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Colour of the hunters' clothing and the alertness in *Capreolus capreolus* (Artiodactyla: Cervidae)

Ostražitost srnce obecného (*Capreolus capreolus*) na barvu loveckého oblečení (Artiodactyla: Cervidae)

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Abstract. Under the so-called "hunter orange regulation", deer hunters in North America and Scandinavia are required to wear orange garment to maximize hunter's safety. It is argued that this practice does not negatively impact hunting success because deer are (assumed to be) red-green colour-blind. This assumption is based on the retinal immunocytochemical studies, yet the behavioural evidence is sparse and controversial. We studied alert responses of the roe deer towards approaching persons wearing a green and camouflage coat, as customary in hunters in Central Europe, and an orange vest, as prescribed in some countries. We found a significant effect of the garment's colour in that the roe deer were most sensitive to and most alerted by the orange outfit. While the ability of differentiating green and orange colours cannot be fully excluded, the higher sensitivity to brightness (luminosity, lightness) of the orange vest over the dull green coat is highly probable. Extending and deepening studies of visual performance in artiodactyls might have practical implications (such as the question of appropriate camouflage of hunters and wildlife observers) but also help to get better insight into the evolution of mammalian colour vision.

Key words. Roe deer, flight initiation distance, colour vision, dichromacy, hunting.

INTRODUCTION

Under the so-called "hunter orange regulation", deer hunters in North America and Scandinavia are required to wear orange garment to maximize hunter's safety. It is argued that this practice does not negatively impact hunting success because deer are (assumed to be) red-green colour-blind (e.g. https://www.ohep.net/info/hunter-orange). The question thus arises whether the traditional dark green clothing of Central European deer hunters or the camouflage outfit of wildlife photographers and viewers makes really sense.

In fact, however, despite their ubiquity, and their importance for humans, the Artiodactyla in general, and deer in particular, have been surprisingly understudied regarding the visual perception. Nevertheless, it appears that artiodactyls are the least diverse mammalian order in terms of retinal photoreceptors (Peichl 2005). Based on the immunocytochemical examination, artiodactyls have a standard set of rods, M or L-cones and S-cones (Witzel et al. 1978, Jacobs 1994, 1998, Yokoyama & Radlwimmer 1998, 1999, Ahnelt & Kolb 2000, Ahnelt et al. 2006, Schiviz et al. 2008, Raveh et al. 2012) and are thus expected to be dichromatic, being not able to distinguish colours within the green-yellow-red spectrum. Physiological (electroretinograms) and behavioural evidence for red-green colour blindness has been, however, rare and partly controversial.

The cattle differentiated perfectly several colour stimuli within the medium-long wavelength range (between 550 and 700 nm) from grey samples but experienced considerable difficulty in perceiving short-wavelength colours (between 400 and 500 nm) (Riol et al. 1989). The tests whether they could also differentiate between particular colours within the medium/long wave range (e.g. green from yellow or red) were, however, not performed. Green food was more attractive for the cattle than red food (Uetake & Kudo 1994). In other studies, cattle were found to be able to distinguish long (red) from medium (green) or short (blue) wavelength light (Gilbert & Arave 1986, Phillips & Lomas 2001) but, interestingly, showed a very limited ability to differentiate medium (green) from short (blue) wavelength light (Phillips & Lomas 2001). Similarly, sheep seem to have some sort of spectral vision for colours in the longer wavelength range. Ewes learned to differentiate between red, orange, yellow and white, but performed poorly between blue, green, light grey, darker grey, or black (Alexander & Stevens 1979). It was argued, and also demonstrated, in the above mentioned studies, that the understanding of colour perception in domestic artiodactyls may have implications for husbandry (animal welfare and handling).

