

*Leopard-spotted patterns.* The wide variation in leopard-spotted patterns does not allow defining them concisely. On the other hand, they should be considered together, since a leopard-spotted horse of a given pattern may produce a foal of any of the leopard patterns [8]. Many leopard-spotted patterns change with horse age and become others, usually progressively lighter, patterns. Leopard-spotted patterns may occur on any base colour.

One of the descriptions says that a leopard-spotted horse should be regarded as a pigmented horse, covered by a, smaller or larger, more or less transparent white blanket, originating from the loin and the croup [11]. Hence, the blanket in broad meaning may consist of single white hair intermingled in dark areas, or white spots, or a white area on the loin and/or croup, or, finally, of a leopard pattern. Sometimes minimally marked horses occur, in which the pattern may be recognised only by mottled skin around eyes, on a muzzle or genitals, visible sclera and/or striped hooves [7, 11]. The “frosted” pattern is more developed and the least attractive. It consists of white hairs intermingling with darker coat colour, like it is in roans. However, in the former the mixed hairs occur mainly on the loin and frontal part of the croup, as well as sometimes on the whole body, and not like in roans which retain darker head and legs. The frosted pattern sometimes goes together with the “snowflake”, in which the white hairs form spots. The snowflake is sometimes more developed, with a blanket on the croup. In “leopard” pattern dark spots of the base colour occur on white even on the whole body. White hair grows on unpigmented skin, dark hair on pigmented skin, but in this case, like in tobianos, the dark skin sometimes extends further than the dark spot, forming a halo effect covered with white or mixed hair. Areas of base or mixed colour may rest on head, neck, behind elbows and on groins. In “few-spots” the leopard spots are rare. The “blanket” is similar to leopard but covers most often solely the croup and may extend not further than to the withers. If it covers the whole trunk with the withers and includes leopard spots, it is called leopard pattern. The blanket may be also without any spots. The edges of the blanket or leopard patterns are sharp of zigzag line, of mixed hair so-called roaned, or mottled. In most of the patterns discussed so far dark leopard spots, sometimes darker than the base colour, of a coin size, often occur, mainly on the croup.

In the “varnish roan” horses, dark areas of base colour occur on the horse’s head, bottom of neck, of the trunk and where the bones are placed nearest to the skin, e.g. on hips [7, 11]. They can form a net resembling a marble pattern. These are so-called varnish marks, which sometimes appear in adults. Besides them, the varnish roan horses are white, without leopard spots. Exceptionally, the “snow cap” pattern occurs, in which the white horse has a clear triangle of dark hair above each hoof. All-white horses, without any spots, which produce exclusively leopard-spotted offspring when mated to solid colour horses, occur extremely rarely [11].

*Tobiano inheritance.* Tobiano pattern is produced by a completely dominant To allele [9] which belongs to the second linked group of genes localised on ECA 3 [3]. This allele is linked, among others, with those from E locus (Extention) and closely linked with Alb and Gc alleles. The linkage with Alb and Gc allows identifying the homozygosity of individuals in Tobiano locus [1, 2]. According to Sponenberg [7], probably most or all homozygotes have ink spots. Bowling [2] did not assume this to be absolute. As suggested by Sponenberg [7], the homozygotes do not have a larger proportion of unpigmented coat areas than heterozygotes do. Thus, the reason of considerable differentiation of the tobiano pattern is not known.

*Inheritance of leopard-spotted patterns.* The leopard-spotted patterns are also inherited in dominant way. They are produced by an autosomal Lp allele, which genetic linkage is not known. As far as now, it is not known either, how the great differentiation of leopard-spot patterns is controlled. It may be supposed that all-white horses, which produce solely Lp offspring, are homozygous. Sponenberg [6] on the basis of studies on Noriker horses stated the few-spots were homozygous. However, in a later paper, Sponenberg *et al.* [8] reported they had found some heterozygous few-spots. The authors suggested that the pattern differentiation was caused by modifiers, which limited to various extend the Lp allele expression. More extensive white areas without dark spots might result from the homozygosity in Lp locus.

