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Analysis of the influence of environmental enrichment on the behaviour of wild cats kept in captivity

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Abstract: *Analysis of the influence of environmental enrichment on the behaviour of wild cats kept in captivity.* The purpose of this study was to examine the influence of environmental enrichment on cats in captivity. The observation was carried out in Warsaw ZOO. Three species: puma (1:1), jaguar (1:1) and ceylon leopard (1:1) were observed. The data were collected from May to November 2000. Each week of observation was represented by three consecutive days (I – baseline, II and III – with enrichment). As regards to inactivity and activity time ratio was recorded. In reference to stereotypy also incidence of pacing was noted. The results based on 5400 minutes of observation time. Out of the six observed cats one, the male puma, did not show any form of stereotypical behaviour. The female jaguar devoted less than 2% of time to stereotypical behaviour. With four out of six observed cats, the introduction of the enrichment had a major influence on their behaviour. The study confirmed the different motivational background of stereotypical behaviours and the necessity of fitting the type of enrichment to the animals' desires.

Key words: environmental enrichment, puma, jaguar, leopard, stereotypy, stereotypical behaviour.

INTRODUCTION

Keeping animals in zoological gardens inevitably involves their exposure to numerous unnatural factors, limitations

and constraints. Enforced unnatural gathering or dividing into groups, herds or pairs precludes natural selection of partners causing deviations from the natural social and sexual behaviour typical for given species. The stress in animals is further intensified by shabby or unsuitable furniture and poor design of the enclosure, incompatible with the needs of the species. Lack of variety in fodder composition and feeding regime additionally influences the repertoire of hunting behaviours. Furthermore the above limitations are accompanied by exposition of the animals to contacts with human beings such as visitors, keepers etc.

The resulting unfulfilled social, hunting, sexual and other needs outstripped the animals' ability to adapt and cause their frustration. The animals' apathy, evident decrease of natural activity, and the appearance of stereotypical behaviours typical for captive animals demonstrate the level of frustration. Stereotypies, are defined as "series of movements of the whole or parts of the animal's body which are repeated regularly and which serve no

apparent function” (Shepherdson, 1991; after Dantzer and Mormede, 1983). An animal walking along the outlined path and repeating identical movements, is also a questionable attraction for visitors in a zoological garden. The introduction of elements of diversity in the captive animals’ environment appears to provide a chance of a marked improvement to the welfare of animals in zoological gardens. The story of enrichments used in zoological gardens is quite a long one (Bassenge et al., 1998).

There are many papers, in which different forms of enrichment have been analysed: from nutritional (Markowitz, 1995), environmental (Langenhorst, 1998) to training or grouping with other representatives of the same or different species (Langenhorst, 1997; Kolter and Zander, 1995). The influence of enrichments on behaviour of species as different as: polar bears (Law et al., 1991), otter (Hawke et al., 2000), and primates (Marriner and Drickamer, 1994; Wood 1998) has been observed. Much attention has also been given to big cats: lions, tigers and leopards due to their tendency to express stereotypy usually caused by unfulfilled hunting instinct (Bassenge et al., 1998, Schiess-Meier and Wiedenmayer, 1994).

The aim of this study is to establish the influence of environmental enrichments on behaviour of cats of three species: the Ceylon leopard (1:1), the jaguar (1:1) and the puma (1:1) kept in Warsaw Zoological Garden. This study also seeks to answer the question how stable

the effect of a given enrichment is, whether an animal does not get used to a new element of the environment and does not start to ignore it.

MATERIAL AND METHODS

Observations concerned three species of predatory cats: pairs of puma (*Puma concolor*), ceylon leopard (*Panthera pardus kotiya*), and jaguar (*Panthera onca*) kept in the Warsaw Zoological Garden.

Animals

The observed male and female of puma were 13 and 7 years old accordingly. Both cats were born in captivity. The female comes from the zoo in Tallin and was brought to Poland in 1998. The male was born in the Warsaw Zoological Garden. For the whole observation period the animals were kept in separate cages because of cubing. In June 2000 the young were separated from the mother and transferred to a nearby enclosure (they were not included in the research).

The female leopard was wild-born in Sri Lanka in 1985 and a male was born in captivity also in 1985. Both animals were brought to the Warsaw Zoological Garden in 1987. The pair of leopards was sharing the same enclosure for the first 2 months (till 18 July 2000) of the observation.

Both observed jaguars were born in captivity. The female (20 years old) was brought from Copenhagen in 1982. The male (17 years old) was born in Łódź

and transferred to Warsaw in 1984. Throughout the observation period the jaguars were kept together.

Housing

The animals were fed every evening (except for Sundays) after they had been locked in individual inside cages sized 4.0×2.5 m. Shelves were the only equipment in the cages. The animals were given portions of beef or skinned rabbit, from time to time one-day-old chicken or young quail.

At about 8 a.m. the animals were let into outside enclosures. The enclosures of jaguars and pumas were rectangular of about 27 m^2 (4.5×6.0 m) each. The enclosure of leopards was bigger. Some 60 m^2 shaped as a semicircle. After separation they were put into smaller cages, and a pair of jaguars took their place. The floor of all cages was covered with sand. The equipment consisted of trunks, ropes and branches leading to shelves placed high above. Neighbouring enclosures were separated with bars and wooden walls making an eye contact impossible for the animals.

Enrichments

In the experiment, different forms of toys were used as environmental enrichments. These were placed in the central parts of the enclosure on the branches or hung on the ropes. The following enrichments were used:

- A jute sack (approximate size: 40×70 cm) stuffed with hay and hung;

- A toy made of tanned sheep skin stuffed with sawdust and wood chips, hung or laid down;
- A hedgehog made of a half of a jute sack, stuffed with sawdust and wood chips and pierced with thin sticks (20×35 cm + 20 cm sticks), laid down;
- A round toy made of woollen material stuffed with sawdust and wood chips (diameter 20 cm), laid down;
- A piece of horse-cloth (area: 1 m^2) with a strong smell of a horse, hung;
- Raw rabbit skins (2–3 pieces) sawn together and stuffed with sawdust and wood chips, laid down;
- A small jute sack (15×25 cm) stuffed with hay or dry leaves and pierced with feathers, laid down;
- Linen sacks hidden in the enclosure (5×7 cm, 4 pieces) stuffed with sawdust and wood chips soaked in rodents' urine
- A rattlebox made of cardboard box and chestnuts, sawn into a jute sack (20×35 cm) stuffed with hay, laid down.

Experiment design

1. Providing the enrichment

Observations were carried out from 20 May to 9 November 2000 according to the following schedule: during each of the 12 weeks of the experiment observations were carried out for 3 consecutive days.

Day I – without the enrichment was treated as a baseline. During that day no

action was taken except for the observation of the cats' behaviour.

Day II – with the enrichment introduced was used to observe the reactions of the animals to the new element placed in the enclosure.

Day III – on which the same enrichment as on day II was provided to enable judging the persistence of the enrichment's effect and possible boredom of the animals with the new environmental element.

During each subsequent observation week a different enrichment was used in order to guarantee the effect of novelty.

2. Observations scheme

The animals were observed twice a day: in the morning – after being released, and in the afternoon before being locked in, in view of expected intensification of stereotypical behaviours before locking in (identified with the feeding time).

The cats were observed 3 times in each part of the day. The observations were conducted over 5-minute-periods with a 15-minute-break in between.

The applied observation schedule was based on a previous survey on stereotypical behaviour carried out by Złamal in 1997–1998 (not published). It allowed to choose the most appropriate duration for a single observation, as well as the optimum break between repetitions (according to the average duration and frequency of a stereotypical series).

Three main categories of behaviour were defined: normal behaviours, ste-

reotypy and inactivity. Normal behaviours contained all forms of activity typical for the species' activity: locomotor, hunting, sexual as well as social or playing (form of interaction with enrichment – physical, visual). Stereotypical behaviours included locomotor – continuous walking along a certain path. Inactivity was considered as lack of any of the above categories of behaviour, which was identified with sleep of the animal.

The incidence of marking was also recorded, as well as frequency of the interaction with the toy or a cage mate.

Methods of data analysis

1. Traits

Considering the three predefined categories of behaviour, that is normal activity, stereotypy and inactivity, the ratio of time spent on a given form of behaviour to whole period was applied as a measure. To analyse the effect of each single factor on the frequency of stereotypical series, the incidence of pacing during a single 5-minute-period was taken into consideration. Because of serious interdependence of neighbouring minutes measured as 1st class autocorrelation (from 0.799** to 0.872**) the whole 5-minute-period was treated as a single observation.

2. Effects

Beside the enrichment effect (E), the following other fixed effects have been taken into consideration:

- Time of the day (T) – morning, afternoon;
- Weather (W) – 8 weather categories reflecting combination of cloudiness and the temperature – rainy cold, rainy warm, rainy hot (no observation recorded), cloudy cold, cloudy warm, cloudy hot, sunny cold, sunny warm, sunny hot;
- Number of visitors (V) – average number of people in front of the enclosure during the observation;
- Separation of animals (S) – in the case of the pair of leopards and the female puma separated from the cubs.

3. Statistical analysis

Statistical analysis of the data has been carried out individually for each of the cats.

The ratio of time spent on a given form of behaviour had a discrete distribution with relatively numerous extreme values. Those properties did not allow to carry out any transformation to obtain normal distribution. That is why non-parametric statistics was used enabling the simultaneous analysis of single effects. This was appropriate when the collinearity between effects did not exist. This method has also another limitation. Testing of any interaction between factors is not possible. Significance of the effect (time of day, visitors, separation and weather) on time ratio of inactivity, stereotypy and activity was estimated with the Kruskal-Wallis test. Comparison of each

pair of the effect levels was carried out using Mann-Whitney u-test.

In view of the character of the incidence of stereotype a multi-factor analysis was used in accordance with the following logistic model:

$$y_{ijklmn} = e^{\varphi} / (1 + e^{\varphi})$$

$$\varphi = m + T_i + W_j + V_k + S_l + E_m + e_{ijklmn}$$

where:

y_{ijklmn} – incidence of stereotypical serie,
 e_{ijklmn} – error.

Because of their lack of significance all the two-way interactions have been removed from the model.

Other traits, like incidence of marking and interaction with a partner were not taken into consideration because of their occasional character.

RESULTS

The average level of activity, inactivity and stereotypical behaviours on control days are shown individually for each of the cats, on the figures (Figs. 1–6.).

Out of the six observed cats one, the male puma, did not show any form of stereotypical behaviour. The female jaguar devoted less than 2% of time to stereotypical behaviour. In the analyses which were carried out on the observed cats a clear influence of time of day, visitors and separation as well as weather on the time devoted to each of the previously defined behaviour categories have been shown (Tab. 1).

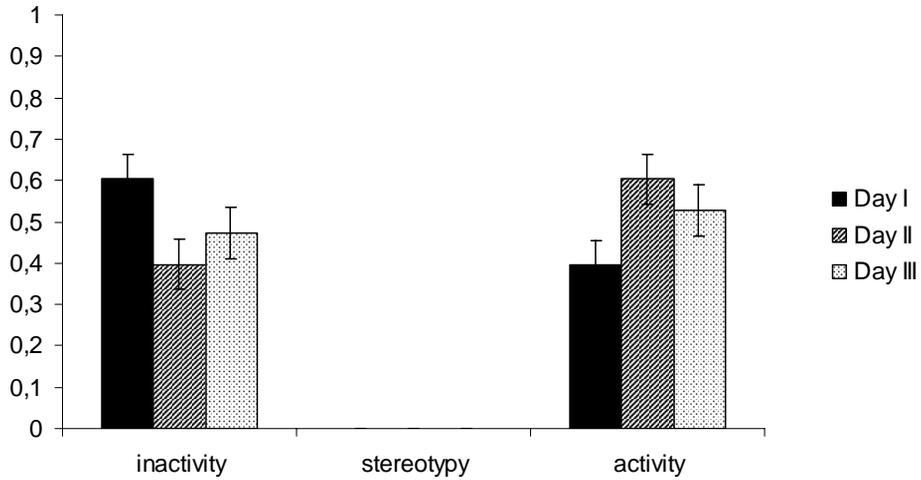


FIGURE 1. Male puma. Comparison of levels of inactivity, stereotypy and activity during three consecutive days of experiment (significance marked with * – $p \leq 0.05$ and ** – $p \leq 0.01$; at the name of behaviour category – significance of the effect)

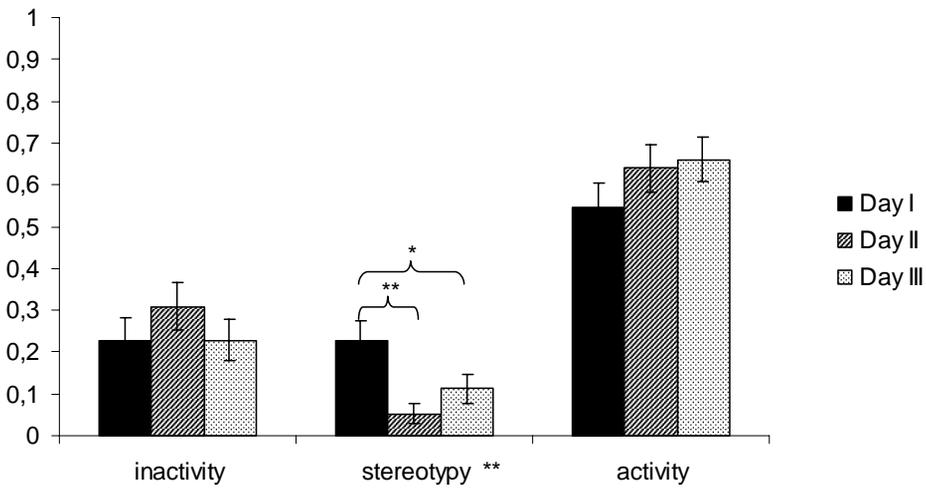


FIGURE 2. Female puma. Comparison of levels of inactivity, stereotypy and activity during three consecutive days of experiment (significance marked with * – $p \leq 0.05$ and ** – $p \leq 0.01$; at the name of behaviour category – significance of the effect)

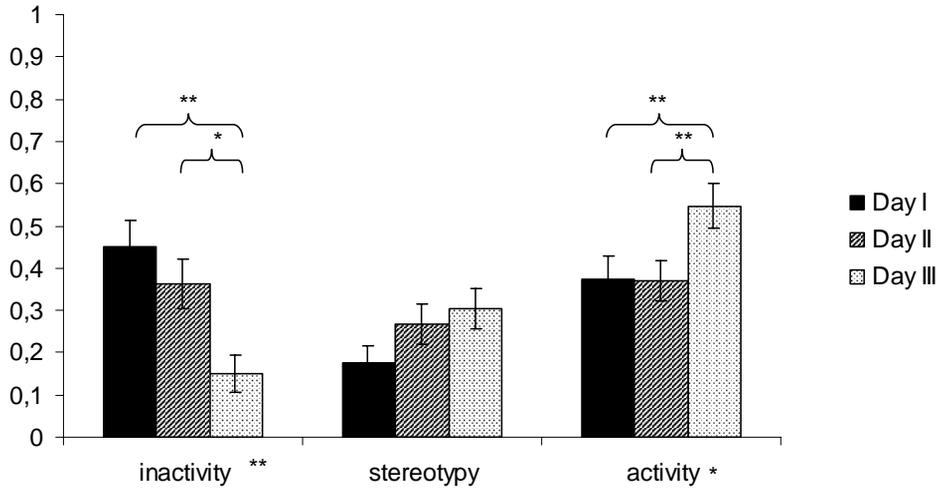


FIGURE 3. Male jaguar. Comparison of levels of inactivity, stereotypy and activity during three consecutive days of experiment (significance marked with * – $p \leq 0.05$ and ** – $p \leq 0.01$; at the name of behaviour category – significance of the effect)

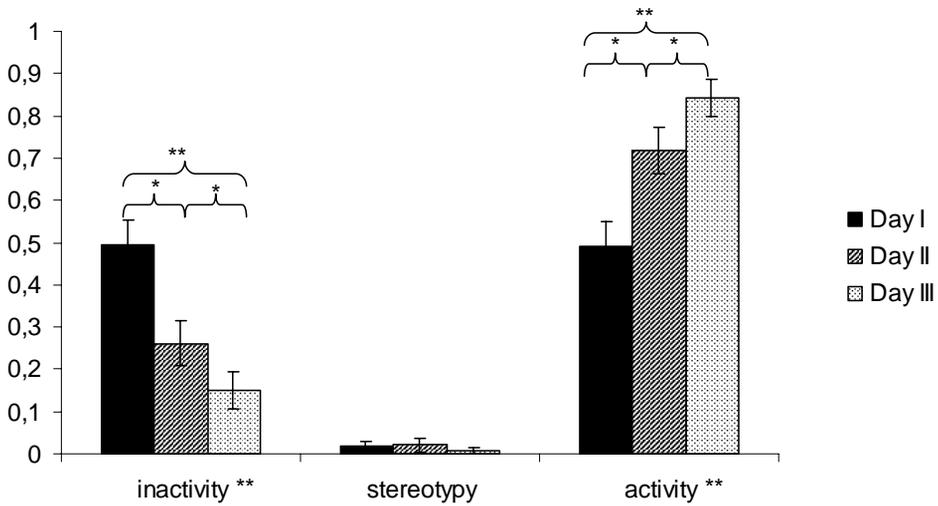


FIGURE 4. Female jaguar. Comparison of levels of inactivity, stereotypy and activity during three consecutive days of experiment (significance marked with * – $p \leq 0.05$ and ** – $p \leq 0.01$; at the name of behaviour category – significance of the effect)

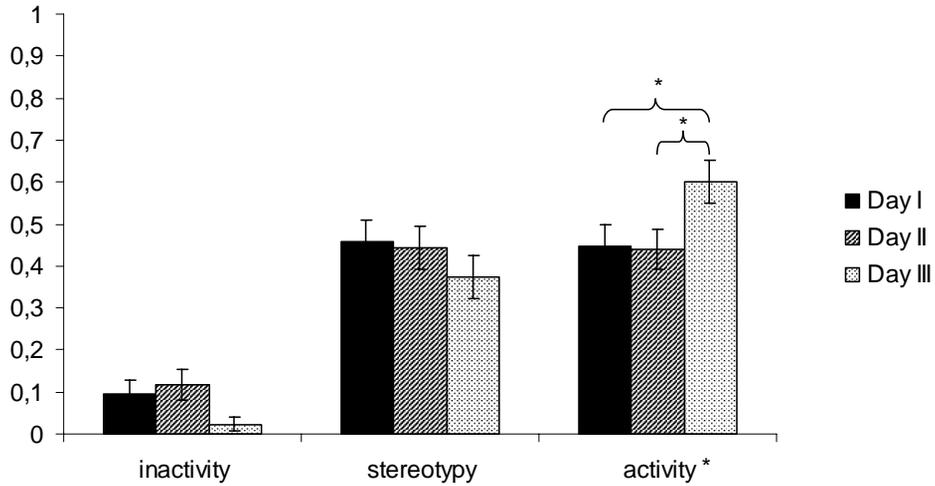


FIGURE 5. Male leopard. Comparison of levels of inactivity, stereotypy and activity during three consecutive days of experiment (significance marked with * – $p \leq 0.05$ and ** – $p \leq 0.01$; at the name of behaviour category – significance of the effect)

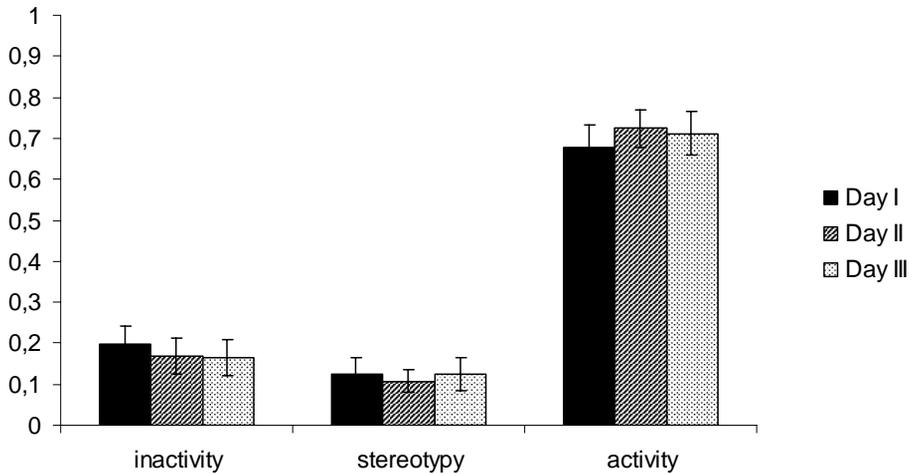


FIGURE 6. Female leopard. Comparison of levels of inactivity, stereotypy and activity during three consecutive days of experiment (significance marked with * – $p \leq 0.05$ and ** – $p \leq 0.01$; at the name of behaviour category – significance of the effect)

TABLE 1. The K-W statistics and significance of time of day ($df=1$), visitors' ($df=3$), separation ($df=1$) and weather ($df=7$) effect on time ratio of inactivity, stereotypy and activity of observed cats

Species	Sex	Time of day			Visitors			Separation			Weather		
		inactivity	stereotypy	activity	inactivity	stereotypy	activity	inactivity	stereotypy	activity	inactivity	stereotypy	activity
Puma	male	24.022**	29.047**	4.339*	41.326**	41.326**	41.326**	0.438	2.097	0.023	35.863**	35.863**	35.863**
	female	33.098**	29.047**	4.339*	55.867**	12.277**	26.308**	0.438	2.097	0.023	20.181**	35.354**	13.438
Jaguar	male	0.541	1.006	0.057	18.442**	9.750*	7.432				37.998**	45.257**	18.108*
	female	0.322	0.205	0.609	20.707**	5.534	20.764**				14.781*	6.798	13.944
Leopard	male	17.779**	5.235*	0.033	19.092**	0.826	6.416	17.037**	37.659**	14.469**	17.292*	36.181**	17.315*
	female	4.658*	13.166**	21.415	6.461	21.837**	16.059**	18.820**	53.116**	2.139	24.404**	19.298**	17.704*

* $p \leq 0.05$, ** $-p \leq 0.01$, $n = 180$.