Learning the visual capacities of free-ranging artiodactyls can provide a foundation for understanding their ecology as well as the development of more effective camouflage for hunters and wildlife viewers or strategies to reduce vehicular collisions with game and other game-human conflicts (Cohen et al. 2014). Thus, Raveh et al. (2012) have not found any differences in flight reactions of the chamois (*Rupicapra rupicapra*) to differently coloured (blue, red, and yellow) raincoats (of comparable brightness) of the approaching persons, while, in another study, the roe deer (*Capreolus capreolus*) did not specifically react on warning blue light (thought to have repellent effects) compared to white light (Brieger et al. 2017). On the other hand, in two other behavioural studies on colour vision in cervids, the white-tailed deer (*Odocoileus virginianus*) were shown to be more sensitive to shorter wavelengths than to longer wavelengths (Cohen et al. 2014), and the fallow deer (*Dama dama*) were trained to differentiate between green and grey (Birgersson et al. 2001).

According to general belief within the hunters' community and predictions of the retinal studies, we expected that the most common hunters' clothing, either green, camouflaged (as traditional in most European countries) or complemented with orange (as customary in Scandinavia or North America) will not differently affect the alert distance in the roe deer.

MATERIAL AND METHODS

Sampling

Data were collected from April 2015 till September 2018 in an open agricultural landscape (vegetation cover under 50 cm height): in the Pardubice region (49°32'N, 16°15'E) in 2015 and 2016 by one observer (PW) and in South Bohemia (49°23'N, 13°58'E) in 2017 and 2018 by another observer (PO), at altogether 131 localities. Both observers, experienced wildlife biologists and game managers (PO, man 176 cm, 2017–2018; PW, woman 165 cm, 2015–2016), were equipped with binoculars (Nikon monarch 8×42), rangefinder (Nikon Laser Prostaff 7; ±1 m), luxmeter Testo 540 (±1 lux), and anemometer (Technoline EA3000; ±0.1 km/h). Experiments were performed in outdoor clothing of three colour variants (Fig. 1): camouflage (hue 153°, saturation 10%, brightness 52%); olive green (hue 189°, saturation 15%, brightness 43%), and combination of orange and red (prevailing hue 3°, saturation 78%, brightness 85%).

Researchers scanned for the roe deer during walks constituting routine activities of game managers. Only those animals were followed which apparently did not notice the person before she/he had spotted them and animals that did not show any signs of agitation. Further conditions for starting the experiment were that the wind did not blow in the direction from the observer to the animal and that there were no terrain obstacles on the way to the animal.

The following variables were protocolled for each observation: garment's colour (camouflage, green, or red), Observer (PO / PW), locality (South Bohemia / Pardubice region), habitat of the observer and habitat of the animal (8 levels), month of observation (12 levels, January to December), daytime (5:15 to 21:34), light intensity (0.005 to 180.032 Klx), strength and direction of wind (0 to 26.9 km per hour), air temperature (–2 to 31 °C), distance of the animal to the nearest shelter (0 to 1700 m), type of the shelter (10 levels), sex (male / female) and estimated age of the deer (adult being older than one year, juvenile being under one year old), and number of animals in the group (1 to 100). The observer measured the *starting distance* when she/he first spotted the animal. Then, the observer approached the animal with a constant walking pace (on average 5.2 km/h) until the roe deer evidently noticed the person, raised its head and started to observe her/him. This distance was measured and denoted as *alert distance* (= *reaction*

Fig. 1. Colours of the outdoor garment used by the observers.
Obr. 1. Barvy terénního oblečení používaného pozorovateli.