However, the studies on the leopard-spotted patterns inheritance did not consider the changes undergoing with the age of horses. For instance, it is difficult to consider separately the frosted and snowflake horses, since after some time the first pattern often changes to the second one. The horses that are white with spotted hindquarters when they come of age, usually start out dark with spotted blanket, and later the dark areas become mixed, and eventually whiten. Likewise, the few-spot pattern sometimes derives from a roaned blanket.

*Aim of the study.* As far as now, the simultaneous effect of tobiano and leopard alleles activity, the pattern called by Americans pinto [2, 4], has not been studied. The aim of the present paper has been to study the occurrence of these alleles and to describe the phenotypes in tobiano-leopard individuals in Felin Pony population.

## MATERIALS AND METHODS

The phenotype has been described in 19 tobiano-leopard horses of various age. The coat colour of 8 out of them has been observed during a few years. In tables, the results of the latest observation are included. The pattern ratio has been analysed in 55 foals sired by Waron, 11 in that born in related pony breeding in Czechówka near Cracow. The actual ratios have been compared with the expected ones for the dominant tobiano and leopard inheritance. The differences have been verified with  $\chi^2$  test.

Most of Felin Ponies, beside the conducted register of matings and births, had their parentage tested with the blood test (routine erythrocytes and polymorphic proteins test).

## RESULTS

The so-called painted colours, in which on the base colour a pattern of white patches occurs, are particularly spread in ponies. Shetland ponies and Huculs often have the pied pattern tobiano, whereas to Felin Pony population and horses related to it, in addition, the leopard-spotted patterns were introduced. Hence, among others in the latter, Tobiano and Leopard alleles sometimes occur simultaneously and produce the joint tobiano-leopard pattern. Such horses are usually identified as tobiano or rarely, when the leopard-spotted pattern is more distinct – as leopard-spotted or tobiano-leopard.

*Tobiano-leopard pattern.* According to the results of the phenotypic description, the tobiano pattern in tobiano-leopard horses can be identified mainly thanks to the presence of big white patches arranged in vertical direction and usually crossing the upper trunk line ([Tab. 1](#)). The white legs or very extensive markings on the legs are an additional property, which allows to be sure, that a horse carries the Tobiano allele. Quite big markings are most often also present on the head and the eye in few cases is wall, i.e. blue. In most tobiano-leopard horses some edges of the patches, particularly at the front of the trunk, are smooth as it is in tobianos.

**Table 1. The frequency of tobiano pattern properties in tobiano–leopard horses (*the properties not usual for tobiano pattern are marked with italics*)**

	Property	Per cent of horses
White patch arrangement	arranged in vertical direction, crossing the vertebrae line ( <i>possibly with the blanket</i> )	100.0
	<i>lack of white patches arranged in vertical direction</i>	0.0
White patch edges in frontal part of body	smooth	68.4
	<i>mottled, jagged or roaned</i>	31.6
Markings on head	quite big	94.7
	<i>small or lack of markings</i>	5.3
Eye colour	wall eyes	15.8
	<i>dark</i>	84.2
Markings on legs	white legs or with extensive markings	100.0
	<i>small or lack of markings</i>	0.0

A leopard–spotted pattern in the tobiano–leopard horse is often more difficult to recognize. Particularly in foals it may be overlooked. In some cases, only “human” eyes, i.e. with visible narrow stripe of white sclera, and mottled skin on the muzzle, eyes or genitals, indicated its presence (Tab. 2). However, the mottled skin sometimes appeared later. Sometimes, the edges of white tobiano patches in sucklings’ hair seemed to be smooth and only after the change of hair they appeared to be not straight. Soon, roaning appeared, i.e. white hairs intermixed in dark ones around the white patches on the croup. The white patches grew and penetrated the dark ones (Fig. 1 and 2 – Firma). The patch edges became jagged and after some time in these passing areas the white hairs formed dots (Fig. 3). In some horses the dots and not the roaning, appeared from the beginning of the leopard–spotted pattern development. Thus, on the previously dark areas, along the white patches, the frosted or snowflake pattern was appearing.