Time of day

The level of inactivity was lower in the morning for all the observed cats. Only with the pair of jaguars this effect was not significant statistically. The male leopard and the female puma spent much less time on stereotypical behaviours in the afternoon. Fewer numbers of minutes spent on stereotype was accompanied by lower frequency of stereotype incidence as well.

On the other hand with the female leopard a reverse tendency was observed both as far as time and frequency of stereotypical series was concerned. An increase in stereotypical behaviours was observed with her in the afternoon in reference to both of the above measures (time and frequency). The result of the changes in proportions of stereotype and inactivity was a major decrease in the activity of cats for which the effect turned out to be a significant one.

Visitors

On the basis of one-way analysis it has been established that a higher number of visitors in front of the enclosure caused the increase in inactivity of all animals at the expense of their normal activity. Moreover, with female leopard and puma, an influence of that factor on the amount of time spent on stereotype has been observed. However, the reaction of each of the animals was different. The female leopard, contrary to the female puma, spent much more time on stereo-

type in the presence of a higher number of visitors.

The above differences were not reflected in the multi-way analysis of the stereotypy frequency. The influence of the number of visitors on that feature has not been proved with any of the cats.

Separation

The separation of the couple of leopards had a great influence on the level of their inactivity and stereotypical behaviours. Both of them spent much less time inactively. Both the female and the male after separation spent much more time on stereotypical paths. Increase in stereotype was accompanied by decrease in their natural activity. However, with the female this difference was not significant statistically.

With the female puma the separation from the cubs did not influence greatly the level of any of the categories analysed or the frequency of stereotype incidence.

Weather

With all the cats the weather effect had a great influence on each of the behaviour forms. Lower temperature caused increase in both normal and stereotypical activity. With reference to frequency of stereotypy series, the influence of weather categories was shown with two cats – the male jaguar and the female puma, and the observed tendency was similar. Incidence of stereotypy was more frequent in lower temperature.

Enrichments

With four out of six observed cats, the introduction of the enrichment had a major influence on their behaviour (Figs. 1–6). However, with reference to stereotypy, measured as the frequency of its incidence, a major effect of the enrichment was observed only with the female puma (*Wald statistics* = 12.387, *df* = 1, $p \leq 0.01$).

With the female puma after the introduction of the enrichment (day II) reduction in stereotypical behaviours was accompanied by increase in both activity and inactivity (Fig. 2). On the next day (day III) still a higher level of activity (in comparison with the baseline) was observed. Her inactivity fell down to the level of the control day, and the level of stereotypy increased slightly.

Introduction of the enrichment (day II) caused a major reduction of that feature. On the next day with the enrichment, the frequency of stereotypy although lower than without the enrichment, increased sharply. It could also be shown that the female puma relatively frequently had contact with the enrichment (25% of active minutes).

With the female jaguar in the subsequent days following the introduction of the enrichment, the increase in activity was accompanied by the decrease in inactivity (Fig. 6). The highest frequency of contact with the enrichment has also been observed (35% of active minutes). With the male leopard (Fig. 3) and jaguar the difference in the level of the catego-

ries of behaviour was observed only on day III. With the former cat a clearly higher level of activity was observed, with the simultaneous decrease in the other categories. The male jaguar on the other hand showed increase in both natural activity and stereotypy (Fig. 5). Both cats had very rare direct contact with the toy (less than 10% of active time).

DISCUSSION

Time of day

A lower level of inactivity in the morning was connected with release of the animals into the outside enclosure. Immediately after being released, the cats were exploring the enclosure intensively. With two of them (the male leopard and the female puma) initial exploration of the enclosure turned into walking along stereotypical paths, which was the reason for a higher level of stereotypy in the morning compared to the afternoon. Intensification of stereotypical behaviour with the female leopard in the afternoon might be explained by stimulation of the animal connected with the approaching feeding time.

Visitors

Significant influence of the visitors' effect seems to be the hidden effect of time of the day. This appears to be confirmed of collinearity of the factors: in the afternoon more people visited the garden (Tab. 2). The multi-way analysis effects of stereotypy frequency, in which for

TABLE 2. Spearman correlation coefficients between fixed effects (exemplary values for female leopard)

	Visitors	Separation	Weather	Enrichment
Time of day	-0.636**	0.000	-0.068	0.000
Visitors		0.147*	-0.043	-0.188**
Separation				
Weather			-0.328**	0.000
				-0.004

* $-p \leq 0.05$, ** $-p \leq 0.01$, $n = 180$

each of the cats the visitors' effect turned out to be non-significant, also confirm such interpretation.

Separation

Separation of the leopard pair was connected with a placing them into smaller enclosures. Lack of the partner and limited space contributed to decrease in the animals' welfare reflected in higher level of stereotypy at the expense of normal behaviours. Lyons et al. (1997) observed in captive felids in smaller enclosures, a higher, but not significantly higher, level of stereotypy. Kolter and Zander (1995) reached similar conclusions when comparing the levels of stereotypy in female polar bear placed in different enclosures (18 m²; 117 m²; 720 m²). However, in a pair of cheetah (Lyons et al., 1997) stereotypical behaviours appeared only when the cats were separated from each other in different halves of the enclosure.

Weather

High temperature made the animals unwilling to show any activity, both normal and stereotypical. Moreover, on ra-

iny days a change in stereotypical paths has been observed with leopards – they preferred parts of the enclosure sheltered from the rain.

Enrichments

The toys were characterised by low endurance because of the materials used. The male puma playing with them intensively destroyed them within the first minutes of the observation. In the afternoon, in the cage there were only the remains of the toys that did not attract the cat's attention. The above fact was used to explain the lack of clear influence of the enrichment on this cat (Fig. 1), and simultaneously, the highly significant influence of the time of day.

The other cat, the female leopard, on which there was no clear influence of the enrichment, showed relatively high normal activity regardless to the observation day (Fig. 4). The interest in the toy (more frequent contact and increase up to 15% of time spent active) appeared in her after separation from the male. Also Altman (1999) observed the influence of the toys on the behaviour of a male spectacled bear in the presence of the female or without it. The use of the enrichment caused the decrease in stereotypical behaviours only when the male was isolated, which seems to confirm the our results.

Numerous previous observations of cats confirm the reduction of stereotypical behaviours level as a result of environment enrichment (observed in the

female puma) (Bassenge et al., 1998). On the other hand the increase in stereotypy level on the third observation day in the female puma was explained as the result of getting used to the toy, which is confirmed by the fact of less frequent contacts with the enrichment on that day. Langenhorst (1998) observed that the brown bears are much more interested in objects thrown into the pool by visitors than a well-known toy made of wood. Poulsen (1995) considers that the use of the enrichments alone will not eliminate long-term stereotypy and has only a short-term effect.

The observed female puma was also encouraged to play by a pair of cubs accompanying her, and then more frequent contacts with the toy occurred. The female jaguar showed no decrease in the interest in the toy and higher activity than on the control day. Twice higher level of activity in comparison with control day was observed also by Altman (1999) in polar bears. In our studies the female jaguar devoted much time to contact with the toy not allowing the male to play, which was the reason for an increase of stereotypy in the male. A similar observation was made by Kolter and Zander (1995) in a group of polar bears. The incidence of stereotypy in a subordinate female was considered by the authors to be a reaction of avoiding a specimen higher in the hierarchy, which was confirmed by separation of the group – stereotypy in the female decreased sharply.

It is difficult to explain the increase in activity level in a male leopard only on the third day. The cat showed the highest stereotypy level and ignored the enrichments introduced, with the exception of the ones with a strong smell. On the basis of earlier observations carried out by the staff of the zoological garden it has been observed that it reacted only to enrichments of feeding nature (Zlamal, 2001). The smell of a potential victim turned out to be enough to stimulate feeding behaviours of a leopard.

Play

After getting used to a new element in the cage, the animals started to play. The toys laid down on branches were thrown into the air and carried around the enclosure. Then, in case of toys with fur and feathers, the cats started to get rid of these elements, which were similar to natural feeding behaviours (Hutchins et al., 1984). In the end the toys were torn to pieces, and their parts dragged about the enclosure. The hung toys, being less accessible, were a bigger challenge. Moreover, when struck they remained in motion much longer retaining the attention of the animal.

CONCLUSIONS

1. It is difficult not to notice the fact that except for the positive influence of the enrichment on the behaviour of the cats, the playing cats attracted more interest of the visitors in the zoological garden.

2. Motivation background of stereotypical behaviours of these cats needs further research. Finding out the reasons for stereotypy would help to find a suitable type of enrichment.

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Streszczenie: Analiza wpływu wzbogaceń środowiskowych na zachowanie dzikich kotów utrzymywanych w niewoli. Obserwacje wpływu wzbogaceń środowiskowych na zachowanie kotów prowadzono w ZOO Warszawa na trzech gatunkach: puma (1:1), jaguar (1:1) i lampart cejloński (1:1). Dane zostały zebrane w okresie od maja do listopada 2000. Każdy tydzień obserwacji był reprezentowany przez trzy kolejne dni (I – bazowy, II i III – ze wzbogaceniem). W odniesieniu do nieaktywności oraz naturalnej aktywności odnotowano ich udział czasowy. W przypadku zachowań stereotypowych zapisywano również ilość ich wystąpień. Wyniki oparte są na podstawie 5400 minut obserwacji. Spośród sześciu obserwowanych kotów, tylko samiec pumy nie wykazywał jakiegokolwiek formy stereotypii.

Samica jaguara poświęcała mniej niż 2% czasu na zachowania stereotypowe. U czterech spośród sześciu obserwowanych kotów, wprowadzenie wzbogacania miało znaczący wpływ na ich zachowanie. Badania wykazały różne tło motywacyjne stereotypowych zachowań i konieczność indywidualnego dostosowywania typu wzbogacania do zwierząt.

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Social behaviours in Siberian huskies

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Abstract: *Social behaviours in Siberian huskies.* The behaviour of a pack of 14 Siberian huskies dogs of different sex and ages was observed. Based on observation of different dominant, submissive and affiliate behaviours of each dog, pack hierarchy was described. Behavioural patterns and frequencies of different behaviours were compared with those, previously described in wild wolves. Social structure and linear hierarchy proved very similar, whereas there were some differences as far as single specimens are concerned. The alpha male did not display overt aggression towards the omega male, and he was – at least to some extent – involved in controlling and ceasing conflicts. Husky pack displayed several behaviours, common in wolves, yet seldom reported in domestic dog, e.g. feeding area marking, leg lifting during urination in females and full range of vocal patterns.

Key words: affiliate behaviour, *Canis familiaris*, canine, dominance, dog, pack, social behaviour, submission,

INTRODUCTION

According to Wood-Gush (1983), behaviours of domesticated species differ from those of their wild ancestors only due some modifications, related to levels of threshold stimuli, and their actual expression. Goodwin et al. (1997) classify all breeds of dogs in terms of changes in their morphology, body posture and facial expression, by comparing to

those of wolves. They conclude that these changes influence both intra-species communication and social behaviours in different breeds of dogs. They also claim that dogs, morphologically similar to wolves, such as Siberian huskies, display greatest variety of signalling, resulting in modulations of agonistic interactions. Additionally original utility of Siberian huskies brings some added importance.

This breed descends from sledge dogs, bred by Chukchi tribes in Eastern Siberia and Kamtschatka. Dogs worked and were fed in winters, but in summers they were roaming free and had to care for themselves. All year they lived in packs and their survival depended mainly on well functioning pack structure. Even today, albeit many huskies are kept as companion dogs, they are often kept in packs and trained for sledge sports.

The aim of the study was to test the hypothesis that although huskies form strong bonds with the owners, their social behaviours in the pack are close to those of wolves.

MATERIAL AND METHODS

A pack of Siberian huskies, composed of 14 dogs – 8 males and 6 females was observed. There were four unrelated adults:

ten years old male SK, eight years old bitch SO, one and half years old male KO and eight years bitch CH. The remaining dogs were children of the latter female: five years old male TA, three years old bitches WE and WA, six years old males BO, BJ and BU, six years old bitches BA and BY and her children three years old male SO and one and half years old male HA (Fig. 1).

All dogs lived permanently in a grass-covered yard, measuring approximately 400 m². They were provided with 11 m² shelter and two-storey kennel with 6 m² bedding. The yard was shaded by several trees and bushes.

First the preliminary observations was conducted. It lasted for a period of one year, and was carried several times a day at different times. Pack hierarchy was

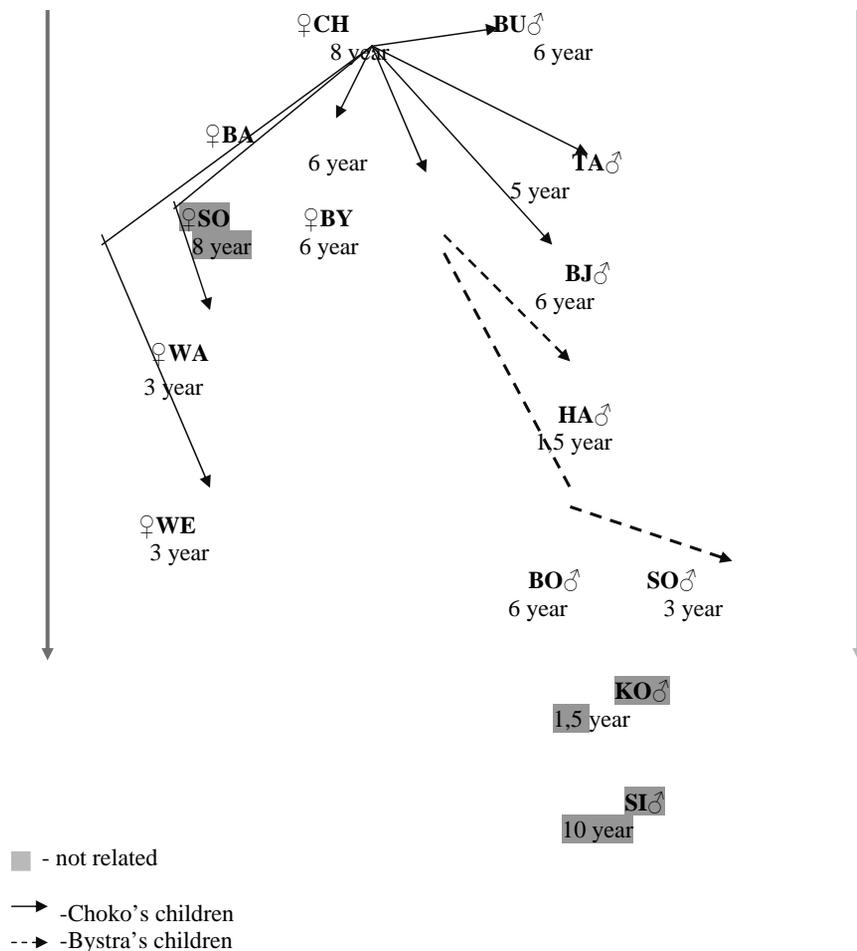


FIGURE 1. Family relation and hierarchy in pack

recognized with these observations. The more detailed observations of individual dogs with two VHS cameras followed.

Overall observations lasted for 105 days and consisted each of 5 sessions, divided into 6 sequences 5 minutes each. Each sequence was followed by 1-minute break. Each dog was observed twice at same times, therefore total observation time for each dog was 5 hours. Observations were conducted at resting time and never during feeding, grooming, exercising or training.

RESULTS AND DISCUSSION

Observations on dominant and submissive behaviours enabled to establish pack hierarchy. It is more clearly seen in males (Figs 2, 3).

The dominant **alpha** position is held by BU male, which displayed the gre-

atest number of dominant behaviours and never appeared submissive. **Beta** dog remains TA male; he was the one usually challenged by BU yet very seldom reacted in same way and never went into real fight. However, he was often dominant towards other males. The next male in hierarchy was BJ. Occasionally he displayed dominant behaviours towards Beta – TA, but more frequently was challenged and dominated by him. He also happened to be challenged by BO male, placed lower in rank, what gives an extra proof to his **gamma position**. The next male is HA. It was difficult to establish positions of two males, namely BO and SO, exhibiting similar behaviours. Such situations were observed in wolves (Ginsburg, 1987), their relations sometimes not being clearly defined. Males of uncertain positions are

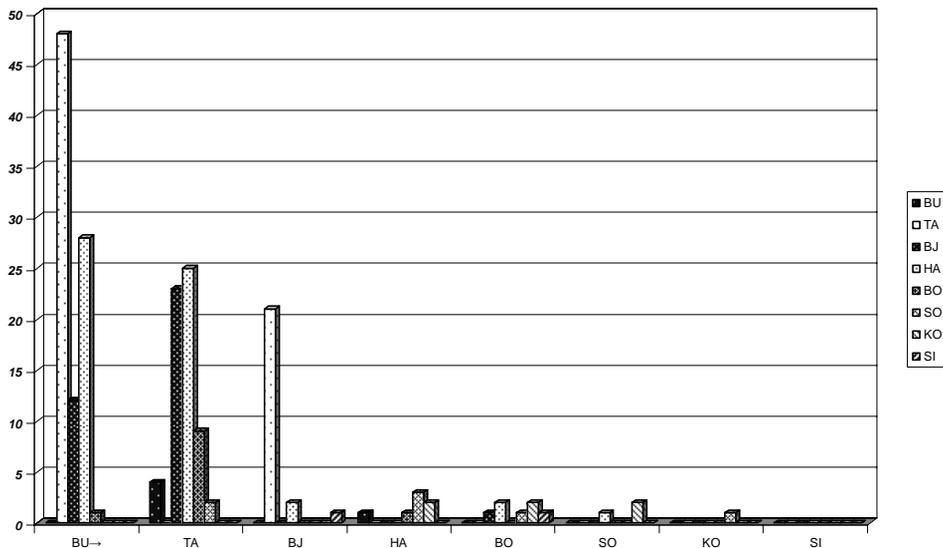


FIGURE 2. Dominant behavior among males

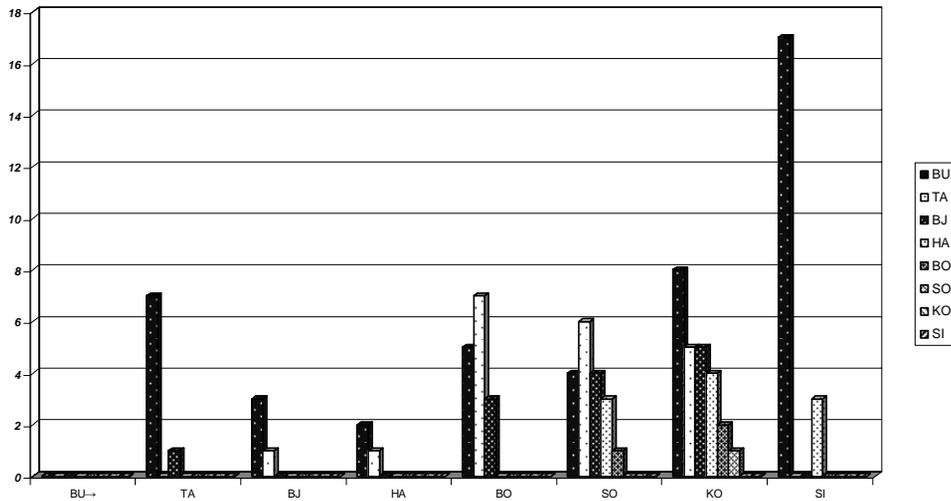


FIGURE 3. Submissive behavior among males

more likely to engage in conflicts. The lowest male in hierarchy was KO (one year old), showing mainly submissive behaviours.