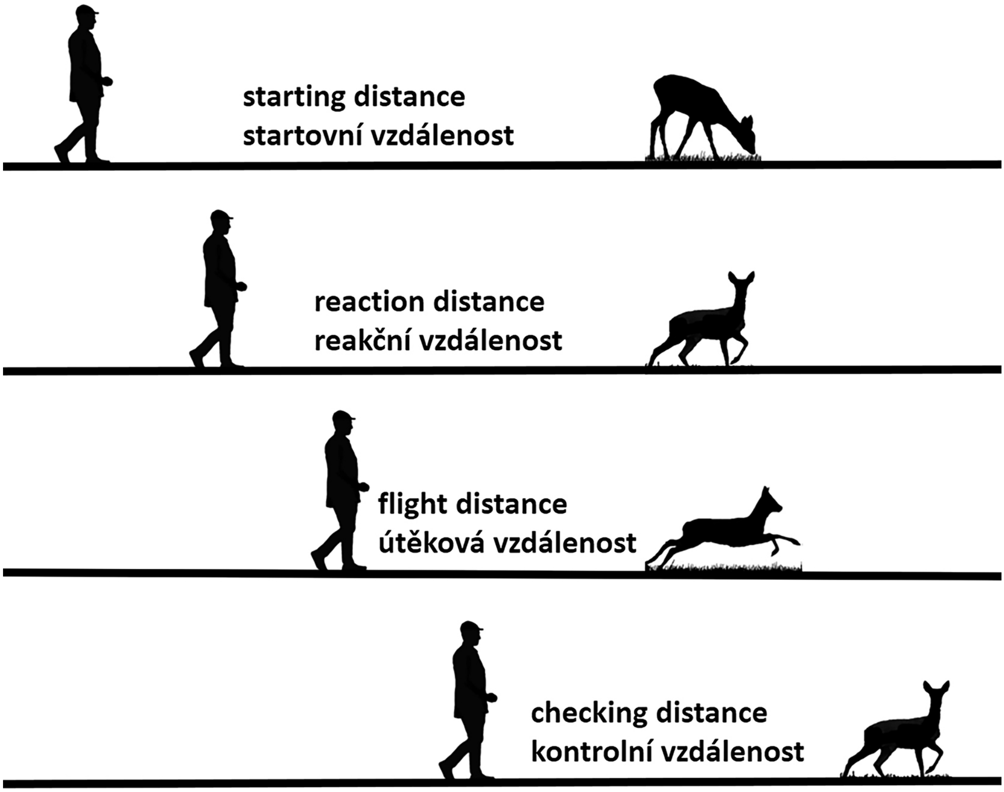

Fig. 2. Illustration of the distances measured as the behavioural response of the focal roe deer.
Obr. 2. Ilustrace vzdáleností měřených coby behaviorální odpovědi sledovaných jedinců srnčí zvěře.

distance). Then the observer continued walking towards the animal until it started to flee. The distance, denoted as *flight initiation distance* (= *flight distance*), was measured and the walk continued. If the animal stopped during the escape to check whether it was followed, the *checking distance* was measured (Fig. 2). In order not to disrupt continuity of the walking pace through stopping and measurements and since it is not possible to measure the distance of a fleeing animal by means of a rangefinder, the flight distance was calculated from the number of steps made after the reaction distance, considering the individual step length (0.72 m and 0.80 m, respectively). Reaction distance minus flight distance was subsequently calculated and denoted as *evaluating distance*.

We documented reactions of 131 focal individuals (54 males, 69 females, and 8 juveniles of undetermined sex). Out of them, 47 animals were singles, 35 were from pairs, 20 from triads, 4 from quartets, 7 from quintets, and 18 groups were larger but within each group we measured reactions of the animal which was reacting as the first one in the respective group (focal individual). Each individual or each group was provoked to flight at a different locality and a different time. Wearing of clothes of different colours was randomly and evenly distributed in time and space.

S t a t i s t i c s

The data were analysed using Statistical Analysis Systems (SAS) version 9.4. In order to check for possible multicollinearity, we first calculated correlations between the individual variables involved as described above. Significant correlation was found between the distance variables. Starting distance was correlated with reaction distance (r=0.65, P<0.0001), flight distance (r=0.58, P<0.0001), and evaluating distance (r=0.33, P=0.0002). We chose the flight distance as a dependent variable for the further analysis. Moreover, strength of wind was correlated with air temperature (r=-0.42, P<0.0001), light intensity (r=0.36, P<0.0001), and distance of the animal to the nearest shelter (r=0.63, P<0.0001). Associations were sought between the flight distance and the environmental variables using a multivariate General Linear Mixed Model (GLMM, PROC MIXED). To account for the use of repeated measures on the same individuals, all analyses were performed using the mixed model analysis with ID of the individual as a random factor. We constructed the GLMM entering first the garment's colour and the factors expected to have the most significant effect (month of the year, reaction distance, habitat of the deer and some of the weather characteristic). Subsequently we checked the model with addition of other factors described above which might contribute. The significance of each fixed effect in the GLMM was assessed by the F-test. Non-significant factors (P>0.05) were dropped from the model. Where appropriate, we tested interaction terms. We fitted the best model according to default information criteria (all being in a smaller-is-better form):