**Table 2. The frequency of leopard–spotted pattern properties in tobiano–leopard horses (*the properties not usual for leopard–spotted pattern are marked with italics*)**

	Property	Per cent of horses
Pattern variant	round leopard spots spread regularly on white patch on croup/croup–loins/croup–loins–back/loins–back	21.1
	less regular single dark spots around dark patch of jagged edge on groin	15.8
	mixed hair or mottled areas situated on dark patches, emerging from white patches, growing with age	52.6
	dark patches mottled, whitening with age	10.5
	<i>lack of pattern</i>	0.0
White patch edges on croup	mottled	52.6
	jagged and/or roaned	31.6
	<i>smooth</i>	15.8
Leopard spots on croup	present	15.8
	<i>lack of leopard spots</i>	84.2
Varnish marks	present	10.5
	<i>lack of varnish marks</i>	89.5
Sclera in eyes	visible	47.4
	<i>not visible or wall eyes</i>	52.6
Skin on muzzle, around eyes and genitals	mottled	47.4
	<i>dark or pink in case of marking</i>	52.6
Hoof colour	striped	52.6
	<i>yellowish or dark</i>	47.4

**Fig. 1. Trunk in Rapsodia filly (King Size seal brown - Rakieta dark brown-tobiano-leopard), dark bay-tobiano-leopard, 2 years old (photographer Anna Stachurska)**



**Fig. 2. Firma mare (Wampir black-tobiano-leopard - Figa yellow dun), dark bay-tobiano-leopard, 5 years old, with Fantazja daughter (after Mandaryn black), dark bay-tobiano-leopard (photographer Emil Sasimowski)**



**Fig. 3. Hind part of trunk in Ramzes II colt (Inbred black - Rakieta dark bay-tobiano-leopard), 1 year old (photographer Anna Stachurska)**



However, in two horses the frosted pattern changing into the snowflake fulfilled regularly almost the whole dark patches and left only the darker varnish marks. One of the horses was Rubin sire (Wacat – Różnica) presented at the age of 4 and 5 years in the [Figs 4](#) and [5](#). It was born bay-tobiano, with white and black mane and tail. The presence of a leopard-spotted pattern was identified only by its specific “human” eyes and striped hooves. Afterwards, the dark patches whitened except the rest of dark hairs on the head and bottom parts of the trunk. Thus, the varnish roan combined together with the tobiano, came out to be lighter than a usual varnish roan pattern.

**Fig. 4. Rubin sire (Wacat bay-tobiano-leopard - Różnica buckskin-tobiano), bay-tobiano-leopard, 4 years old (photographer Anna Stachurska)**



**Fig. 5. Rubin sire (Wacat bay-tobiano-leopard - Różnica buckskin-tobiano), bay-tobiano-leopard, 5 years old (photographer Anna Stachurska)**



Some tobiano–leopard horses since birth had an apparent leopard–spotted pattern, e.g. resembling blanket with round spots on the croup (Fig. 2 – Fantazja). In one horse the blanket or leopard pattern was probably covered in this place by dark tobiano patch and was visible only on back and loins (Fig. 6). As it is known, there are usually dark patches on the croup with the centres on the groin and at the base of the tail in tobiano horses [4]. In some horses, dark leopard spots were present on a white area around the dark patch on the groin (Fig. 7). They could have indicated the presence of the few–spot or leopard pattern.

**Fig. 6. Upper part of neck and trunk in Romanka filly (second one from left side of photo) (Rubin bay-tobiano-leopard - Roma yellow dun-tobiano), yellow dun-tobiano-leopard, 1 year (photographer Anna Stachurska)**



**Fig. 7. Wampir colt (Waron bay-tobiano-leopard - Sroka blue dun-tobiano), dark bay-tobiano-leopard, 1 year (photographer Anna Stachurska)**



It was not possible to confuse the leopard spots with ink spots occurring sometimes in the tobiano pattern. The ink spots usually are smaller. These greater are not so round. That is why sometimes they are called paw prints. Confusion with overo, sabino or splashed white patterns can also be excluded. The alleles, which govern them, are not present in the examined population. No overo, sabino or splashed white foal ever appeared in it.