Some interesting observations related to SI male. According to standard grading procedure, he was an **omega** dog. Seldom any dominant behaviours directed towards him was noticeable, but he frequently displayed submissive behaviours. It seems possible that, at time observations started, he had just lost his number one position. Many attacks towards him by other members of the pack were observed. Those attacks started with no apparent reasons and without any prior displays of dominant behaviour. Attacks were mainly initiated by **beta** male, followed by other males as well as by some females. A single attack towards him started by either **alpha** male or female was never observed.

Pack hierarchy among females was not as clear as that among males (Figs 4, 5) Two bitches – CH and BA did not display any submissive and/or defensive behaviour. BA showed the highest level of general dominance, even though it was CH bitch that most frequently displayed dominant postures and T position. Interestingly, she was generally avoided by all other bitches, with the exception of SO bitch. Apparently, most of all dominant behaviours displayed by CH were directed towards SO. It may be presumed that CH bitch is alpha female in this pack.

Similar results for agonistic behaviours were found with Ba bitch, ranked as **beta**. Most of her dominant behaviours, however, were directed towards two young bitches, WE and WA. Their positions were probably not fully established, therefore they were regarded as a potential threat

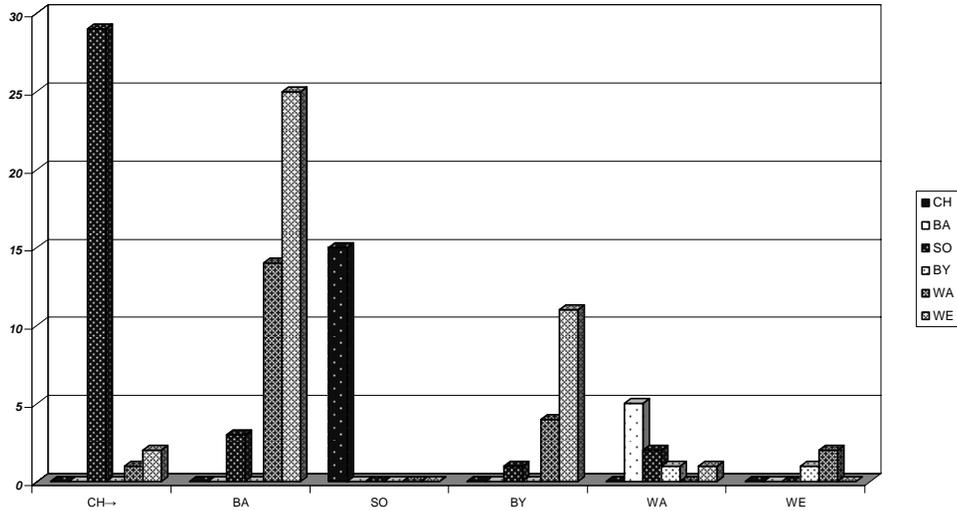


FIGURE 4. Dominant behavior among bitches

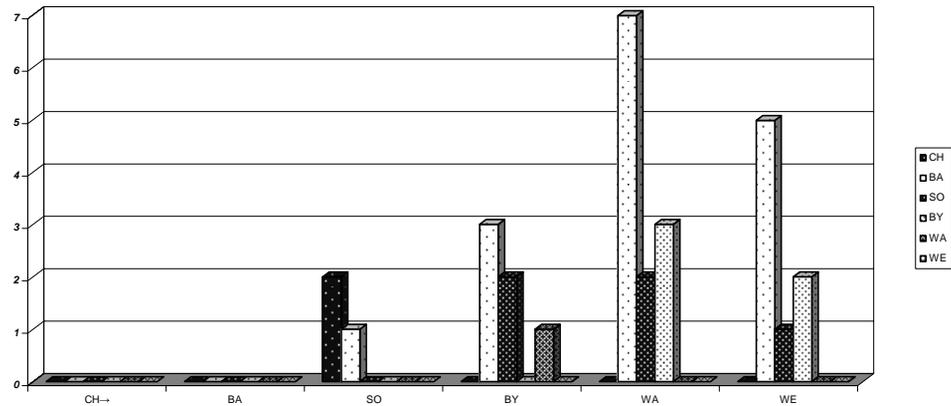


FIGURE 5. Submissive behavior among bitches

to her current status. Similarly to what was observed in males, it was difficult to establish any clear ranks of SO bitch (eight years old) and BY bitch (six years old). They displayed similar levels of dominant behaviours; although they were slightly more frequent in case of SO, she also displayed distinctly more

frequent defensive behaviours. WA and WE bitches were the two lowest ranked. The hierarchy in the observed pack can be presented as double linear and can be presented as follows figure 1.

Contrary to what was reported by Mech (1999) in wild wolves, no dominant and/or submissive behaviours

among dogs of opposite sex was observed. Even in case of **alpha** male, who was a son of **alpha** female, no display of dominant or submissive behaviours between the two was observed.

It is worthy to note that only two dominant behaviours were observed, whereas Scott and Fuller in their classic work (1965) described 5 of them. The only two observed were dominating a laying dog by standing over it and growling, or standing or walking on stiff legs with tail carried high and straight (T-position). Placing feet on other dog's withers, combined with growling, mounting and nipping on neck with tail carried low, or threatening with head and tail carried low was not observed.

In case of dominant behaviours or even fights, which were about to start between two males, it was the **alpha** male (BU) who took an intervention and broke conflicts. He did it either by approaching them and displaying T-position, or by ear-licking, usually that of a dog ranked higher. Similar intervention in case of fighting females was never observed, and neither did the **alpha** female.

Several specific behaviours were observed, and usually they were influenced by the actual ranking (Figs 6, 7).

As for exploitative behaviours, they were displayed mainly by the **alpha** male. They were often engaged in patrolling, sniffing, listening and watching

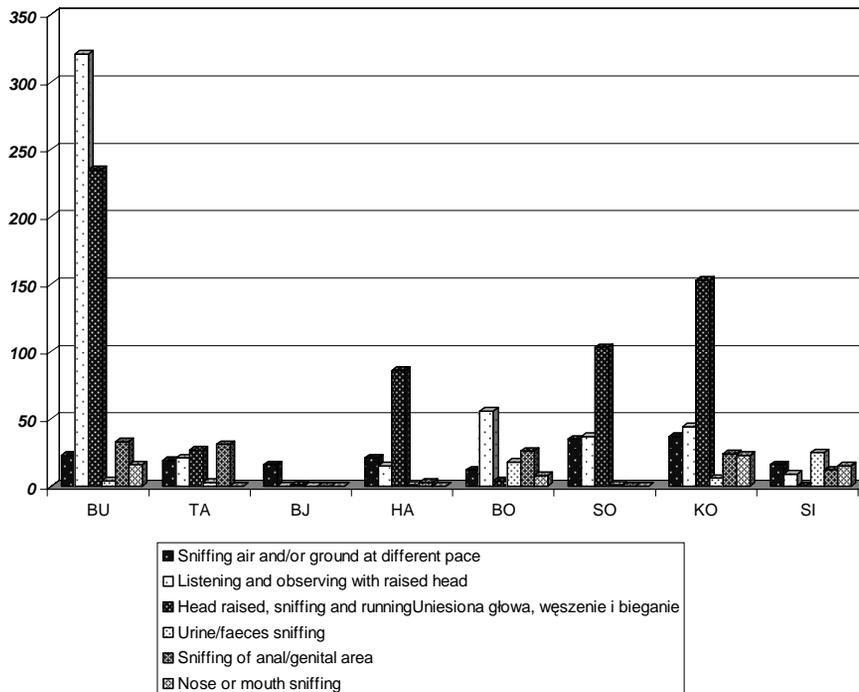


FIGURE 6. Explorative behaviour in males

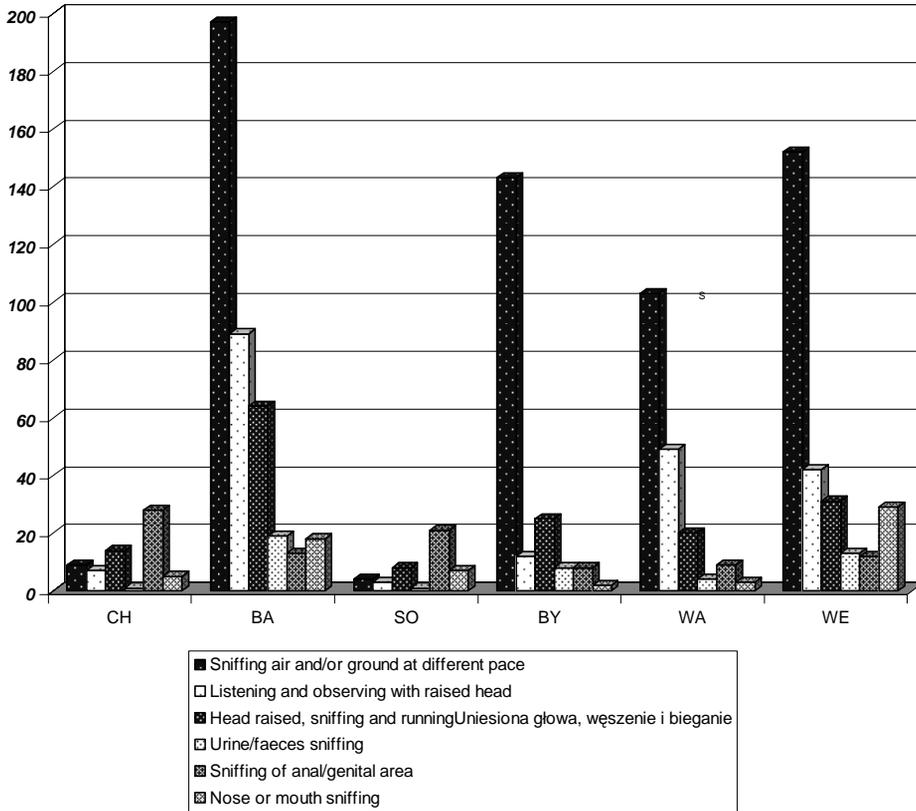


FIGURE 7. Explorative behaviour in females

around. Same activities were observed in case of the **beta** bitch and the youngest ones, whereas the **alpha** bitch was very seldom involved in similar activities, most probably because she was eight years old and felt safe in well-known yard and territory, where she had spent five years. Affiliative behaviours were more frequently observed in males, than in females (Figs 8, 9), the only exception being two bitches – CH and SO. It seems, however, that it was more imitation than real bond forming. Similarly to what was described by Mech (1999) in

relation to wolves, we found that young dogs were likely to keep in closest proximity of other dogs, as shown in Figures 10 and 11.

Some interesting observations relate to urination in females, displayed as territory marking. Highest ranked females tend to lift hind leg during urination, similarly to males. The **alpha** bitch tends to scratch ground with all paws. We also observed frequent urination into empty bowls and onto fences.

Dogs presented great variety of vocalisation, displaying all 11 patterns known

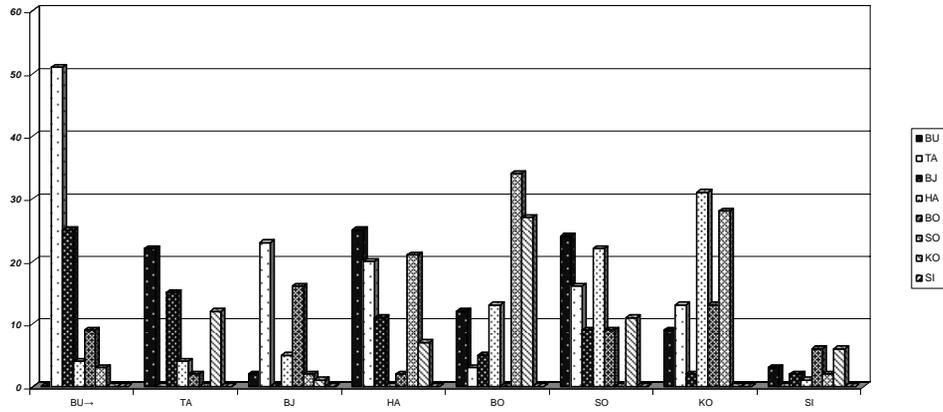


FIGURE 8. Affiliate behaviour among males

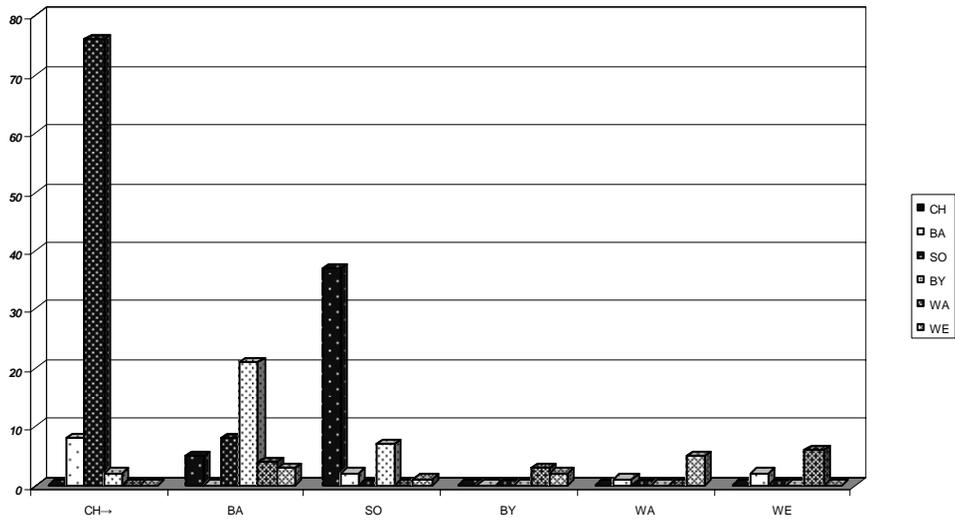


FIGURE 9. Affiliate behaviour among female

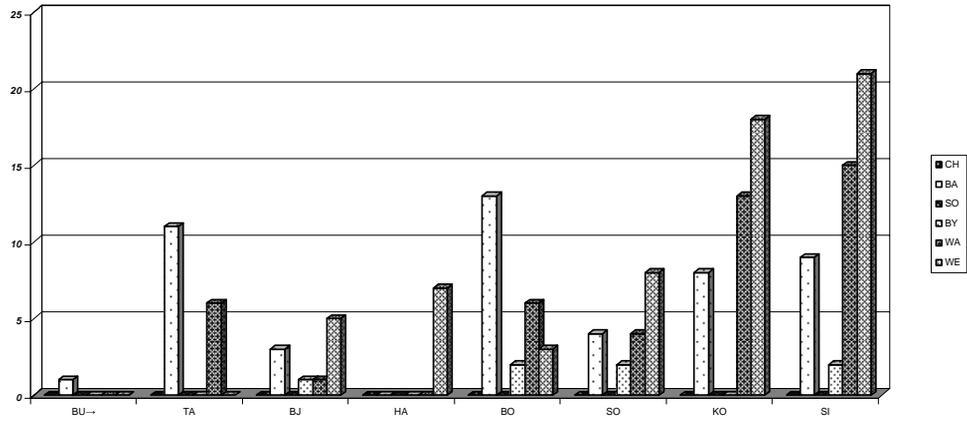


FIGURE 10. Affiliate behaviour males vs. females

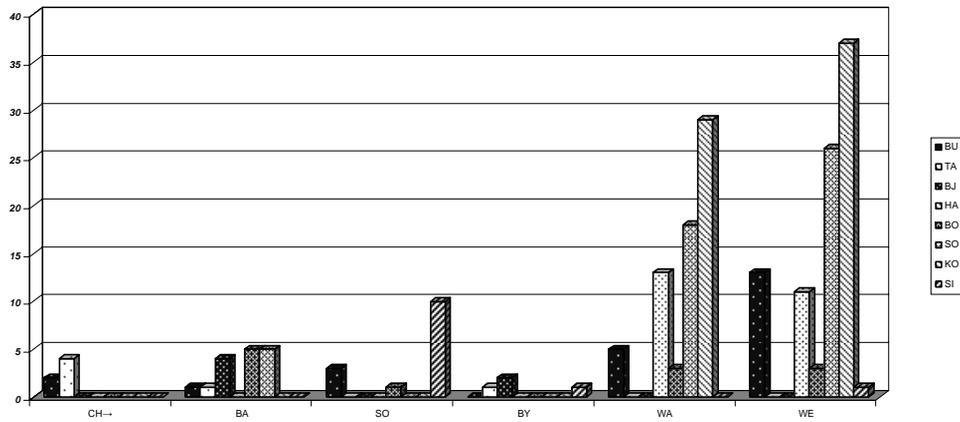


FIGURE 11. Affiliate behaviour females vs. males

in wolves. The most frequent was howling, initiated by a single male; barking seemed of lesser importance and was employed only as alarming signal or attraction seeking behaviour.

CONCLUSION

All of these observations lead to the confirmation of the hypothesis that behaviours and social relations in Siberian Huskies pack show striking similarities to those of wild wolves. Starting from social structure, through affiliate and territorial behaviours, as well as vocalisation, huskies are pretty similar to wolves, probably far more than other, more „advanced” breeds. The pack leader (**alpha** male) was predominantly involved in explorative and territorial behaviours and was instrumental in controlling and ceasing potential conflicts. Feeding area was scent-marked with urine. Urination with lifted leg was part of territorial behaviours typical of high-ranked females (Fig. 7).

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- Streszczenie:** *Zachowanie społeczne u Siberian husky*. Obserwowano zachowanie stada psów rasy husky składającego się z 14 osobników obu płci w różnym wieku. W trakcie obserwacji określano kształtowanie hierarchii stada na podstawie występowania zachowań dominacyjnych, submisyjnych i afiliacyjnych poszczególnych psów. Obserwowano także zachowania afiliacyjne i wokalizacje. Częstość występowania i wzorce poszczególnych zachowań porównywano do opisywanych w literaturze zachowań stadnych wilków. Struktura grupy socjalnej i podwójna hierarchia liniowa okazały się takie same, jak opisywano u wilków, zachowanie poszczególnych osobników było jednak nieco odmienne. Osobnik α nie wykazywał agresji w stosunku do psa ϕ , a także w pewnej mierze kontrolował i tłumił konflikty w obrębie stada. Uboższy był repertuar zachowań dominacyjnych i brak submisji aktywnej między psami. Obserwowano również u badanej sfory zachowania typowe dla wilków, a opisywane jako niewystępujące lub rzadko występujące u psów, jak: znakowanie miejsca posiłku, podnoszenie nogi w czasie urynacji suk i pełen zakres sygnałów wokalnych.

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Kinship in free – living field vole (*Microtus agrestis*) population

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Abstract: *Kinship in free – living field vole (Microtus agrestis) population.* Population of field vole in Southern Poland represents triennial cycle of numerical force. Regarding to this the free living vole population was tested in reference to two hypothesis of Charnov and Finerty (1980) and Lambin and Krebs (1991a), according to which the cyclic changes of sizes in population were dependent on changes of their inter individual genetic relationship. In 1999–2001 when phases of number decrease (low phase) and increase (high phase) took their places we carried out the analysis of inter individual kinship in summer and autumn seasons (1999 and 2000), and spring season 2001 on one hectare of sampling area. Basing on the places of trapping, the individuals were grouped (or not) in aggregations. Nevertheless because of low number of captures, the aggregations could be defined for summers 1999 and 2000 only. The tissue samples of 68 *Microtus agrestis* individuals (50 females and 18 males) were taken. RAPD method with three primers was applied for DNA analysis. The total number of fragments obtained, and the frequency of every single fragment were calculated for each starter. No difference in kinship among the individuals belonging neither to the same nor to the different populations was detected. This may suggest that during summer seasons genetic relations do not play any important role on vole's dispersion. Higher level of kinship was found in the increase phase than in decrease one, in accordance with model of Lamin and Krebs (1991a) but not with that of Charnov and Finerty (1980). Kinship of individuals trapped in spring was twice as high as of that in summer and autumn and kinship among males was lower than among females.

Key words: *Microtus agrestis*, field vole, population dynamics, RAPD.