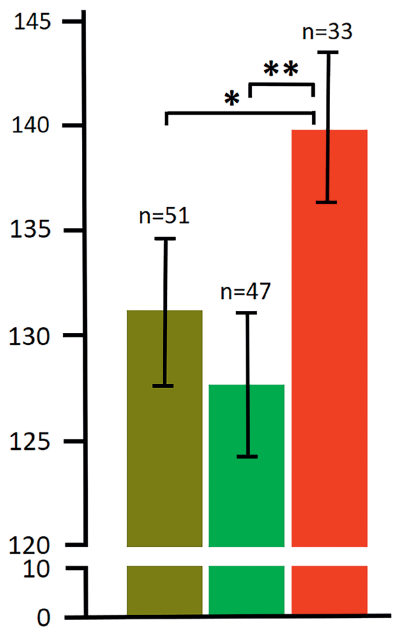

Fig. 3. The flight distance (Y axis in metres) according to the garment's colour (X axis, camouflage, green and red). Means, standard errors and number of observations are given. P=0.05 is marked by *, P=0.007 by **.
Obr. 3. Útěková vzdálenost (osa y, v metrech) ve vztahu k barvě oblečení (osa x, maskáče, zelená a červená barva). Uvedeny jsou průměry, směrodatné chyby a počet pozorování. * značí P=0.05, ** značí P=0.007.

–2 Res Log Likelihood, AIC, AICC, and BIC. Associations between the dependent variable and countable fixed effects were estimated by fitting a random coefficient model using GLMM as described by Tao et al. (2002). We calculated predicted values of the dependent variable and plotted them against the fixed effects with predicted regression lines. Least squares means (LSMEANs) were calculated for categorical fixed effects by computing the mean of each treatment and averaging the treatment means. These means of means were then used to compare the factors. In this way, the means were adjusted for the number of observations in each treatment. This estimate is unbiased because the unequal number of observations is taken into account (Welsh et al. 2000).

RESULTS

The observer spotted roe deer from a mean *starting distance* of 265 m (±97, 70–497 m). As expected, single animals and pairs were usually spotted later (244±104, 70–497 m) than larger groups (298±74, 179–422 m). The animals noticed the observer from a mean *alert distance* of 160 m, which means on average some 100 m later than they themselves had been spotted by the observer (mean *distance to reaction* = 104 m). After having noticed the approaching observer, the roe deer allowed the observer to approach for on average further 21 m (*evaluation distance*) and started to escape when the observer was about 140 m away (*flight distance*, *flight initiation distance*). About 30% of the fleeing roe deer stopped after running about 110 m to check the situation (*checking distance*).