The colour of eyes and hooves also came out to be a good indicator, which helped to identify the tobiano and leopard-spotted patterns in tobiano-leopards. As it was told, the eyes with visible sclera indicated the leopard-spotted pattern, whereas the wall eyes sometimes occurred in tobianos. The striped hooves characteristic of leopard-spotted horses were found in more than half of the examined horses.

*Tobiano-leopard pattern inheritance.* The results of matings are consistent with the dominant mode of inheritance of tobiano pattern (Tab. 3). A certain excess of tobiano foals produced in test matings did not cause a significant difference with the expected proportion. The test matings of leopard-spotted ponies also resulted in coat colour ratio consistent with the expected proportion for dominant mode of inheritance. Overlooking this pattern in foals, particularly tobiano-leopard, during an early identification, could have caused an inconsiderable deficit of leopard-spotted individuals.

Considering simultaneously Tobiano and Leopard alleles, the pattern ratio in progeny was also in agreement with the expected one (Tab. 4).

**Table 3. Mating results with respect to tobiano inheritance and leopard-spotted pattern inheritance**

Parents		Progeny				chi <sup>2</sup>
		actual number		expected ratio		
Toto	toto	29 Toto	20 toto	1 Toto	1 toto	p > 0.20
Toto	Toto	6 To_	0 toto	3 To_	1 toto	–
Lplp	lplp	23 Lplp	31 lplp	1 Lplp	1 lplp	p > 0.20
Lplp	Lplp	1 Lp_	0 lplp	3 Lp_	1 lplp	–



**Table 4. Mating results with respect to simultaneous tobiano and leopard-spotted pattern inheritance**

Parents		Progeny				chi <sup>2</sup>	
TotoLplp	totoLplp	actual number	12 TotoLplp	15 Totolplp	4 totoLplp	14 totolplp	p > 0.05
		<i>expected ratio</i>	1	1	1	1	
Totolplp	totoLplp	actual number	2 TotoLplp	0 Totolplp	0 totoLplp	2 totolplp	–
		<i>expected ratio</i>	1	1	1	1	
TotoLplp	TotoLplp	actual number	1 To_Lp_	0 To_lplp	0 totoLp_	0 totolplp	–
		<i>expected ratio</i>	9	3	3	1	
TotoLplp	Totolplp	actual number	4 To_Lp_	1 To_lplp	0 totoLp_	0 totolplp	–
		<i>expected ratio</i>	3	3	1	1	

**Table 5. Test mating results of double heterozygotes: Waron, Wampir and Wacat sires**

Sire	Progeny			
	TotoLplp	Totolplp	totoLplp	totolplp
Waron	2	2	2	4
Wampir	4	5	0	4
Wacat	1	7	0	1
<i>Expected ratio</i>	1	1	1	1

The similar pattern ratio in progeny produced in test matings with the expected proportion indicates that Tobiano and Leopard alleles are not genetically linked. The ratios in foals sired by Waron, Wampir and Wacat show it, as well (Tab. 5). Waron sire got Tobiano allele from its sire and Leopard allele from its dam. If these alleles were linked, two tobiano-leopards and four foals without any pattern should not be produced in test matings. Wacat sire, which was one out of these two tobiano-leopards, sired in test matings 7 tobiano-not-leopard foals. It would not have been possible, either, if the alleles had been linked. Wampir sire could have got Leopard allele from Waron sire and Tobiano allele from its tobiano dam. Then, four tobiano-leopard foals and four foals without any pattern would not be expected. Instead, if Wampir had got both dominant alleles from its sire, it should not have sired five tobiano-not-leopard foals. Thus, the mating results indicate an independent inheritance of tobiano and leopard patterns. This conclusion should be documented in a more extensive material.

## CONCLUSIONS

The conducted investigation allowed describing the phenotype in tobiano-leopard horses with regard to specific properties of both patterns and changes undergoing with age. The results should facilitate the proper identification of foal coat colour. Nevertheless, in some cases it will be necessary to alter the phenotypic description of the colour later in life.

In the light of the results, the tobiano pattern is inherited independently from the leopard pattern.

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