INTRODUCTION

Kinship is one of main factors influencing the evolution of social behaviors at animals. This factor seems to be highly essential above all in interindividual relations, where the level of kinship can play the substantial role in the quality of these contacts (a cooperation, an altruism). Social interactions among individuals have the huge influence on the reproduction success and thus the rate of a growth in population (Chitty, 1967, Mappes et al., 1995, Pusenius et al., 1998). On account of the fact that reproducing females do play the main role in dynamics of population, factors influencing the occurring interactions among these females to large extent also influence the shape and the rate of population growth. In populations of small rodents, particularly at species from the *Microtus* genus, the females are filopatric, i.e. young maturing (almost active sexually) females settle in neighbourhood of their mothers, in the vicinity of sisters (Boonstra et al., 1987, Lamin and Krebs, 1991,

Pussenius et al., 1998). This phenomenon has therefore considerable influence on the level of kinship between the closest neighbour's i.e. reproducing females and for their density, as their territories very often overlap. Increasing of kinship can be beneficial for the reproductive success of related specimens (Mappes et al., 1995, Pusenius et al., 1998) both by lowering the probability of infanticide by neighbouring unrelated females (Wolff, 1993, Lamin and Yacoz, 1998, also Agrell, 1994), by reducing costs of the defense of territory and advantages of cooperative breeding. But growth of kinship generates also certain costs caused by higher probability of inbreeding and the competition between related specimens (Wolff, 1995). In the connection with all above Charnov and Finerty (1980), basing on differences in kinship between specimens, created the hypothesis trying to explain the cause of cycles – the kinship selection hypothesis. ACCORDING TO THIS HIPOTHESES the kinship among the neighbors in the population changes in reference to the density. In population being in low and growing phase neighbours are related – in the opposite one when phase peak is reached, the unrelated specimens dominate.

Unfortunately the verification of this hypothesis has brought the unanimous results. Although it was confirmed by Boonstr and Hogg (1988) and Ylonen et al. (1990) but not by Pugh and Tama-

ryn (1990), neither by Lamin and Krebs (1993), Dalton (2000).

As the determination of kinship in free living population is often misleading, most of the evaluations of this parameter in vole families were done in controlled fenced conditions. Additionally all of them focused on kinship among females, regarding to their main role in population dynamics, thus neglecting the kinship of males.

In the present paper we would like to trace the coefficient of kinship in free living population, in both sexes, throughout the seasons and following the phases of growth.

MATERIALS AND METHODS

The investigations were carried out in West Sudety Mountains (south of Poland) close to the city Szklarska Poręba on the population of field vole subject to seasonal number fluctuations (Borowski, 2001). Capturing was done on one hectare forest plantation situated within 80 years old spruce trees. The sampling surface was grown mostly by grasses (primarily *Deshampsia flexuosa*, s, *Juncus effuscus*, *Carex canescens*), *Vaccinium myrtillu* and tree seedlings (*Picea abies*, *Alnus incana*, *A. glutinosa* and *Fagus silvatica*). Within area we situated 11 rows of wooden live catching traps (15 in each) dispersed 10 m apart from one another. The voles were lured into them by oat seeds and apple pieces. The traps were controlled daily twice at 9 am and 6 pm Each catching session

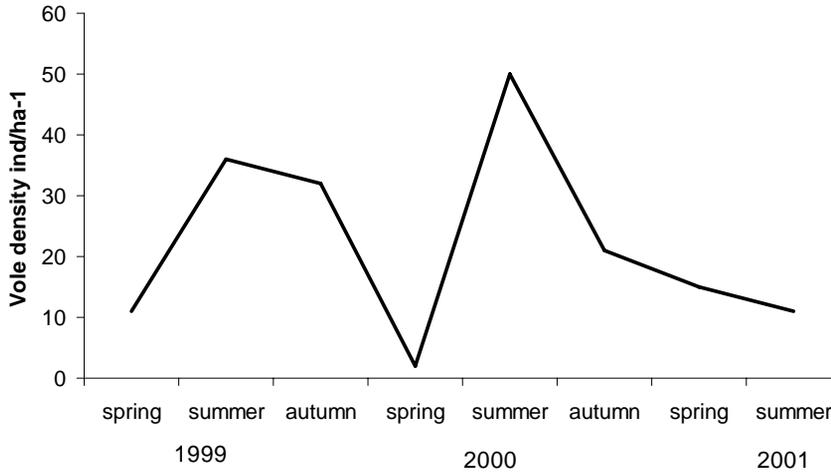


FIGURE 1. Field vole (*Microtus agrestis*) population density calculated by MNA method during the trapping period (spring – May, summer – August, autumn – October) in Sudety Mountains, Poland (source: Borowski 2001).

went on 8 days. The animals were captured by CMR (catch, mark and release) method in August (summer) and October (autumn) in 1999 and 2000 and in May (spring) 2001, then marked by toe clipping and weighted (with the accuracy to 0.5 g). The sex, sexual activity and place of capture were noted. Clipped toes were collected for further DNA analysis.

Totally 106 individuals were captured and 68 of them (50 females and 18 males) were used for genetic analysis. In order to describe the distribution of voles we created the co-ordinate system basing on the net determined by traps positions (140 squares). Localization of each catch in every session was noted. The individual caught more than once was assigned to many groups, depending on each catch localization. Assigning each vole to the aggregation and de-

fining the aggregations themselves were done by Nearest Neighbor Distance method, according to Krebs (1989). The number of aggregations defined was not constant – in summers 1999 and 2000 we found 5 and 4 respectively but in remaining seasons no aggregations could be defined because of low number of animals caught. In 1999 the trapping were carried out in low (decreasing) phase of vole population growth and in 2000 and 2001 in high (increasing) phase (Borowski, 2001; Fig. 1). The sizes of population were evaluated by the Minimum Number of Alive method MNA (Krebs, 1989).

DNA extraction

DNA was isolated from claws, which were stored after clipping in moisture absorbing silica gel to prevent decay.

To the fragmented Tissue 500 μ l Lysis buffer (tris/sucrose/MgCl₂/triton X), and 25 μ l proteinase K (10 μ l/ml) was being added. Next incubated through 24 h in the 55°C. DNA was extracted by phenol-chloroform method and resolved in TE buffer in 65°C and stored in -20°C.

PCR-RAPD

Three RAPD primers were used. Their lengths don't exceeded 10 nucleotides and the G/C pairs content amounts to 66–78%. (A-11, B-2, N-2) (TIB Mol-Biol): A-11 5'CAA TCG CCG T3'; B-8 5'GTC CAC ACG G3' i N-2 5'ACA ACG CCT C3'. The PCR reaction was carried in the final volume 30 μ l. To single PCR reaction the following amounts of reagents were used: 3 μ l (10 \times) PCR buffer, 1 μ l MgCl₂, 0.3 μ l dNTP (POL-GEN), 5 pmol primer, 1.5 unit RedTaq Polymerase (SIGMA), 8 μ l template DNA, MilliQ water (MILLIPORE) to the final volume. The PCR conditions for 45 cycles, for each primer were given in Table 1.

Electrophoresis

Electrophoresis separation of PCR products was done on the 6% poliakrylamide gel (no denatured), supplemented with glycerol to finale 5% concentration. Fragments were separated in 1x TBE buffer at 15 W constant power. Visualization was done by silver staining method. Dyed gels were dried overnight in 80°C and scanned.

Statistic

For all primers applied, a total number of fragments and their frequencies were calculated. Genetic distance between individuals was calculated according to Neighbor-Joining Method, (Nei, 1987). For calculating the genetic distance we used SPSS 10.1. The relationship of particular aggregations was performed by non-parametric Kruskal-Wallis test.

RESULTS

Molecular analysis

The A11 primer started amplification of 49 different fragments with the lengths 177–801bp. The highest frequency of a single

TABLE 1. PCR conditions used in experiment

Name	Primer sequence	Reaction conditions
A-11	5' CAA TCg CCg T 3'	denaturizing 94°C, hybridization 35,5°C, extension 72°C
B-8	5' gTC CAC ACg g 3'	denaturizing 94°C, hybridization 30°C, extension 72°C
N-2	5' ACA ACg CCT C 3'	denaturizing 94°C, hybridization 36°C, extension 72°C

allele amounted to 0.074, while the lowest to 0.012. With the usage of B8 primer we obtained 161 different fragments. The shortest identified fragment had 110bp and the longest 2805bp. The fragments' frequency was within 0.0028–0.048 range. Primer N2 gave us 147 fragments with their lengths ranging from 63bp to 2514bp. The fragments' frequency ranged within 0.0024–0.041.

kinship among females but not among males (Tab. 2).

In autumn seasons 1999–2000 and in spring 2001 no aggregations were defined because of low number of voles caught and the genetic relationship evaluation was done for all individuals together. In autumn 1999 the values of genetic distance were set within 0.039 to 0.316 scopes. The analysis of genetic

TABLE 2. Comparison among females and males of field vole (*Microtus agrestis*) in August 1999 and 2000

Sex/year	females 1999	males 1999
Females 2000	$\chi^2 = 9.533$ $p = 0.002^*$	–
Males 2000	–	$\chi^2 = 0.022$ $p = 0.883$

* $p \leq 0.05$; ** $p \leq 0.01$ Kruskal Wallis test.

Genetic distance analysis

In the summer season 1999 the highest value of genetic distance for all individuals amounted to 0.047 and the lowest to 0.403 in the next year the same values amounted to 0.066 and 0.417 respectively. The comparison of relationship within aggregations and among them for each season was done by Kruskal-Wallis test. The kinship values within and among aggregations were on the similar level. In both seasons (1999 and 2000) the genetic distance was calculated separately for males and females. The analysis revealed significant differences in

distance of animals captured in spring 2001 showed 0.074–0.347 scopes.

In order to analyze the kinship of free living vole population in spring, summer and autumn seasons, the genetic distances of animals captured in mentioned periods were compared. The values obtained for spring 2001 differed highly significantly from those of summer and autumn 1999 and 2000. The individuals caught in spring 2001 created almost uniform, homogenous group as far the kinship coefficient is concerned. The comparison between 1999 and 2000 seasons done by median test revealed

that the kinship of voles captured in 1999 was twice as low as that in 2000 (Tab. 2). Basing on these data the logarithmic model describing the seasonal changes of kinship of field vole in the population of Sudety Mt. in 1999–2001 was created (Tab. 3). Because

of non parametric character of data describing the genetic distance in seasons analyzed, we used standard deviation from median calculated for population (SD – Me) which illustrates clearly the ups and downs of kinship within seasons of our interest.

TABLE 3. Comparison of kinship level among captured field vole (*Microtus agrestis*) in seasons 1999–2001

Season/year	Summer 1999	Autumn 1999	Summer 2000	Autumn 2000	Spring 2001
Summer 1999	–	$\chi^2 = 0.207$ p = 0.65	$\chi^2 = 4.261$ p = 0.039*	$\chi^2 = 0.036$ p = 0.849	$\chi^2 = 22.982$ p = 0.000**
Autumn 1999	$\chi^2 = 0.207$ p = 0.65	–	$\chi^2 = 0.420$ p = 0.517	$\chi^2 = 0.497$ p = 0.481	$\chi^2 = 8.653$ p = 0.003**
Summer 2000	$\chi^2 = 4.261$ p = 0.039*	$\chi^2 = 0.420$ p = 0.517	–	$\chi^2 = 3.208$ p = 0.073	$\chi^2 = 0.324$ p = 0.000**
Autumn 2000	$\chi^2 = 0.036$ p = 0.849	$\chi^2 = 0.497$ p = 0.481	$\chi^2 = 3.208$ p = 0.073	–	$\chi^2 = 15.859$ p = 0.000**
Spring 2001	$\chi^2 = 22.982$ p = 0.000**	$\chi^2 = 8.653$ p = 0.003**	$\chi^2 = 0.324$ p = 0.000**	$\chi^2 = 15.859$ p = 0.000**	–

*p ≤ 0.05; ** p ≤ 0.01 (Kruskal – Wallis test).

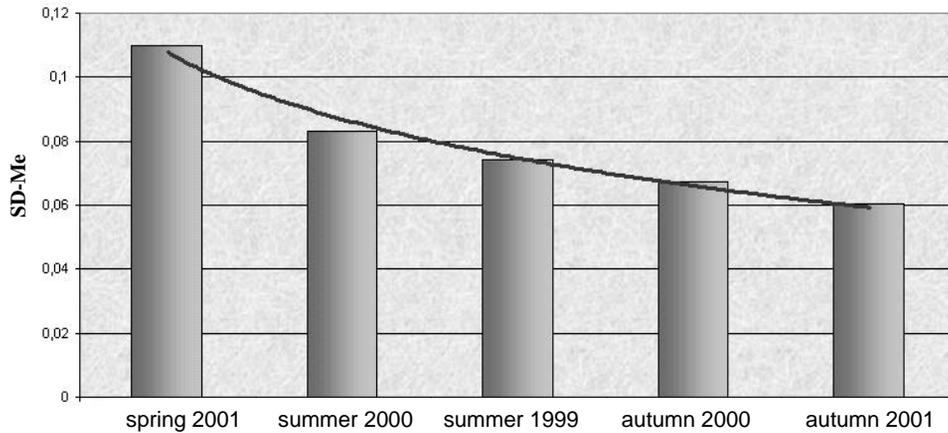


FIGURE 2. The curve linear trend of kinship among the seasons in 1999–2000 ($y = -0.31 nx + 0.12$)

DISCUSSION

Coefficients of kinship among voles differed significantly between low and high growth phases (the higher in the subsequent one) what is in accordance with model of Lamin and Krebs (1991) in which the kinship should grow together with density increase but opposite to Charnov and Finerty (1980) who claimed reverse connexion. We noted higher kinship during the reproduction season than in post reproduction one. We believe that the decrease of kinship was caused by late summer migration differentiating structures of populations. It was clear that the aggregations were formed in reproduction seasons only, when the highest density occurred. But as mentioned above we didn't monitored the population in early summer - at the beginning of reproduction season, when according to Pusenius et al. (1998) the aggregations are formed, to fall apart

when the breeding time is over. Nevertheless the lack of differences in kinship among individuals forming the aggregations and others from outside suggests that it was not the kinship what caused the forming of summer aggregations.

One (despite the kinship) of the factors influences aggregating of animals is preference to certain types of habitats. In our sampling area the pivotal role in forming of aggregation played the density of plants, which provided the natural protecting shelter against natural predator enemies (Borowski, 2001). Thus we conclude that the environment preference and high density caused the aggregation in August.

Twice as low values of kinship among males than among females seem to be quite obvious as at voles the males are the main dispersion factor (Aaars and Ims, 2000 among others). It could be caused both by migration of the males

from areas of higher density to those of lower ones and by immigration of unrelated males from outside (Borowski and Owadowska, 2000). The higher kinship among females could be caused by their settlements close to the natal places.

It is worth to notice to the fact that the high kinship values among the winter survivals (the highest in our analysis) was observed also in other species (Tait and Krebs, 1985). Remembering the relatively low kinship values at autumn period we conclude the presence of selective pressure during winter time, directed for the certain phenotypes, probably uniformed by common ancestry. The role of migration can also influence the level of genetic relation in open population like this one but we know that the level of dispersion during winter and early spring has the lowest values throughout the seasons.

The results presented herewith are in accordance with the hypothesis of Lambin and Krebs (1991) saying that the increase of population density causes the increase of kinship. Basing on our finding we can not confirm neither denies that voles spend winter in related groups.

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Streszczenie: Spokrewnienie u wolno żyjących norników burch (*Microtus agrestis*). Populacja nornika burego w południowej Polsce wykazuje trzyletnie cykle liczebności. W wolno-żyjącej populacji nornika przetestowano słuszność dwóch hipotez (Charnov i Finerty, Lamin i Krebs) tłumaczących cykliczność wahań liczebności zróżnicowaniem w spokrewnieniu osobników. W latach 1999–2001, przypadających na fazę niskiej liczebności i fazę wzrostu populacji, przeprowadzono analizę spokrewnienia osobników

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Polymorphism of RPE65 gene in the Polish briard population

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Abstract: *Polymorphism of RPE65 gene in the Polish briard population.* In order to identify the possible mutations in exon 5 RPE65 gene in the Polish population of French sheepdogs – briards – we have studied 51 dogs of this breed (26 males and 25 females). The population studied consisted of about 80% of the key breeding stock animals in Poland. PCR reaction was performed after isolation of genomic DNA. The resulted product was – with no exception – of 107bp length, suggesting that no 4 bp deletion (485 del AAGA) occurred. PCR-SSCP reaction was performed to search for any possible mutations, resulting in three SSCP patterns obtained: *a*, *b* and *c* at 0.157; 0.549 and 0.294 frequency, respectively. After DNA sequencing we were able to prove that all specimens, showing pattern *c* were heterozygous and combined patterns *a* and *b*. Only slight single nucleotide polymorphism (SNPs) of RPE65 gene was detected. The difference found between both DNA sequences studied was restricted to 459 nucleotide (T459C) of RPE65 gene. When both sequences studied were compared to those, already published in GeneBank, we found they differed in 432 (T432C), 459 (T459C) and 505 (T505C) nucleotides of RPE65 gene. However, none of those differences resulted in either of (N135N), (Y144Y) and (L160L) protein product change. Having conducted the fore mentioned study, we come to conclusion that neither affected animals, nor carriers of mutated RPE65 gene are present in the Polish briard population.

Key words: RPE65 gene, polymorphism SSCP, *Canis familiaris*.

INTRODUCTION

Progressive Retinal Atrophy (Dystrophy) is a hereditary disease, found in many dog breeds, of different onset and development (Dekomien end Epplen, 2000; Petersen-Jones et al., 1999). Generally, it leads to photoreceptor degeneration. One of the main functions of retinal pigmented epithelial cells (RPE) is degradation of photoreceptor segments as well as their production. Has disease occur, dystrophic RPE cells are not longer able to fulfil their functions and in result photoreceptors of both cones and rods undergone progressive degeneration, leading to deteriorated central vision. However, peripheral vision remains unchanged. Deterioration originally manifests itself as night blindness and is gradually developing at different rate, in some cases leading eventually to total blindness.

Gu et al. (1997) in their study on clinically diagnosed PRA found that – in contrast to other genes whose defects have been implicated in degenerative retinopathies – RPE65 is the first disease gene in this group of hereditary disorders that is expressed exclusively in the

RPE, and may play some roles in vitamin A metabolism of the retina.

Studies in mice on association with 11-cis-retinal dehydrogenase suggests that RPE65 is a component of visual cycle pathway, the process in which the 11-cis-retinal chromophore of visual pigments is photoisomerised to the all-trans-isomer, which is subsequently regenerated in darkness (Redmond et al., 1998).

In human RPE65 gene was located in 1p31.2 chromosome (Hamel et al., 1994; Narfström et al., 1989), in mice in 3 chromosome (Hamel et al., 1994), in rats in 2 chromosome (Manes et al., 1998), in cattle in 3 chromosome (Golapalli et al., 2003), whereas in dogs it is registered in GenBank (accession number AF084537).

Aguirre et al. (1998) found that the normal canine RPE65 cDNA spans 1724 nucleotides and includes 1606 nucleotides of coding sequence predicted to encode a protein of 533 amino acids (61 kDa). They also showed that the deduced amino acid sequence of canine RPE65 gene shared 98%, 97% and 93% identity with homologous human, bovine and rat sequences, respectively. The above mentioned authors proved that a homozygous 4 nucleotide (AAGA) deletion was only found in *csnb* and retinal dystrophy affected Swedish briards; heterozygous animals had normal and mutant alleles. This mutation produces frameshift causing a deduced mistranslocation with a premature stop codon. The mutation

causes retinal dysfunction and RPE accumulation of lipid vacuoles (Aguirre et al., 1998). The increasing interest in researching genetic background of PRA in this breed dates since 1984, when results published in Great Britain showed that 31% of dogs aged 18 months and over were clinically affected (Bedford, 1984). Assuming the trait follows random distribution pattern, those results established the frequency of the recessive gene in British population at 55% (Bedford, 1984). Narfström et al. (1989) described the disorder as a stationary one and analogous to human congenital stationary night blindness (CSNB) and later the disorder was eventually described as having a progressive component and termed hereditary retinal dystrophy (Wrigstad et al., 1994).

Additionally, Gu et al. (1997) proved the relation between the occurrence of *csrd* (children-onset severe retinal degeneration) and mutations in the RPE65 gene, whereas Marlhens et al. (1997) and Morimura et al. (1998) showed the relation between LCA (Leber's congenital amaurosis) and mutations in the same gene. Common features of the affected patients include loss of vision from birth or early childhood, associated with total night blindness, all suggesting a role of RPE65 in retinal functions (Redmond et al., 1998). Dogs blinded by an inherited retinal degenerative disease are an excellent animal model for human disease. In 2001 Acland et al. used the gene therapy technique to treat 3-month-old

briard-beagle mix dogs with defective RPE65 gene and had been blind since birth. Gene therapy with recombinant adeno-virus carrying wild-type RPE65 gene restored vision to the treated dogs. Recently the same technique was used to treat three adult patients with Leber's congenital amaurosis caused by mutations in RPE65 gene (Maguire et al., 2008). Although the normal vision was not achieved, this study provides the basis for further gene therapy studies in young patients with LCA.

Considering the severe consequences of RPE65 mutations, we found it necessary to study the Polish briard population.

MATERIAL AND METHODS

Blood samples were collected in co-operation with the breed club from 51 pedigree briards (26 males and 25 females) from the whole country. All dogs belonged to 16 families, stemming from 25 studs (7 of them living actually in Czech Republic) and can be considered a representative group. It originates from the Czech and Slovakian lines and also from several imports brought directly from France.

Genomic DNA was extracted from peripheral blood according to standard protocol. DNA concentration and purity was evaluated with Genequant spectrophotometer (Pharmacia).

Polymerase-chain reaction (PCR) was performed in Trio-Bloch thermocycler (Biometra) in 0.5 ml Eppendorf-type tubes. The following components were

used: 5 µl of *Taq* DNA polymerase (10x) buffer (SIGMA), 200 µM dNTPs (Ter-Pol), 20 pmol of each primer (TIB MOL-BIOL), 1 µg DNA (genomic DNA) and 2.0 U *Taq* DNA polymerase (SIGMA). Reaction volume was adjusted to 20 µl with de-ionised water. RPE65-1 and RPE65-3 primers were used in PCR reaction (1) and the reaction itself was run under the established amplification conditions : (94°C/3 min; 61°C/1min; 72°C/3 min) × 1; (94°C/30s; 61°C/1 min; 72°C/1 min) × 30 ; (94°C/30 s; 61°C/1 min; 72°C/5 min) × 1.

PCR reaction product was tested by 10% polyacrylamide gel electrophoresis in Dcode™ Universal Mutation Detection System (BioRad) vertical electrophoresis set in 1xTBE buffer at 15°C and 300 V voltage for 2 hours.

SSCP analysis was performed for all dogs studied. Amplification products after denaturation were separated with electrophoresis in native 12% polyacrylamide gel in the same set in 1xTBE buffer at 10°C, 300 V and 40 W for 2.5 hours. After electrophoretic segregation gels were stained with silver. DNA fragments length was analysed with the use of ScanPack 3.0 program (Biometra).

PCR products were cloned in pGEM-T Easy vector (Promega). Selected clones, after plasmid DNA isolation using standard techniques (Sambrook et al., 1989), were sequenced using the Sanger dideoxy chain termination method. Cycle sequencing reactions were performed using the thermo sequenase fluorescent-

labelled primer sequencing kit (Amersham Pharmacia Biotech) and Cy5 labelled universal primers. Sequencing reactions were run in 5.5% polyacrylamide gel and analysed with ALFexpress DNA sequencing system (Amersham Pharmacia Biotech).

RESULTS AND DISCUSSION

All samples from the Polish briards produced identical cleavage pattern as observed with normal samples, indicating all were negative (–/–) for csnb form of PRA. PCR reaction produced product of 107 bp length in all 51 cases. The length of the amplified fragment shows that

In order to find the possible mutations in gene sequence, PCR product were analysed for single strand conformation polymorphism (SSCP). Three different SSCP patterns (*a*, *b*, *c*) were found with frequencies at 0.157; 0.549 and 0.294, respectively. In males as well as in females the most frequent was pattern *b* (0.50 and 0.60, respectively), whereas pattern *a* remain the least frequent and was found in two females only (0.08) yet it was more frequent in males (0.23). Pattern *c* was found in 27% of males and 32% of females. Single strand conformation polymorphism (SSCP) of exon of the 5 RPE65 gene fragment is shown on Figure 1.

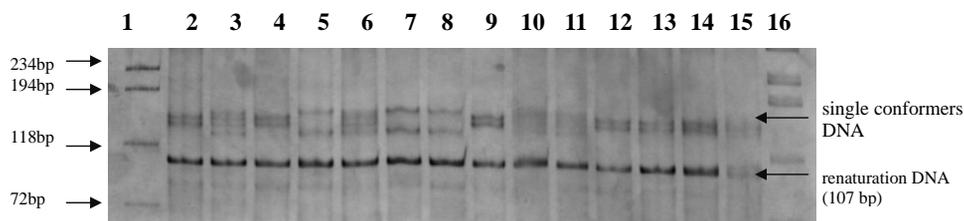


FIGURE 1. Electrophoretic segregation in 12% native polyacrylamide gel of the studied RPE65 gene single conformers DNA (lines 1 and 16 – marker Φ X 174 HaeIII, lines 5, 7, 8 – sequence *a*, lines 2, 4, 9, 12–15 – sequence *b*, lines 3, 6 10, 11 – pattern *c*)

no 4 bp deletion occurred in the RPE65 exon 5 (485del AAGA). Such deletion was found in the Swedish briard population (Aguirre et al., 1998; Veske et al., 1999). Other authors, having studied 26 breeds of dogs known for the incidence of PRA, did not find such deletion, either (Dekomien and Epplen, 2003).

After sequencing DNA samples we found that *c* pattern specimen were heterozygous and combined *a* and *b* pattern. We did not detect much single nucleotide polymorphism (SNPs) of the RPE65 gene. The only difference found between the two studied DNA sequences (SSCP *a* and *b* patterns) was restricted to 459 nucleotide (T459C) of the RPE65 gene (Fig. 2).

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Seq.a: 1 caatgcacctgttaatgtctaccagtaggggaagattactatgacctgcacggagaccaa 60
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Seq.b: 1 caatgcacctgttaatgtctaccagtaggggaagattactacgacctgcacggagaccaa 60

Seq.a: 61 cttcattacaagattaatcctgagaccttggagacaattaagcagg 107
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Seq.b: 61 cttcattacaagattaatcctgagaccttggagacaattaagcagg 107
    
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FIGURE 2. Comparison of *a* and *b* studied sequences

The identified fragment of amplified DNA was confirmed to be identical with RPE65 by direct sequencing of the PCR product, and was subsequently compared with previously published homologous sequences using BLAST service. It was found that both sequences (*a* and *b*) differ from the ones published previously for *Canis familiaris* in GeneBank in 432 (T432C) and 505 (T505C) nucleotides of the RPE65 gene (Fig. 3). Additionally, the difference was found in exon 5 459 (T459C) nucleotide of *a* and AF084573.1, as well as in *b* (C459T) and NM_001003176.1, AJ506754.1, Y16567.1 sequences. Veske et al. (1999) in the Swedish briard population and Dekomien and Eppelen (2003) in their study on 20 different breeds found similar SNPs (T459C).

The above described differences in the studied fragment of RPE65 gene did not result, however, in any changes of protein product (N135N), (Y144Y) and (L160L), respectively (Fig. 4).

Sequence <i>a</i>	caatgcacctgttaatgtctaccagtaggggaagattactatgacctgcacggagaccaa 107 caatgcacctgttaatgtctaccagtaggggaagattactatgacctgcacggagaccaa 523 caatgcacctgttaatgtctaccagtaggggaagattactatgacctgcacggagaccaa 523
Sequence <i>b</i>	caatgcacctgttaatgtctaccagtaggggaagattactatgacctgcacggagaccaa 107 caatgcacctgttaatgtctaccagtaggggaagattactatgacctgcacggagaccaa 523 caatgcacctgttaatgtctaccagtaggggaagattactatgacctgcacggagaccaa 523
NM_001003176.1	caatgcacctgttaatgtctaccagtaggggaagattactatgacctgcacggagaccaa 107
AJ506754.1	caatgcacctgttaatgtctaccagtaggggaagattactatgacctgcacggagaccaa 523
Y16567.1	caatgcacctgttaatgtctaccagtaggggaagattactatgacctgcacggagaccaa 523
AF084537.1	caatgcacctgttaatgtctaccagtaggggaagattactatgacctgcacggagaccaa 107

FIGURE 3. Comparison of sequence of *a* and *b* sequences of RPE65 gene with those published in GeneBank

Sequence a:	1	DNALVNVYPVGEDYYACTETNFITKINPETLETIKQV	111
Sequence b:	1	DNALVNVYPVGEDYYACTETNFITKINPETLETIKQV	111
AAC72356:	130	DNALVNVYPVGEDYYACTETNFITKINPETLETIKQV	166
CAA76290.1:	130	DNALVNVYPVGEDYYACTETNFITKINPETLETIKQV	166
CAD45010.1:	48	DNALVNVYPVGEDYYACTETNFITKINPETLETIKQV	84

FIGURE 4. Comparison of aminoacid sequences of *a* and *b* sequences of RPE65 gene with those published in GeneBank

CONCLUSIONS

None of the dogs studied is either affected or carries the mutated RPE65 gene. The population studied represents about 80% of the key breeding stock animals in Poland. This population consists mainly of Czech and Slovakian bloodlines with some addition of direct French imports.

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Streszczenie. Polimorfizm genu RPE65 w polskiej populacji briardów. Analizowano częstość występowania mutacji genu RPE65 (delecja 4 par zasad w 5 eksonie) w polskiej populacji owczarka francuskiego – briard. Badaniami objęto grupę 51 psów (26 samców i 25 samic), która stanowiła około 80% populacji tej rasy psów w Polsce. Identyfikacja genotypu w locus RPE65, przeprowadzona z zastosowaniem metody PCR, wykazała, że u wszystkich badanych psów długość amplifikowanego fragmentu wynosiła 107 par zasad, co wskazuje na brak zmutowanego allelu (485delAAGA) w tej grupie zwierząt. W celu identyfikacji ewentualnych innych, nieopisanych dotychczas mutacji, zastosowano metodę PCR-SSCP. Zidentyfikowano trzy wzory konformatorów: *a*, *b* i *c*, występujące w badanej grupie psów z częstością odpowiednio: 0,157, 0,49 i 0,294. Na podstawie analizy sekwencji badanego fragmentu, przeprowadzonej metodą sekwencjonowania w automatycznym sekwenatorze, stwierdzono, że wszystkie próby DNA wykazujące wzór konformacyjny *c* były heterozygotami złożonymi ze wzorów *a* i *b*. Oba wzory *a* i *b* różniły się w 459 zasadą w 459 nukleotydzie (T459C) genu RPE65. Analiza porównawcza homologii sekwencji eksonu 5 genu RPE65 w badanej populacji briardów z sekwencjami zarejestrowanymi w GeneBank wykazała różnice typu SNP w nukleotydach: 432 (T432C), 459 (T459C) i 505 (T505C). Różnice te nie powodowały jednak zmiany sekwencji aminokwasowej białka kodowanego przez gen RPE65.

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Effect of egg storage temperature on uniformity of embryo development during the first day of incubation and hatchability in Japanese quail (*Coturnix japonica*)

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Abstract: *Effect of egg storage temperature on uniformity of embryo development during first day of incubation and hatchability in Japanese quail (*Coturnix japonica*). A total of 358 incubating embryos and 575 chicks produced by 36 Japanese quail parental pairs were used to determine the effect of temperature applied during short-term storage on uniformity of embryo development after 24 h of incubation and on hatchability. Embryos from eggs laid during the night reached at average the higher level of development (4.81 points) than from day deposited eggs (4.34 points), but differences were not statistically confirmed. This insignificance could result from lack of ability to apply a proper time scale to deal with eggs that were laid at night. No impact of investigated storage temperature on hatchability was found. Nevertheless, significant differences between the lines and nests occurred.*

Key words: storage temperature, incubation, development alignment, embryo, hatchability, Japanese quail.

INTRODUCTION

One of the most important economical factors in poultry production is ability to obtain brood in forecasted time with uniformity of chick hatching. The initial stage and the rate of embryo development during incubation can have an impact on the whole hatching process.

According to studies by various authors (Romanoff, 1960; Buttler, 1991), the temperature above which embryogenesis starts, called ‘physiological zero’, is 20–28°C in poultry. Placing the eggs in the lower temperature immediately after laying causes total cessation of blastoderm divisions and provides the baseline for initiation of development upon subsequent incubation.

The aim of this experiment was to determine the influence of temperature used in short-term period of egg storage on progress and variability of embryo development after 24 hours of incubation and on reproductive traits in Japanese quail.

MATERIAL AND METHODS

The material comprised three lines of Japanese quails: K1, K2 and K3, kept separately from 10 generations. Within each line there were 16 parental pairs randomly mated using the computer program developed in Department of Genetics and Animal Breeding, which allowed to minimize the inbred level (Olech and Michalska, 1990). In current studies there

were created 12 nests (parental pairs) within every line. Both experiments (1 and 2) were based on eggs obtained from nests represented by the same quails.

Both males and females used for reproduction were at the age of 8 months. Day light length (14 hrs/day) and temperature (24°C) were controlled in the breeding facility. Incubation was carried out in incubator with automatical egg turning every hour, in temperature of 37.8–37.9°C and 65% of relative humidity.

Experiment 1: Embryo development after 24 hours of incubation

Studies were carried out on 358 Japanese quail embryos obtained from 36 nests. All eggs were collected every 1–2 hours during the day and stored in 16°C ($\pm 1^\circ\text{C}$) for not longer than 24 hrs. Eggs laid during the night were treated as a separate group, because they were not collected and in consequence staying in the room temperature for a longer period (up to 10 hrs). Incubation was carried out for 24 hrs.

Observations of embryos were conducted using 25 \times magnifying binocular. To estimate a level of embryo development (stage) an original morphological evaluation point system was applied (Grzegorzółka and Michalska, 2005). This system was based on the Japanese quail development classification by Zacchei (1961), build on earlier work regarding chicken embryo development by Hamburger and Hamilton (1951).

Experiment 2: Hatchability

This experiment was based on 575 set eggs obtained from 36 nests. The observation of reproductive traits was performed in 3 weekly hatches: I – eggs collected 2–3 times per day and stored up to 7 days with no cooling during storage period (stored in breeding facility temperature); II and III – proceeding with eggs was the same as in the experiment 1, eggs were stored in 16°C ($\pm 1^\circ\text{C}$) up to 7 days.

All eggs were incubated for 17 days. The eggs were tested for fertility on the 14th day of incubation by candling (the number of eggs with developing and dead embryos). The hatchability was expressed as the per cent of chicks hatched to the fertile eggs and chicks hatched to all eggs set.

Statistical analyses of embryo development and hatchability were carried out using SPSS 12.0 PL package. The model of analysis in the case of the two experimental factors included the effect of the egg storage temperature ($i = 1, 2$; in experiment 1) or hatch ($i = 1..3$; in experiment 2), line ($j = 1..3$), nest within the line ($k = 1..12$) and interaction between storage temperature and line.

The post hoc methods in hatchability analysis were used to evaluate significance of differences between any two pairs of lines' means. Logistic regression applied in the second experiment analysis included the hatch and line effects.

RESULTS AND DISCUSSION

Embryos from eggs laid during the night reached at average the higher level of development (4.81 points) than from day deposited eggs (4.34 points) after the 24 hours of incubation. The variability of development level of embryos from eggs laid during the day was little higher than of eggs laid in the night ($SD = 0.871$ vs 0.818 points) (Tab. 1). Nevertheless, no relationship between the level of early embryo development and investigated temperature was statistically confirmed ($p = 0.085$). These results differ from conclusions presented by Stepińska and Olszańska (1983). In studies on quail eggs conducted by these authors, at the time of laying egg the blastoderm consisted of 32.500–46.250 cells. After several hours of storage at room temperature, the number of cells increased to 47.200–55.800 significantly.

In present study an influence of genotypes of lines ($p = 0.042$) and parental pairs ($p < 0.001$) on embryo development observed after 24 hours of incubation was proved. No interaction between line and storage temperature was found ($p = 0.641$).

Table 2 presents the number of hatched quail chicks, mean values (\bar{x}) and standard deviations (SD) of investigated reproductive traits for each hatch and each line.

Analysis of variance showed the significant line effect ($p = 0.036$) and highly significant effect of nests within lines ($p < 0.001$). The post hoc analysis revealed significant differences ($p = 0.042$) in hatchability only between lines K1 and K3, both in Scheffe test and Bonferroni test.

The significant effect of hatch ($p = 0.039$) was proved in analysis of variance and lack of line \times hatch interaction occurred ($p = 0.444$).

TABLE 1. Number of observations, mean (\bar{x}) and standard deviation (SD) of stage of embryo development after 24 hrs of incubation for both storage conditions and each line

Storage conditions	Line	Number of embryos	Stage 24 h [points]	
			\bar{x}	SD
24/16°C (night deposited eggs)	K1	39	4.87	0.758
	K2	33	5.07	0.662
	K3	23	4.34	0.943
Total		95	4.81	0.818
16°C (day deposited eggs)	K1	107	4.43	0.720
	K2	78	4.55	1.023
	K3	78	4.01	0.807
Total		263	4.34	0.871

TABLE 2. Number of hatched quail chicks, mean (\bar{x}) and standard deviation (SD) of fertility and hatchability (chicks hatched to the fertile eggs and chicks hatched to all eggs set) for each hatch and each line

Hatch	Line	Number of chicks	Fertility		Hatchability			
					hatched /fertile eggs		hatched /set eggs	
			\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
I	K1	64	0.850	0.359	0.941	0.237	0.800	0.402
	K2	51	0.861	0.348	0.823	0.385	0.708	0.458
	K3	36	0.844	0.366	0.667	0.476	0.562	0.500
Hatch I total		151	0.821	0.356	0.821	0.385	0.699	0.460
II	K1	52	0.820	0.386	0.812	0.393	0.667	0.474
	K2	28	0.745	0.441	0.800	0.406	0.596	0.496
	K3	31	0.808	0.398	0.738	0.445	0.596	0.495
Hatch II total		111	0.797	0.404	0.787	0.411	0.627	0.485
III	K1	53	0.851	0.359	0.930	0.258	0.791	0.410
	K2	43	0.833	0.376	0.860	0.350	0.717	0.454
	K3	36	0.854	0.356	0.766	0.428	0.654	0.480
Hatch III total		132	0.846	0.362	0.857	0.351	0.725	0.448
Line K1 total		169	0.840	0.367	0.894	0.308	0.751	0.433
Line K2 total		122	0.821	0.384	0.830	0.377	0.682	0.467
Line K3 total		103	0.836	0.371	0.720	0.450	0.602	0.491

Logistic regression showed the significant line effect ($p = 0.002$), that confirmed the result of analysis of variance. Nevertheless, no relationship in hatchability between the hatches was statistically confirmed. Lack of ability to apply to the logistic model the interaction of line \times hatch and nests within line factor could be the reason.

In the III hatch, there was in average the highest ratio of fertilized eggs and hatched chicks while in the II hatch all these reproduction parameters were the

lowest. In both hatches, as opposed to the first hatch, eggs were stored in reduced temperature.

CONCLUSIONS

1. The influence of storage temperature on level of embryo development after 24 hrs of incubation was not statistically confirmed, but there were some differences that could occur to be important in the influence of various factors verification on the basis of early embryos stage.

2. No significant impact of storage temperature on level of early embryo development could result also from lack of ability to apply a proper time scale for dealing with eggs that were laid at night.
3. No impact of investigated storage temperature on hatchability was found. Nevertheless, significant differences between the lines and nests occurred.

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Streszczenie: Wpływ temperatury przechowywania jaj na wyrównanie rozwoju zarodkowego w pierwszej dobie inkubacji oraz wyniki lęgu przepiórki japońskiej (*Coturnix japonica*). W pracy wykorzystano łącznie 358 zarodków i 575 piskląt pochodzących od 36 par hodowlanych przepiórki japońskiej celem określenia wpływu temperatury zastosowanej podczas krótkotrwałego przechowywania jaj na wyrównanie rozwoju zarodków po 24 h inkubacji i wyniki lęgu. Zarodki z jaj zniesionych w ciągu nocy osiągnęły przeciętnie wyższy stopień rozwoju (4,67 pkt.) niż z jaj zniesionych w ciągu dnia (4,35 pkt.), jednakże różnice nie zostały potwierdzone statystycznie. Mogło to wynikać z braku możliwości utworzenia skali czasowej dla jaj zniesionych w ciągu nocy. Nie stwierdzono wpływu temperatury przechowywania jaj na wyniki lęgów. Istotne były natomiast różnice między liniami i gniazdami.

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The comparison of behaviour in two macropodid marsupials: red kangaroo *Macropus rufus* and red-necked wallaby *Macropus rufogriseus* kept in adjacent enclosures at Warsaw ZOO

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Abstract: *The comparison of behaviour in two macropodid marsupials: red kangaroo *Macropus rufus* and red-necked wallaby *Macropus rufogriseus* kept in adjacent enclosures at Warsaw ZOO.* Keeping two groups of macropodid marsupials in adjacent enclosures at Warsaw ZOO created an opportunity to compare their behavior. Red kangaroo (*Macropus rufus*) and red-necked wallaby (*Macropus rufogriseus*) are two species common in zoological gardens which in the wild are crepuscular and nocturnal. The observations were carried out in November 2006 – July 2007 period. They lasted for 14 days and 100 hours. The nearly full spectrum of behavior was recorded and the differences between species in resting, eliminative, comfort, agonistic and reproductive behavior was ascertained. Red-necked wallaby kept at Warsaw ZOO turned out to be animal active in the daytime. It seemed that as a whole the red kangaroo was less active. In the reproductive behavior copulation and the birth were not recorded. On the basis of observations it was not possible to determine the type of social organization of kangaroo and wallaby (cohesion and subtle relations between individuals). It should be the subject of further investigations.

Key words: Red kangaroo, red-necked wallaby, behaviour, ZOO.

INTRODUCTION

Red kangaroo (*Macropus rufus*) and red-necked wallaby (*Macropus rufogriseus*) are two marsupial species quite common in zoological gardens. The main differ-

ence between them is body size, type of habitat and ecological niche. Red-necked wallaby needs some shrubs for cover while the red kangaroo is dweller of open grasslands. The ecological status of red-necked wallaby is similar to leporids, on the contrary, red kangaroo fits well rather in great herbivores group. The type of activity of both species is crepuscular and nocturnal. Both species in the wild seem to be also well adapted to environmental changes and accustomed to the man presence. Red-necked wallaby actually lives in Great Britain as a feral species (Nowak, 1999; Yalden, 1999).

There is some discussion concerning social organization in kangaroos. It was described as rather loose. Kind of dominance hierarchy was sometimes established in males or females wallabies. In the red kangaroo small group with one adult male was typical structures. However, the greater aggregations of kangaroos particularly near the water holes and solitary individuals as well were also observed (Nowak, 1999; Ganschlosser, 2000).

At Warsaw ZOO both marsupials are kept in adjacent enclosures. Their beha-

viour could be observed simultaneously. Then, there was unique opportunity to compare their behaviour under given environmental conditions. In the recent literature the information concerning comparative behaviour of captive marsupials is rather sparse. It was interesting also taking into account that red-necked wallaby may be seen as perspective pet animal in Europe and the red kangaroo is also very popular in zoos and various animal parks.

In this paper only the behaviour of two macropodid marsupial species was described. Quantitative analysis of various factors which effect on marsupials behaviour will be presented later.

MATERIAL AND METHODS

In the observation period there were two groups: 13 red kangaroos and 12 wallabies on display at Warsaw ZOO. They were kept in the rectangular enclosures fairly well enriched (grass, trees, stones, and a pool in the case of red kangaroo).

The data concerning individuals history and demography were available only in the case of the red kangaroo (Tab. 1). In red – necked wallabies group there

were 3 adult males, 5 females and four joeys in various age. In wallaby females there were two handicapped individuals: one blind, another anorectic probably recovering after disease.

Taking into account specific European conditions under which lived marsupials the authors tried to cover during research period summer and the winter as well. Thus, the observations were carried out for 14 days from November 2006 to July 2007. Single observation lasted for 2–10 hours (10 am – 6 pm) Total time of observation was 100 hours: 80 in summer and 20 in the winter.

The weather condition was also recorded in each observation as a potential cause of marsupial activity. However, effect of environment on the macropodid marsupials behavior will be discussed in the other paper.

The method was direct ad libitum observation of marsupial enclosures from various points enabling better recording of behavior. The works of Dawson (1995) and Curtis (2006) were basic source of information to identify and describe behaviour of wild kangaroos, The classification of marsupials behavior observed in this study was based on Kaleta (2003).

TABLE 1. Short characteristic of red kangaroo group observed at Warsaw ZOO

No of individuals	Sex	Born – Year	Born-Place	Transfer to the Warsaw ZOO
2	Female	1998	Warsaw	–
1	As above	2001	As above	–
1	As above	2004	As above	–
3	As above	2005	As above	–
1	As above	2006	As above	–
1	Male	1999	Riga (Latvia)	2004
1	As above	2004	Blackpool (UK)	2006
1	As above	2005	Warsaw	–
1	As above	2006	Warsaw	–

RESULTS AND DISCUSSION

The individuals of both species kept in Warsaw ZOO showed following main types of behavior : resting/sleeping, locomotory, comfort, eliminative, feeding, anti predatory, exploration, agonistic and reproductive behavior, In individuals of both species no sign of territorial behavior and very weak signs of social hierarchy. These findings are rather consistent with literature (Dawson, 1995; Nowak, 1999). In the Table 2 short description of behavior and main differences between species were shown.

The whole activity of red kangaroo and red-necked wallaby observed at Warsaw ZOO was rather different. Wallaby as nocturnal species turned out to be active in daytime. There was no tendency to seek shelter like in the wild (Curtis, 2006). Moreover, wallaby behavior was quite complex. On the other hand, in the life of the red kangaroo resting in daytime played important role just like in the wild (Russell, 1971). There were pronounced periods of resting in red kangaroo particularly in the middle of the day. The peak of feeding activity in red kangaroo in afternoon-evening hours also fitted

TABLE 2. The Comparison of behavior types and forms shown by red kangaroo and red-necked wallaby at Warsaw ZOO

Type of behavior	Red kangaroo	Red-necked wallaby
1	2	3
Resting/sleeping	Recumbency – sternal position Side position	Recumbency – on the back position Side position
Locomotory behavior	Hoping, “crawl-walking” (Penny, 2002)	
Comfort behavior	Scratching self, grooming, licking, head, genitals (male) and pouch (female), yawning, rolling	Scratching self, grooming, licking, head, (more frequently resorted) genitals (male) and pouch (female), stretching, yawning, stretching
Eliminative behavior	In females appeared also in resting position	Visible also during hoping In females appeared also in resting position
Feeding behavior Food	Predominately grass, sometimes leaves, twigs	Tendency to intake wider range of foods like fruits even mushrooms
Feeding activity distribution in summer	One peak of activity in afternoon and evening hrs (5–6 pm)	Activity more evenly distributed with the small peak around 1–2 pm
Body posture during feeding	Four-legs posture, hunching down, laying on the side, sometimes erect posture, paws use	Four –legs posture, laying on the side, sometimes erect posture (rarely seen), paws use
Anti predatory behavior	Rarely seen; flight and alert posture; sudden loud noise and disturbance by approaching persons were common causes	
Exploration	Approaching and sniffing new objects (especially in young kangaroos)	Rarely recorded; approaching and sniffing

TABELA 2, cd.

1	2	3
Agonistic behavior	Threatening “stand up” posture, boxing, kicking, flight, whole sequences just as was described in literature (Curtis, 2006)	Rarely observed; in females chasing blind individual which unintentionally provoked the others, aggressive vocalization (growling)
Reproductive behavior Courtship	Male: following female, poking female with a paw, sniffing anogenital area and urine, flehmenr, licking her back, head and anogenital area; female avoid contacts when not ready	Male assistance, Mutual inspection of anogenital area, male “undulates” with the tail chasing female by male, no poking
Copulation	Not recorded	Not recorded
Pre parturition period	Not recorded	Cleaning pouch and anogenital area, adopting birthing position with the tail pointing between the legs
Parental care	Licking pouch, licking and grooming young	Licking pouch, licking and grooming young

well in this pattern. On the contrary, red necked wallabies showed even distribution of this activity during daytime. Specimens of both species foraged freely in the enclosure using various accessible plants but showing preferences to their natural feeding niches. From ethological point of view there were some interesting differences between both macropodid marsupials in resting, eliminative, comfort and agonistic behaviour. Since ethological studies of captive kangaroos and their relatives in the recent literature are rather sparse it was not possible to compare these findings.

On the basis of observation in Warsaw ZOO it could be said that the signs of dominance status in kangaroo and wallaby were rather weak and were revealed in the time of an aggressive confrontations. In red kangaroo the threat and fight displays in males were visible only in reproduction context. In the result of rivalry the male body size was probably important

factor. Biggest male monopolized mating at Warsaw ZOO. In red-necked wallaby only chasing in female group was observed. It was difficult to find signs of linear hierarchy and submissive displays in these groups. Whether the groups of these species observed at Warsaw ZOO were really cohesive or rather more loose it should be confirmed by further observations.

Red kangaroo and red-necked wallaby seemingly paid little attention to surroundings of an enclosure. However, in red kangaroo vivid reactions to strong stimuli e.g. versus workers in enclosure were recorded. Barry and Croft (2004) argued that flight distance and vigilant behavior in reaction to humans is visible in kangaroo species even if they were accustomed to man's presence. They may still treat humans as predators. In the present study observed individuals of macropodid marsupials also seemed to be rather cautious in reaction to new inanimate objects.

The reproductive activity in mentioned species has taken place at Warsaw ZOO during study period. Most of kangaroos and relatives are able to breed all year round and usually have one young each year (Nowak, 1999). As was shown in Table 1, red kangaroo group at Warsaw ZOO consisted also of several individuals born there. The same is also in the case of red-necked wallaby (oral information). Therefore, it may be said that the local conditions in Poland were convenient for regular reproduction of mentioned species. In spite of this fact, the authors of present study were able to observe only some forms of reproductive behavior. The most conspicuous reproductive sequence at Warsaw ZOO was the courtship. In the case of red kangaroo this behaviour was very similar to described in the literature (Dawson, 1995). On the other hand, there were some differences in courtship between red kangaroo and the red-necked wallaby (e.g. mutual anogenital inspection absent in red kangaroo). Unfortunately, the cases of copulations in both species occurred beyond the scope of observation.

The authors had opportunity to observe some forms of pre parturition and parental behavior. In wallaby typical parturition posture was recorded but not parturition. Females of both species showed parental behavior as comfort acts directed to the growing joey.

The authors not identify any abnormal behavior in the red-necked wallaby. In the red kangaroo probably attempt to suckle lactating female performed by the other adult female was recorded.

CONCLUSIONS

1. The greater changes of the activity pattern in red-necked wallaby than in red kangaroo were observed at Warsaw ZOO. Red-necked wallaby showed the daytime activity there as opposed to the wild specimens.
2. In the both species making of ethogram of mentioned species at Warsaw ZOO turned out to be possible. Nine types of behavior were described. Apart from locomotory and anti predatory behavior some differences were recorded in the other types between kangaroo and wallaby.
3. The authors were not able to observe complete reproductive behavior of studied animals. Present observations were also non-conclusive as regards social structure in red kangaroo and red-necked wallaby kept at Warsaw ZOO. These drawbacks should be eliminated in further studies.

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Streszczenie: Porównanie zachowania się u dwóch kangurów *Macropus rufus* i *Macropus rufogriseus* utrzymywanych na sąsiadujących ze sobą ekspozycjach w ZOO Warszawa. Utrzymywanie dwóch grup należących do różnych gatunków kangurów z rodzaju *Macropus* na sąsiednich wybiegach w ZOO Warszawa stworzyło okazję do porównania ich zachowania się. Kangur rudy (*Macropus rufus*) i walabia Benetta (*Macropus rufogriseus*) to dwa popularne w ogrodach zoologicznych

gatunki, które w naturze prowadzą raczej nocny tryb życia. Obserwację przeprowadzono w okresie listopad 2006 – lipiec 2007 przez 14 dni i 100 godzin. Odnotowano niemal pełne spektrum zachowań i stwierdzono różnice między gatunkami w behawiorze spoczynkowym, w wydalaniu, zachowaniach komfortowych, agonistycznych i rozrodczych. Walabia Benetta trzymana w ZOO Warszawa okazała się zwierzęciem wykazującym aktywność dzienną. Generalnie mniej aktywny wydawał się być kangur rudy. W zachowaniach rozrodczych nie odnotowano kopulacji ani momentu urodzenia. Na podstawie obserwacji nie można też było określić organizacji społecznej kangurów i walabii (spistość i subtelne relacje między osobnikami). Powinno to być przedmiotem kolejnych badań.

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The inbreeding influence on Hucul mares reproduction results

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Abstract: *The inbreeding influence on Hucul mares reproduction results.* The Hucul horses, native breed of Carpathians, are valued for their strongly consolidated features, like high fertility, fecundity and foaling rate. The relation between the inbred and the reproduction results of Hucul mares was analyzed using two-way ANOVA and one-way ANOVA with regression. The inbreeding level is negatively correlated with fertility and fecundity. Increase of inbred level of 1% causes loss of 0.98% fertility and loss of 1.03% fecundity. Average fertility was equal to 88.5% and fecundity 86.2%. There is no relation between inbred and foaling rate. The average foaling rate was very high, equal to 98.1%.

Key words: fertility, fecundity, foaling rate, Hucul horses, inbred.

INTRODUCTION

The Hucul horses is a native breed in mountainous environments. Typical for this breed is very high fertility, fecundity and foaling rate. The population of this breed is small and closed, so the inbreeding depression could be a problem. The aim of this paper is the analysis of inbred influence on reproduction results of Hucul mares.

MATERIAL AND METHODS

The relationship between inbreeding coefficient of mares and their reproduction results (fertility, fecundity and foaling

rate) was studied. The analysis included mares born between 1934 and 2003 which ended their breeding career (288 heads). The fertility is proportion of pregnancies to servings in mare's full time use in breeding. The fecundity is proportion of deliveries to servings and the foaling rate is proportion of foals which survived up to 6 months of age.

The source of pedigree data was Polish Hucul Horses' Stud Book (volumes I–VII) completed with information from Hucul Horses Studs Gładyszów and "Skarbiec", as well as publications (Hackl, 1938; Holländer, 1938; Radvan, 2003a, Radvan, 2003b).

The inbreeding coefficient was calculated using the Quaas-Henderson method by own computer program written in Delphi 5.0. For purposes of this calculation the pedigree was analyzed down to the founders.

The two-way ANOVA and the one-way ANOVA with regression on inbreeding were used to analyze the inbred influence on reproduction results. In the two-way ANOVA model the inbred level was treated as factor and all animals were divided into groups according their inbreeding coefficient values. The

length of the inbreeding partition was equal to one standard deviation (0.04) and non inbred animals were included in first group. Within both models the time of birth was included but because of small number of horses the five years birth periods was used in both models. Mares were born between years 1945 and 2004, so 13 periods were analyzed.

RESULTS AND DISCUSSION

The value of inbreeding coefficient for almost half of the mares (136 out of 288) was very small – below 4%. The large part of mares are not inbred and all of them were born in the first period after World War II (Tab. 1).

It was noticed that the rise of inbred level causes decrease of fertility and fecundity. The fertility level fluctuated from 71.2% (for mares with inbred level above 12%) to 90.2% (mares with inbred level between 4% and 8%). The average fertility was equal to 88.5%. The fecundity fluctuated from 69.7% (mares with inbred level above 12%) to 87.4% (not inbred mares). The average value of fecundity was equal to 86.2%. The foaling rate was very high (98.1%) and similar for all inbred level group. The range of foaling rate started from 97.5% (mares with inbreeding value between 8% and 12%) to 99.3% (mares with inbred level above 12%) (Tab. 1).

MODEL 1

The birth period as well as inbred level had significant influence on fer-

tility and fecundity of mares. The third trait, foaling rate is not influenced by none of analyzed factors (Tab. 2).

Radomska et al. (1988) proved increase of sterile mares from 15% (with inbred level 0.1%–5%) to 20% (with inbred level above 5%). These authors did not notice relationship between the inbred level and the abortions rate. Budzyński et al. (2000b) in their studies on Wielkopolski horse showed that the inbred level was positively correlated with number of sterile mares, and the inbreeding coefficient above 1% had negative influence on fertility and fecundity of thoroughbred mares (2000a). Budzyński (2001) did not notice the influence on reproduction results of Arabians mares. Moreover he demonstrated that moderate inbred level is profitable for reproduction results. Jezierski (1993) proved that high inbred level, even higher than 10%, does not change the number of sterile Polish horse mares and the number of abortions. It is interesting that the inbreeding level below 10% has positive influence on the reproduction results. Mahon and Cunningham (1982) show that inbred does not influence reproduction results of thoroughbred mares. They explain lack of inbreeding depression as a result of low inbred level of analyzed mares (mean inbred coefficient was 0.01%).

None of the factors had influence on foaling rate (Tab. 2). The average foaling rate was very high (98.13%). This result wasn't surprising, because Gancarz et al. (2002) demonstrated

TABLE 1. The results of reproduction (fertility, fecundity and foaling rate) of Hucul horse mares divided into groups according to inbreeding coefficient level

Inbred level [%]	Fertility [%]		Fecundity [%]		Foaling rate [%]		Number of animals
	mean	s.d.	mean	s.d.	mean	s.d.	
0	89.2	18.0	87.4	18.8	98.3	6.4	80
(0-4)	89.4	14.2	87.4	15.9	98.1	6.6	136
(4-8)	90.2	16.9	86.4	18.7	97.7	8.9	50
(8-12)	82.3	31.7	78.6	34.2	97.5	7.9	11
> 12	71.2	33.5	69.7	34.9	99.3	2.1	11
All	88.5	17.9	86.2	19.3	98.1	6.9	288

TABLE 2. The mean square values of two-way ANOVA (Model 1) presenting the influence of inbred level and birth period of reproduction traits

Factor	d.f.	Fertility	Fecundity	Foaling rate
Birth period	12	743.97 **	837.04 **	17.99
Inbred level	4	756.34 *	1 112.99 **	13.08
Interaction	21	434.127	414.51	29.41
Error	250	299.183	352.56	51.21

* p < 0.05 ** p < 0.01

that the most frequent cause of death of Hucul foals kept without stable was being killed by wolfs. However, Gancarz (2001) noticed that rise of inbred level causes decrease of foaling rate of Małopolski horse mares.

MODEL 2

Using ANOVA with regression, it was noticed highly significant influence of inbred value on fertility and fecundity (Tab. 3). The increase of inbreeding level by 1% causes decrease of fertility by 0.98% and fecundity by 1.03%. The foaling rate was not related to inbreeding coefficient. Interesting is lack of relation between birth period and analyzed traits. The model with regression better determined the inbred influence. It's probable

that in two-way model there was some dependence between factors because inbred level and time are proportional.

Klemensdal and Johnson (1989) demonstrated for trotters in Norway that the increase of inbred level by 1% caused rise of abortion rate by 1.27%. Kownacki and Jaszczak (1968) showed decrease of fecundity by 0.13% per every percent of inbred level. Burzyńska (1991) found 0.06% inbreeding depression on still births within Polish Arabian horses.

For Hucul horses the influence of inbreeding on the foaling rate is not noticed. Within other breed the results were different, for example Burzyńska (1991) founded inbreeding depression for foaling rate within Polish Arab breed.

TABLE 3. The mean square values of one-way ANOVA (Model 2) and regression coefficient presenting the influence of inbred level on reproduction traits

Factor	d.f.	Mean squares			Regression coefficient		
		Fertility	Fecundity	Foaling rate	Fertility	Fecundity	Foaling rate
Inbred coefficient	1	2 540.31**	2 802.51**	30.64	-0.98	-1.03	0
Birth period	12	348.92	447.14	25.41			
Error	274	310.70	358.01	49.08			

* $p < 0.05$ ** $p < 0.01$

CONCLUSIONS

The increase of inbred level causes decrease of fertility and fecundity of Hucul mares (fertility decreased by 0.98% and fecundity by 1.03% per 1% increase of inbreeding). The average fertility level was equal to 88.5% and fecundity was 86.2%.

The inbreeding value does not influence the foaling rate. The average foaling rate was very high (98.1%).

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Streszczenie: *Wpływ inbrodu na wyniki rozrodu klaczy huculskich.* Konie huculskie charakteryzują się cennymi, silnie utrwalonymi cechami, w tym bardzo dobrą kondycją, żywym temperamentem, łagodnym charakterem, długowiecznością, doskonałym wykorzystaniem paszy i dobrym zdrowiem. Obliczono współczynniki inbrodu metoda Quassa-Hendersona przy pomocy własnego programu napisanego w Delhi 5.0. W celu przeanalizowania wpływu inbrodu na parametry rozrodu klaczy (źrebność, płodność i odchów źrebiąt) wybrano samice, których

kariera reprodukcyjna się zakończyła. Analizę przeprowadzono przy użyciu pomocy dwóch modeli analizy wariancji. W modelu pierwszym, dwuczynnikowej analizie, uwzględniono poziom inbrodu po podziale klaczy na grupy, zależnie od wysokości tego współczynnika. W drugim modelu współczynnik inbrodu był traktowany jako współzmienna. W obydwu modelach uwzględniono wpływ roku urodzenia klaczy. Analizy przeprowadzono przy użyciu pakietu statystycznego SPSS 12.50. Analizowane były rodowody i wyniki rozrodu 288 klaczy użytkowanych między 1945 a 2004 rokiem. Stwierdzono, że inbred wpływa wysoko istotnie na żrebność i płodność. Wzrost współczynnika inbrodu o 1% powoduje spadek żrebności o 0,98%, a płodności o 1,03%. Nie stwierdzono natomiast wpływu inbrodu na odchów źrebiąt.

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Verification of phylogenetic hypothesis concerning the evolution of genus *Bison*

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Abstract: *Verification of phylogenetic hypothesis concerning the evolution of genus Bison.* In our research we tried explain the high genetic similarity among modern Wisent and domestic cattle. The genetic material was taken from different species of *Bovidae* family including aurochs. The control region of mitochondrial DNA and the sequence of gene 16S rRNA were subject to analyses. To compare the species we estimated genetic distance and constructed phylogenetic tree. The high level of similarity between wisent and aurochs found in our work suggests that hybridization and crossing of wisents and aurochs must have had their place before domestication of the *Bos taurus* ancestor.

Key words: *Bison bonasus*, *Bovidae*, evolution, mtDNA, phylogenetic.

INTRODUCTION

The Genus *Bison* is nowadays represented by two species: European bison *Bison bonasus* (wisent) and American bison *Bison bison*. There is no unanimous theory about its evolutionary path. The separation of the *Bison* from family *Bovidae* has been defined as early as at the end of Tertiary period and took its place in South and East of Asia. In late Pliocene three forms of *Bison* i.e. *Bison sivalensis*, *Bison paleosinensi* and *Bison hanaizumiensis* were widely distributed in Asiatic regions of temperate climate

(Pucek et al., 2004). In Quaternary glaciations (Pleistocene) the *Bison* inhabited Asia and Europe. In the latter it was described as a small form called *Bison schoetensacki*, living in forest regions. During the first glaciations its sizes increased. In the middle of Pleistocene on the big area of Europe and Asia – from today's England to Mandzuria, the big, long horn and solid body species *Bison priscus* was described. At the end of glaciations period this species' sizes started to diminish, particularly in west of Europe and afterwards the short horn form *B. priscus mediator* was described, much smaller than Asiatic *B. priscus gigas* (Pucek et al., 2004).

There are four different theories on the origin of modern species' of this genus. The oldest one claims that in the 15 000–10 000 years B.C. the *B. priscus* became extinct without living any descendants. Thus the *Bison schoetensacki* having crossed the frozen Straits of Bering, due to allopatric speciation evolved in America into *B. bison*, and in Europe was the transient form to modern *B. bonasus* (Hilzheimer, 1910). Degerbol and Iversen (1945) have introduced the additional element to their theory of the

origin of modern *Bison*. They defined the transient form between *B. priscus* and *B. bonasus* called *B. bonasus arbustotundrorum*. Gromova (1965) derives *B. bonasus* from *Bison schoetensacki* through *B. priscus*.

However none of these theories assumes the contrarwise sequence of origin of both modern *Bonasmus* species as did Verkaar et al. (2004). According to him not *B. priscus* and *B. schoetensacki* but *B. bison* is the ancestor of *B. bonasus*. In the middle of Pleistocene approximately 500 000–250 000 years B.C. after crossing the Straits of Bering *B. antiquus* evolved to become the direct ancestor of *B. bison*. The latter one retreated many times into Caucasus regions living the *B. bonasus* as it's descendant. Verkaar et al. (2004) assumes also that during the speciation of both species the new European form of *B. bison* could integrate with inhabiting the same forest regions ancestors of cattle i.e. *Bos primigenius* – aurochs. In Holocene and historical time wisent ranged southern England and Sweden, whole central Europe down to Italian Alps.

The aim of our work was to verify the theory about introgression of genus *Bos* into *Bison* and to prove that hybridization between wisent and domestic cattle has not taken place again in modern times.

MATERIAL AND METHODS

The genetic material was taken from fourteen aurochs (*Bos primigenius*) and 259 wisents (*Bison bonasus*) which

were born in 1950–1970 (84 bones) and after 2000 (175 tissue). Scratches of aurochs bones were sampled from skulls belonging to collection of Department of Animal Anatomy from Poznań Agricultural University. One of the skulls from Holocene period was collected at Nowy Jaromierz. Information about age and place of collection of remaining aurochs's skulls was not available. The wisent old tissue samples (bones) were obtained in Department of Veterinarian Medicine at University of Life Sciences – SGGW in Warsaw. The whole blood from the animals born in the present time was taken after animals reduction in primeval Białowieska Forest.

DNA extraction

Approximately 5–10 g of bone meal was harvested with a wolfram drill under sterile conditions for DNA extraction and stored at –20°C. DNA extraction was carried out in a laminar flow hood. The DNA extraction was performed according to Kalmar et al. (2000) with minor modifications.

PCR amplification

The control region of mitochondrial DNA (375 bp/400 bp) and the sequence of gene 16S rRNA subunit (594 bp) were subject to analyses. The primers' sequences and terms of multiplex PCR reaction are shown in Table 1.

Electrophoresis and visualization of PCR product was carried out in 1.5% agarose gel (Sigma) dyed with Ethidynium

TABLE 1. The primers' sequences and terms of multiplex PCR reaction

NAME	SEQUENCE	HYBRIDIZATION TEMPERATURE	QUERY
16srRNAf	5' CCCGCCTGTTTATCAAAAACAT 3'	62°C	Ward et al 1999
16srRNAr	5' CCCTCCGGTTTGAACTCAGATC 3'	68°C	
ctrlRf	5' AGCTAACATAACACGCCCATAC 3'	64°C	
ctrlRr	5' CCTGAAGAAAGAACCAGATGC 3'	62°C	
ctrlRf'	5' CAGCATAACCCCCATACACA 3'	60°C	Nowak et al 2005
ctrlRr'	5' TATGCTGGTGCTCAAGATGC 3'	60°C	

bromide. For precise evaluation of fragments' obtained sizes the standard pUC-8Mix (ABO Fermentas) was used. The horizontal electrophoresis lasted 30 min at 60 V voltage. We were eluted fragments of interest from agarose gel. The purification of fragments (only of those of 375 bp) was done with the method using GFX PCR DNA and Gel Band Purification Kit (Amersham Bioscience).

Sequencing

The purified product PCR was subject to ligation with pGEM®-T Easy (Promega) vector and than, after multiplication in *E. coli* bacteria of JM109 strain and minilysis (Wizard® Plus Minipreps DNA Purification System – Promega) was sequenced by Sanger method (Sambrook, 2001) with usage of Thermo Sequenase Cy5 Terminator Cycle Sequencing Kit (Amersham Bioscience). The sequencing of fragments cloned was done in automatic sequencer ALFexpress II (Amersham Bioscience). In sequencing reaction the

universal, tagged primers pUC/M13 were used. For high voltage electrophoresis we used the denaturing 8% gel, polymerizing in UV light (ReproGel™ – Amersham Bioscience). Electrophoresis was carried with constant power of 25W and constant temperature 55°C during 6 h. Intensity of laser sampling was 2 sec.

Data and phylogenetic analysis

In comparative analysis done by MEGA (Kumar et al., 2001) program we used all fragments sequenced and sequences available from NCBI data base i.e.:

- 6 aurochs (*Bos primigenius*), U50946, U50945, AF336748, AF336747, AF336746, AF336745,
- 6 domestic cattle (*Bos taurus*), AB065127, DQ520589, AB085926, AJ871629, DQ020118, L27730, L27716,
- 2 zebu (*Bos indicus*), L27732, L27729,
- Yak (*Bos grunniens*), DQ007224,

- 3 wisents (*Bison bonasus*),
U34294, AF083356, AY428860,
- 3 American bison (*Bison bison*)
AF083364, AY428859, AF083366,
- hybrid wisent x domestic cattle
F₁ – material in disposal of Depts.
Genetic and Animal Breeding
(SGGW),
- hybrid American bison x domestic
cattle F₁ – material in disposal of
Depts. Genetic and Animal Breeding
(SGGW).

Genetic distance was evaluated by Tamura-Nei method which considers the rate and number of substitutions (Kumar et al., 2004). The different rate of nucleotide substitutions is caused by the fact that in evolution process transitions are more frequent than transversions.

RESULTS

As a result of multiplex PCR reaction one or two fragments of different length were obtained. The product of 594 bp (sequence of gene from 16S rRNA subunit) shows the control and amplifies at all species from *Bovidae* family. The second fragment – 357 bp theoretically should

amplify at domestic cattle, aurochs and hybrids with the two species mentioned only. The products of amplification are shown on Figure 1. The control fragment is present at all animals' samples. The second one as found at 62% of wisents which were born in year 1950–1970 and at 67.7% animals born after 2000. Because we found it necessary to compare all animals in whose samples

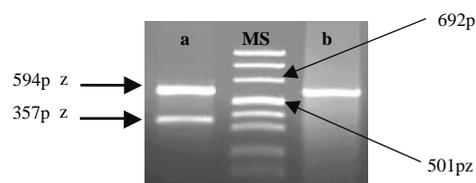


FIGURE 1. Product of multiplex PCR: a – full product of multiplex PCR, b – lack of region characteristic for *Bos taurus*, MS – mass standard

the primers for chosen control region did not hybridize to, we constructed the new pair of primers allowing to obtain longer (400 bp) fragment which include the sequence of interest.

We found four haplotypes for aurochs (4% differences) and six haplotypes for wisents (2–4% differences). The sequences of control region allowed the phylogenetic analysis for chosen species. Table 2 shows genetic

TABLE 2. Tamura-Nei genetic distance

	<i>B. bonasus</i>	<i>B. bonasus</i> *	<i>B. bison</i>	<i>B. taurus</i>	<i>B. bison</i> *	<i>B. grunniens</i>
<i>B. bonasus</i>						
<i>B. bonasus</i> *	0.042					
<i>B. bison</i>	0.142	0.126				
<i>B. taurus</i>	0.143	0.120	0.122			
<i>B. bison</i> *	0.146	0.123	0.124	0.002		
<i>B. grunniens</i>	0.144	0.121	0.107	0.178	0.179	

* – individuals with introgression or suspicious.

distance among domestic cattle, wisent, American bison and yak. It is worth mentioning that distance between domestic cattle and it's hybrids with bison is close to zero. The value of distance between these hybrids and pure bison amounts to 0.124. Distance between wisent in which the "cattle fragment" was amplified and domestic cattle amounts to 0.120. This value is comparable to those between other species of *Bovidae* (0.107–0.179). On the other hand the distance between two groups of wisents – those with "cattle fragment" and without amounts to 0.042 only.

The second step of the study was the comparative analysis of different species from *Bos* genus including aurochs – Figure 2. On the dendrogram we can see clear separation of Polish aurochs and those from British Islands. The wi-

sents subject to our analysis are in close proximity with aurochs. At the same time dendrogram clearly shows bison and wisent as two separated species fairly distant from each other.

On the last dendrogram (Fig. 3) we have generated haplotypes of species and hybrids compared previously. The dendrogram consists of two clades. One of them contains representatives of modern domestic cattle (European and Indian cattle) together with their hybrids. The second is formed by genus *Bison* together with species from genus *Bos* aurochs and yak.

In order to final verification of hypothesis that mutual crossing between wisent and aurochs but not with modern domestic cattle could have place, we constructed two more dendrograms, comparing wisent born in 1950–1970

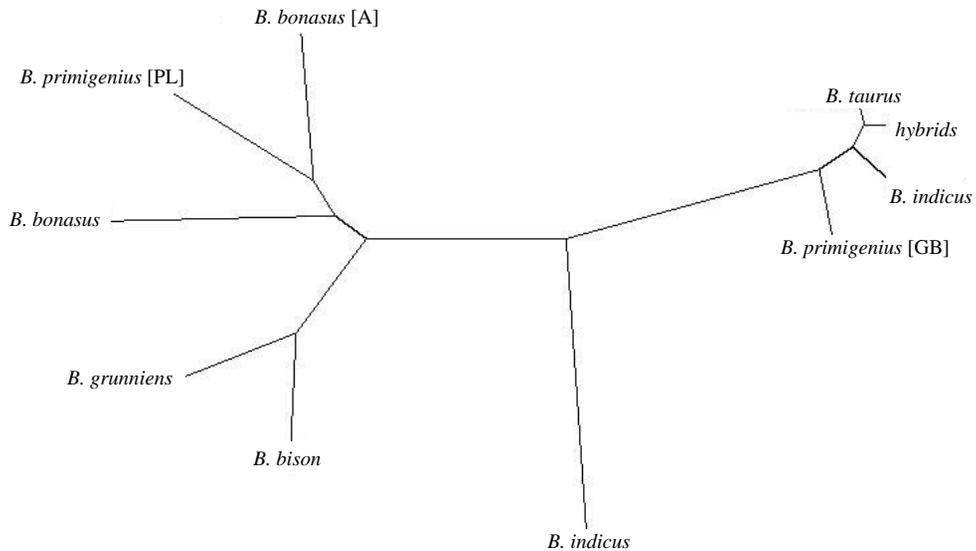


FIGURE 2. Neighbour joining radiance tree, constructed for different species from *Bos* genus

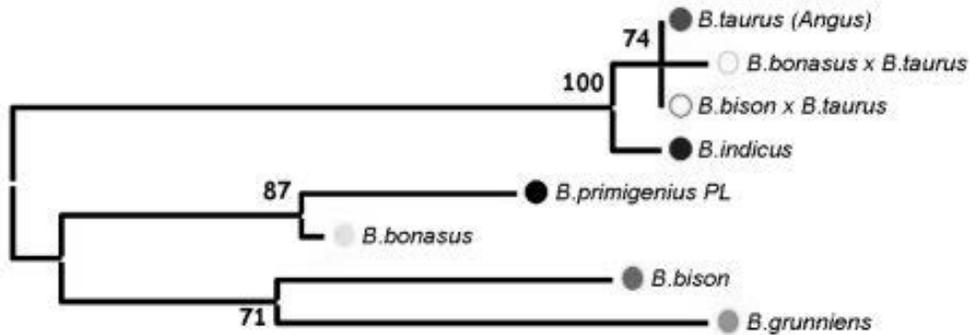


FIGURE 3. Minimum evolution phylogenetic tree

period and after 2001 with haplotypes of aurochsen found in Polish area only. We also included hybrids between wisent and modern domestic cattle. Both dendrograms (Figs. 2 and 3) show clear differences between haplotypes of hybrids and species compared. It is good visible that in two dendrograms aurochsen haplotypes are mixed uniformly with haplotypes of wisents. In each dendrogram haplotypes of hybrids are situated as tree roots (outbred species).

DISCUSSION

The evaluation of mtDNA variation within *Bovinae* family presented bison and wisent as two separate species. But the similarity of bison and yak was confirmed what was in accordance with results of Burzyńska et al. (1999). The similarity of wisent and domestic cattle (*Bos taurus*) was also revealed. Veerkar et al. (2004) stated that similarities of sequences within Y chromosome of wisent and bison are in accordance with their morphologic resemblance and ability

to produce fertile progeny. Like in our results Veerkar's group did not find similarity of mtDNA sequences of these species.

Inter species hybridization between *Bos taurus* and *Bison bison* has been well documented. Researches carried by Ward et al. (1999) proved occurrence of hybridization with usage of mtDNA sequences mentioned before. Halbert et al. (2005) using linked microsatellite markers found introgression of nuclear genome of domestic cattle in five of fourteen investigated bison populations. But the high level of similarity between wisent and aurochs found in our work suggests that hybridization and crossing of wisents and aurochsen must have had their place before domestication of aurochs. The process of hybridization was done by mating wisent males with aurochs female. Confirmation of this phenomenon can not be done by analysis of mitochondrial DNA and must be followed by analysis of nuclear DNA. Nowadays wisent in Europe can represent the parapatric speciation, in which

wisent as the representative of *Bison* genus is formed in population remaining in genetic contact with genus *Bos* in narrow zone of biotope convergence.

Additional evidence of the thesis that genetic similarity of wisent and domestic cattle is caused by wisent hybridization with aurochs not with modern cattle is the fact that representatives of *B. bonasus* and *B. bison* with documented hybridization pedigree are always placed on the phylogenetic dendrogram in close proximity of domestic cattle (Fig. 3). Haplotypes from remaining representatives of these species are placed far apart of them. Early hybridization of *Bison bonasus* and *Bos primigenius* can be the reason of big phylogenetic differences between Polish and British aurochs. We have no evidence that animals skulls found in excavations and hunting trophies collections from XVII century belonged to *B. primigenius* or to the backcrossing generation of hybrids between aurochs and wisent. Basing on the all results obtained in our study we can unambiguously state that most of the ancestors of modern wisents were intergrades with *Bos primigenius* but not with *Bos taurus*. It is possible that due to this introgression the limited genetic scope of modern *Bison* genus got considerable diversification.

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Streszczenie: *Weryfikacja filogenetycznej hipotezy dotyczącej ewolucji rodzaju Bison.* W naszych badaniach spróbowaliśmy wyjaśnić wysokie podobieństwo genetyczne współczesnych żubrów i bydła domowego. Genetyczny materiał pobrany został od różnych przedstawicieli rodziny *Bovidae* z uwzględnieniem turów. Analizom podlegały dwa odcinki mitochondrialnego DNA:

fragment regionu kontrolnego i podjednostka 16S rRNA. Na podstawie analizowanych fragmentów porównaliśmy gatunki i oszacowaliśmy dystans genetyczny oraz skonstruowaliśmy drzewo filogenetyczne. Wysokie podobieństwo żubrów i turów, jakie zostało wykazane w naszej pracy sugeruje, że gatunki te mogły się krzyżować, ale miało to miejsce najprawdopodobniej jeszcze przed udomowieniem bydła.

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Preliminary estimation of longevity for leopard and snow leopard populations

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Abstract: *Preliminary estimation of longevity for leopard and snow leopard populations.* Longevity is the main functional trait, very important in the conservation and restitution breeding programs. This trait is determined by both genetic and environmental effects. The objective of this study was to estimate genetic variance of longevity in leopard and snow leopard populations kept in the zoological gardens. The data were extracted from the International Species Information System (ISIS). The analysis included pedigree information of 1434 and 1256 individuals born in 1915–2003 years (respectively for snow leopard and leopard). The estimation of genetic variance was performed by the Animal Model, using the derivative-free restricted maximum likelihood algorithm. Decreasing genetic variance has been noticed over time in the both populations. It may affect the lifespan and survival in the analyzed populations. Results of this paper suggest that reasonable breeding programs should aid to increasing the genetic variability.

Key words: captive population, genetic variance, Animal Model.

INTRODUCTION

Reduction of the genetic variability is one of the most important threats limiting conservation breeding (Princee, 1998). Occurrence of the high genetic variability is immeasurably advanta-

geous for the process of reintroduction species. Because of the genetic disparity in the species, under environmental pressure individuals with high adaptation ability will be preferred (Crnokrak and Roff, 1999). Natural selection and mating in zoological gardens prefer generally genotypes easily adapted to peculiar environmental conditions. Although, knowledge of genetic variability is the one of the important elements, which can be used to evaluate usefulness of populations maintained in the controlled conditions for future reintroduction procedures (Sternicki, 2002).

Stabilization of genetic variability is one of the most important element for reintroduction of individuals, kept in zoological gardens under conservation of their gene pool procedures. For that reason, monitoring of genetic variability becomes a key element in populations. The aim of this paper was to estimate the genetic variance of longevity for leopard *Panthera pardus* and snow leopard *Uncia uncia*.

MATERIAL AND METHODS

The data were extracted from the ISIS (*International Species Information System*) data base. This base contain information about each individual in population (studbook number, birth and death date, number of sire and dam, transfers, etc.). Data set consisted records of 2080 snow leopards and 2452 leopards. Individuals of unknown death date were eliminated and in result 1434 and 1256 animals left, for snow leopard and leopard respectively. Table 1 presents the size of data and distribution of sexes within analyzed species.

TABLE 1. Number of animals according to species and sex

Species	Number of individuals	Number of		
		males	females	individuals with unknown sex
<i>Panthera pardus</i>	1434	563	619	252
<i>Uncia uncia</i>	1256	560	551	145

Because of the high postnatal mortality, the data set was divided into two subclasses: all individuals (Panthera1, Uncia1) and animals up to 31th day of the lifetime (Panthera31, Uncia31). Table 2 presents the average lifespan in the subsets, according to species and class of longevity.

The data set included long period (1915–2003), and number of records in particular years were relatively small, so random additive predicts were used in the genetic variance, defined as an average predictors in 3 year subperiods. High

TABLE 2. Average life length for analyzed data sets (in days)

Data	Number of individuals	Average life length (in days)	Standard Deviation
Panthera1	1 434	1 739	2 467.53
Panthera31	825	3 019	2 592.55
Uncia1	1 256	1 929	2 420.66
Uncia31	757	3 197	2 382.52

Panthera1, Uncia1 – all individuals, Panthera31, Uncia31 – individuals up to 31th day of life.

level of standard deviations of average lifespan in the analyzed group was caused by postnatal fatality and animals with long lifespan.

The genetic variance was estimated based on the Derivative Free Restricted Maximum Likelihood approach (DFREML) introduced by Smith and Graser (1986), numerically implemented by Meyer (1989).

An assumption of the animal model was used to construct the linear model of lifespan (Henderson, 1988). Known genetic and none-genetic factors were considered. Results of multivariate analysis of variation were used for creation of model because significant factors influenced into longevity were included as

fixed effects. There were: sex, birth date, place of birth, number of transfers, numbers of subspecies (in case of leopard) inbreeding coefficient (as covariable) and random additive genetic effect of individual. The coefficient of inbred (estimated using additive relationship matrix according to Henderson (1988)) was included into partial linear regression. The following linear model was applied:

$$y_{ijkmno} = \mu + s_i + p_j + g_k + t_m + m_n + bx_{ijkmno} + a_{ijkmno} + e_{ijkmno}$$

where:

- y_{ijkmno} – length of life (days) of ijkmo-th individual,
- μ – overall mean,
- s_i – the fixed effect of i-th sex,
- p_j – the fixed effect of j-th period of birth,
- g_k – the fixed effect of k-th subspecies group (for Leopard),
- t_m – the fixed effect of t-th numbers of transfer,

- m_n – the fixed effect of n-th place of birth (country),
- b – linear regression coefficient,
- y_{ijkmno} – the inbreeding coefficient of ijkmo-th individual (included as a covariable),
- a_{ijkmno} – random additive genetic effect of ijkmo-th individual,
- e_{ijkmno} – random error of ijkmo-th observation,

An assessment of the model accuracy was made on the basis of error variance value. Predictors of genetic effects, obtained from individuals grouped in three birth years periods, was estimated based on above-mentioned algorithm.

RESULTS AND DISCUSSION

Generally, changes of genetic variance level within analyzed data sets shown, that the shape of curves for both species is approximately similar. On Figure 1 fluctuation of longevity average

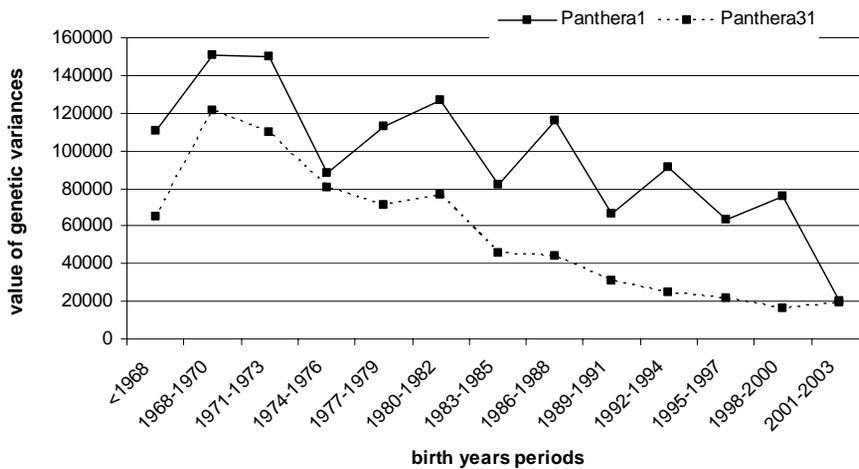


FIGURE 1. Fluctuation of genetic variance for leopard population

genetic variances in leopard population was shown. Curves of genetic variances depict a negative tendency. Larger values were noticed for all leopards (Panthera1), lower – for group which included the youngest animals with lifespan shorter than 31 day (Panthera31). Gradual decrease of genetic variances in time within every analyzed group was also observed.

The higher values with large fluctuation were noticed for all leopard (Panthera1), compared to the individuals that

lead to the reduction of genetic variability. No doubt, this was caused by no or smaller addition of new not related animals. However, transfer of some individuals between zoos in last period was not recorded, which contribute to new unknown breeding lines and gave small fluctuation of genetic variance of lifespan in the years.

Similar course, with decreasing of genetic variance in time, was observed for snow leopard (Fig. 2). Higher genetic variance, in the leopard population than

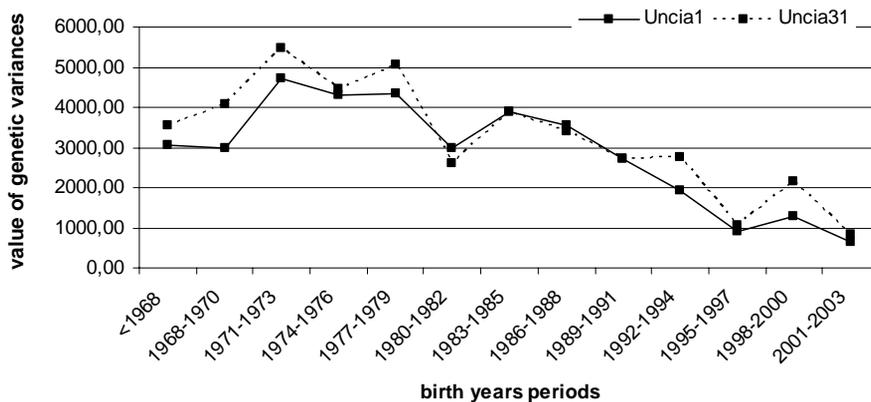


FIGURE 2. Fluctuation of genetic variance for snow leopard population

survived more than 31 days. Probably the large number of animals was the reason for slope change of the above-mentioned value. The high values of genetic variances in the first period of time (since 1970) were obtained due integration of new individuals from the wildlife. Probably, decrease of genetic variability after 1970 was caused by limited selection due to many relatives in population and

snow leopard, could be observed from disparity of researched variability trait, because of coexistence several subspecies in the same data base.

An increase of genetic variance was observed in snow leopard population in first few years (since 1971). After that period, gradual decrease followed. Maximum fluctuation was shown in 1980–1985 with simultaneous insignif-

ificant incrementation in 1996–2001. Big disparities in analyzed data for species were not noticed. Similar course of curve (insignificant change in scale) characterized animals from data set Uncia1, as well as Uncia31. Individuals survived more than 31 days are characterized by the highest value of genetic variance.

For both populations, during the analyzed period, it have been noticed a decrease of genetic variability. Both researched species are protected with international regulations (IUCN – The World Conservation Union and CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora) with prohibition of capturing the animals from their natural environments since 1975. In consequence of this low population maintained in the zoological garden, as small closed populations, are affected by increase level of homozygosity.

Incessant decrease of genetic variability seems to be a serious barrier in furthest breeding of the analyzed species due to erosion of gene pool which appears in populations kept in captivity for many years (Lacy, 1994).

From the point of view of conservation breeding, adverse individuals appear in population, because of decrease of adaptation (Lacy, 1993; Laurence and Smart, 2000) and also increase mortality (Crnokrak and Roff, 1999). In view of genetic variability reduction in analyzed populations in result is possible smaller capability of effective selection. This can

contribute to future breeding. Comparing studies carried over other species, leads to the conclusion, that loosing of genetic variability and increase of inbreeding makes many difficulties for reintroduction programs (Thomas, 1987; Lacy, 1993; Laikre et al., 1996; Frankham, 1997; Olech, 2003). Moreover, negative result of increase of animal's homozygosity and decrease of genetic variability were shown in many small population of mammals kept in captivity (Ryan, 2003). Taking into account animals from zoological gardens under assumptions that environmental conditions are rather stable, the decrease of genetic variance is influenced by inbreeding increase. However, in the populations in which from the long period are relative mating, the increase of population size is often achieved at the cost of genetic variability (Wright, 1920). It may seem, that individuals from zoological gardens are not suitable for reintroduction because their adapting ability to natural biotopes may been lost. Decrease of genetic variances (with environmental effects) influenced deterioration of survival traits (Lacy, 1993). Because of this fact, population of endangered species, which are kept in zoological gardens, maintained a main species gene pool. In case of genetic variability decrease in analyzed populations of leopard and snow leopard with decrease of population size, the future breeding situation seems to be difficult.

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Streszczenie Wstępne oszacowania długości życia populacji lamparta oraz śnieżnej pantery. Długość życia jest bardzo ważną cechą dla ochrony gatunków oraz programów hodowlanych i restytucyjnych. Determinują ją zarówno czynniki genetyczne, jak i środowiskowe. Celem tych badań było oszacowanie wariancji genetycznej długości życia dla dwóch populacji kotowatych lamparta oraz śnieżnej pantery, utrzymywanych w warunkach ogrodu zoologicznego. Materiał badawczy stanowiły rodowody zwierząt skatalogowane w Międzynarodowym Systemie Informacji o Gatunkach (ISIS). Baza danych zawierała informacje o 1434 oraz 1256 (odpowiednio dla śnieżnej pantery oraz lamparta) osobnikach. Szacowania wariancji genetycznej dokonano przy pomocy Modelu Zwierzęcia (Animal Model), stosując algorytm klasycznej estymacji punktowej, największej wiarygodności z ograniczeniem. Zaobserwowano zmniejszający się w czasie poziom wariancji genetycznej w obu populacjach. Może mieć to wpływ na przeżywalność analizowanych populacji. Wyniki tej pracy sugerują konieczność prowadzenia programów hodowlanych w taki sposób, aby zwiększać wariancję genetyczną badanej cechy.

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Morphological and behavioral analyses of the indoors hatched pond turtle *Emys orbicularis* in aspect of prognosis of it's future survivorship

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Abstract: *Morphological and behavioral analyses of the indoors hatched pond turtle *Emys orbicularis* in aspect of prognosis of it's future survivorship.* The clutches of pond turtle *Emys orbicularis*, which have not completed their incubation period were transferred to indoors enclosure to finish the development. Newly hatched turtles were monitored regarding to the body weight and carapace sizes. During each of 24 measurements the turtles were subject to Open Field Test in order to evaluate their mobility and courage. The coefficient of repeatability for OFT performance was calculated as well as the correlation between body measurements and mobility. The trial prognosis about the further prospects of turtles after restocking were suggested.

Key words: conservation *n* situ, *ex* situ, pond turtle, Open Field test, coefficient of repetability, correlations.

INTRODUCTION

One of the methods used in active conservation of European pond turtle *Emys orbicularis* in Poland is the transfer of unhatched eggs, at the end of the season, to the indoors enclosures in order to continue their incubation in artificial, warm enough conditions. Hatched turtles are kept through winter period in captivity and restocked next spring to the natural habitat (Mitrus and Zemanek 2004).

The beginning of such endeavors can be traced to late 90. when in the 1997–2001– 123 one year old turtles were released back. Nevertheless there are no exact data evaluating the efficiency of such activity (Mitrus, 2005). The experience gained in salmonids sea ranching (Larsson P-O, 1977a,b; Larsson H-O., 1977) showed how important is to know the right technique of releasing of captive raised material for it's eventual survival.

In our study we carried out the pilot attempt for prognosis the chances of survival of individual captive raised pond turtle, basing of their morphological and behavioral features.

MATERIAL AND METHODS

Two clutches of eggs (13 and 7 eggs respectively) were transferred from Okólny Ług Reserve (Kozienice Forest) in September 2005 to Warsaw ZOO Garden and placed in hatchery unit. Hatching process started at the end of September and lasted throughout the October resulting in 9 neonates (7 and 2 from both clutches respectively). The turtles

were kept in aquariums filled with water. Islands made of stones and moss enriched the microhabitats and Repti Glo 2.0 provided UV radiation. Hatchlings were fed twice a week at the beginning and every second day from their 2nd month of age.

Every week the turtles were weighed with the accuracy to the nearest 0.1 g (dried weight) and length, width and height of shell were measured with the accuracy to the nearest 0.1 mm.

The test of turtles' activity started at 9th November. Each turtle after taking the measurements mentioned above was placed in Open Field marked with squares (5 × 5 cm) for 5 minutes and the rout walked within that time was drawn to count the numbers of squares passed (Fot. 1, Fig. 1) thus scoring points: 1 for every square along the walls of Open Field area and 2 points for every square in the two middle rows (marked with solid lines – Fig. 1). Test were repeated 24 times.



FOTO 1. Open Field Test. The turtles were placed within the field and numbers of squares walked during each trial were recorded

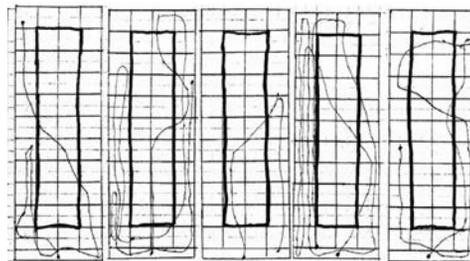


FIGURE 1. Some drawings of routs passed during observations. The solid lines within the fields mark squares scored with double points

The scores of the tests were subject to one way ANOVA according to model:

$$Y_{ij} = a_j + c_j + e_{ij}$$

a_i – the effect of i -th observation,

c_j – the effect of j -th turtle,

e_{ij} – interaction of j -th turtle and i th observation.

In order to normalize the distribution of the scores the $y = \arccos \sqrt{x}$ transformation was used where X was the sum of points of each turtle after trial before transformation.

DISCUSSION

The food chain relations imply big mortality of all it's elements, particularly at young stages. Chelonias are no exception. According to Janzen (1995) the survival rate of common snapping turtle *Chelyra serpentine* during first year amounts to 3%. Out of this just 17% reach 2nd year but since then, two years old turtles almost all reach sexual maturity (93%). So the first two years are essential for final result. The losses start already during incubation and can exceed 70% Congdin et al.(1993), after Janzen, Trucker,

Paukstis (2000b) are increased by foraging predators, including humans. After hatching the neonates have to reach the safe environment and disperse. The question is what factors do have the crucial influence on their survival success. Janzen (1995) focusing on TSD influence found positive correlation between body weight and shell length and width but no correlation between body sizes and survival rate. Interestingly author found negative correlation between propensity to run and survivorship, probably caused by more open exposition of active turtles for predators eyes. Janzen, Tucker, Paukstis (2000a) studied avian predators influence on survival during migration to water of red ear slider's *Trachemys scripta elegans* hatchling. They found positive effect of bigger sizes but when avian factor was eliminated, the bigger sizes became unimportant. They thus disagreed with conclusions of Congdom et al. (1999). Those authors did not find any relation between body size and successful migration at different distances neither between the body sizes and the place of releasing of the hatchlings of common snapping turtle. They concluded that for land predators the hatchlings' sizes are not the obstacle. Size factor can be important during confrontation with predator in shallow water (fish mostly). It corresponds with results of Gyuris (1999). Author found positive influence of body size of green turtle *Chelonia mydas* hatchling on survivorship during swimming across the

reef flat to deep water beyond the reef. Du and Ji (2003) investigating the influence of temperature on body sizes and fitness, understood here as swimming performance on racetrack, found positive correlation between sizes and fitness of soft-shelled turtle *Pelodiscus sinensis* hatchlings.

In our study connected with the strategy of active protection, realized by releasing the indoors hatched turtles, during next spring, we can expect better survivorship just due to bigger sizes of turtles. The animals released are few months older than those hatched naturally in late summer, and additionally were fed during winter period. But in the artificial condition we noted quite high mortality – just 5 turtles were restocked out of 20 eggs collected. Thus the indoors enclosures did not eliminate all reasons of mortality. The compensation of the losses is achieved by restocking the turtle directly to water and thus omitting the crucial land migration period.

From Table 4 we know that in our material we have found positive correlation between carapace length and width and mobility (measured in numbers of squares walked during OP test) but not with carapace height. There was also positive correlation between mobility and body weight. Nevertheless the values of coefficient correlation were not high, though highly significant. In Tables 1–3 we can notice that the heaviest turtles (no 3 and 9) have high ranking both in terms of mobility – propensity

TABLE 1. Final measurements of 5 turtles which have survived the captivity period

	Body weight [g]	Carapace length [mm]	Carapace width [mm]	Carapace height [mm]
\bar{x}	17.26	32.8	29.8	10.2
σ_x	3.9	0.2	0.1	0.1

TABLE 2. Measurements of turtles' mobility based on number of squares walked in Open Field test. Data for the 5 turtles which survived to restocking time

Ordinal no of each turtle Body weights [g]	Total number of squares walked during 24 measurements	Mean number of squares per one observation	Ranking based on total numbers of squares walked
2 (15.5 g)	478	19.9	4
4 (12.0 g)	876	36.5	3
5 (17.8 g)	297	12.3	5
3 (19.4 g)	908	37.8	1
9 (22.3 g)	906	37.7	2

TABLE 3. Evaluation of turtles' braveness based on number of points scored during walking on squares in middle rows of Open Field. Data for 5 turtles which survived to restocking

Ordinal no of each turtle Body weights [g]	Number of squares with "doubled" points	Percentage of "doubled" points in total score [%]	Ranking based on percentage of "doubled" points in total score
2 (15.5 g)	63	23.7	4
4 (12.0 g)	93	19.6	5
5 (17.8 g)	76	40.7	2
3 (19.4 g)	123	23.8	3
9 (22.3 g)	282	45.4	1

TABLE 4. Coefficient of correlations between carapace measurements and number of points scored in Open Field test (data for 9 turtles)

	Mean of 24 measurements	Standard deviation	Coefficient of correlation	Level of significance
Carapace length [mm]	2.787	0.345	0.646	0.000
Carapace width [mm]	2.564	0.337	0.570	0.000
Carapace height [mm]	0.933	0.107	0.318	0.059
Body weight [g]	12.519	3.043	0.505	0.000

TABLE 5. ANOVA results for number of points scored during 24 trials. Data for 9 turtles

Source of variation	df	MSS	EMSS
General	139		
Observations	23	0.738	$\delta^2e + n \delta^2a$
Turtles	8	0.569	$\delta^2e + q \delta^2c$
Interaction	107	0.145	δ^2e

δ^2a – component of variation for observations, δ^2c – component of variation for turtles, δ^2e – component of variation within turtles, n – number of turtles, q – number of observations, coefficient of repeatability $-r = \delta^2c / \delta^2e + \delta^2c$ (Zuk, 1975), $\delta^2a = 0.066$, $\delta^2c = 0.018$, $\delta^2e = 0.145$, $r = 0.11$.

to run according to Janzen (1995) and “braveness”, understood herewith as the percentage of points scored in the middle rows of Open Field. The smallest turtle (no 4) got the lowest braveness rank, though it was quite mobile and active (3rd position in Tab. 2).

Looking through the papers cited above we could predict that according to Congdon et al (1999) and Gyuris (1999) the turtles no 3 and 9 have the biggest chances to survive after releasing to water. Their activity – which would lower their chances (Janzen 1995) on the land migration phase is not the obstacle when they were released to water directly. In our study the mobility trait got very low coefficient of repeatability ($r = 0.11$) what was not with accordance with results of Bennett (1991) after Janzen (1995). Thus the rankings presented in Tables 2 and 3 may have not the constant tendency. So the smaller animals (here the turtle no 2 and 4) should be released in more dense water weeds area while the bigger ones could be put to more open water areas in order to help the dispersion.

CONCLUSIONS

- The highest prediction for success of survival of released turtles are for the bigger animals.
- The biggest turtles can be released to more open water areas (the borders of weeds), while the smaller should be provided with shelter of more dense water plants.
- The approach presented herewith should be repeated on more numerous material and connected with tagging of turtles released in order to confirm the conclusions concerning the restocking technique.

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Streszczenie: *Morfologiczna i behawioralna analiza sztucznie wyklutych żółwi błotnych *Emys orbicularis* w kontekście prognozy dla jego przetrwania w przyszłości.* W ramach pracy odchowano żółwie błotne *Emys orbicularis* pozyskane ze złóż jaj, które nie zakończyły inkubacji w gniazdach naturalnych i zostały doinkubowane sztucznie. Wyklute żółwki zostały mierzone 24 razy (masa ciała, pomiary pancerza) oraz poddane testowi aktywności i odwagi w Teście Otwartego Pola. Obliczono współczynnik powtarzalności dla aktywności w TOP oraz korelacje pomiędzy aktywnością a pomiarami merystycznymi. Na podstawie wyników przedyskutowano prognozy dotyczące losów żółwi na wolności.

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