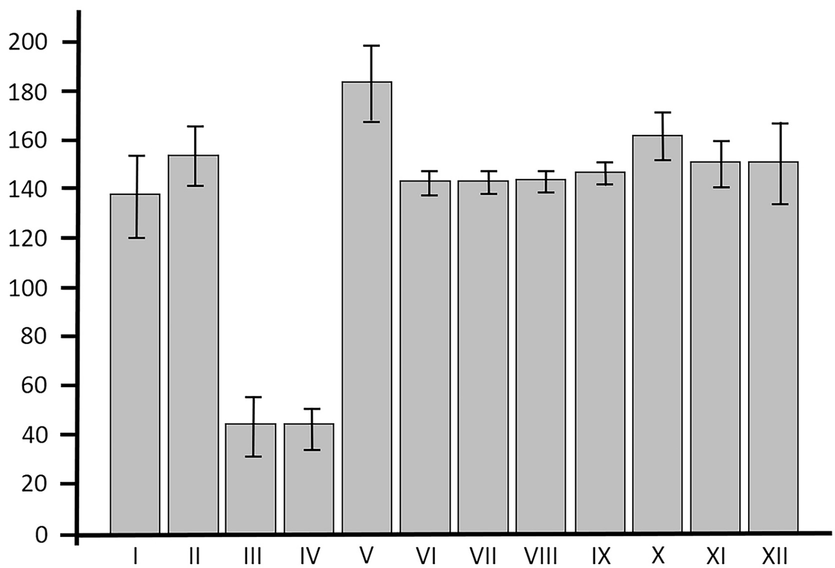

Fig. 4. The flight distance (Y axis in metres) according to the month of the year (X axis), January (I) to December (XII). Means and standard errors are given.
Obr. 4. Útěková vzdálenost (osa y, v metrech) ve vztahu k měsíci roku (osa x), leden (I) až prosinec (XII), uvedeny jsou průměry a směrodatná chyba.

The flight initiation distance was the highest when the colour of the garment was red, followed by green and camouflage ($F_{(1, 129)}$=4.95, P=0.009; Fig. 3). The shortest flight distance was recorded in March and April, while the highest flight distance was seen in May ($F_{(11, 129)}$=15.48, P<0.0001; Fig. 4). There was quite a remarkable variation across different habitats where the roe deer were tested ($F_{(8, 129)}$=10.14, P<0.0001). The flight initiation distance increased with increasing alert distance ($F_{(1, 129)}$=1303.70, P<0.0001) and also with increasing wind strength ($F_{(1, 129)}$=4.63, P=0.03).

DISCUSSION

Contrary to expectation and the hypothesis that the roe deer (and artiodactyls in general) do not distinguish between orange/red and green colours (i.e. long and medium wavelength light), we show that they were more alerted by orange than by both green and camouflage. There was no difference between green and camouflage, however. This means that the roe deer perceived the red colour as more conspicuous. Our findings in roe deer thus conform with the results of the studies on the cattle (Gilbert & Arave 1986, Uetake & Kudo 1994, Phillips & Lomas 2001) and sheep (Alexander & Stevens 1979). The fact that a similar study design applied on the chamois did not reveal any alerting effect of the coat colour (blue, yellow, red) (Raveh et al. 2012) can be explained in a multiple way: species-specific difference or general habituation of the chamois on tourists in the given region. Besides that the authors did not test green colours. It is, however, also probable that the sample size (ten trials per coat colour) was too small to reveal differences which are rather of statistical nature than really categorical. Nevertheless, and perhaps most importantly, the garments in our study differed in brightness (green 43%, camouflage 52%, orange/red 99% / 72%), whereas the coat colours in the study of Raveh et al. (2012) were of comparable brightness. It is therefore possible that the roe deer were actually alerted by the brighter vest rather than by its orange colour. From the point of view of our original hypothesis, it is, however, significant that this is exactly the case of the "hunter orange" which is required to be bright.

The flight distance dropped to seasonal minimum in March and April. This may be associated with the seasonal breakdown of the roe deer groups (Sieber 1995, Mrlík 1998, Villerette et al. 2006), and the period of velvet shedding and establishing the territories (Sempéré & Boissin 1981, Johansson 1996). This accumulation of various vital changes in the life of the roe deer could distract them from alertness to the human being. On the other hand, a sharp increase in the flight distance in May might be due to the start of parturition period in the roe deer dams (Linnell & Andersen 1998, Plard et al. 2013) and subsequent increase alertness.

The habitat-related variation in alertness has been reported in many studies (e.g. Bonnot et al. 2013, 2015). Our study did not show any real link between a particular type of the habitat and the flight distance. This may be due to inter-individual variation in behavioural and physiological responses to a given disturbance or stressor defining personality or behavioural types (Koolhaas et al. 1999). Indeed, Bonnot et al. (2015) demonstrated such a link in the roe deer between an individual's behavioural profile and its willingness to take risk by using an open habitat during daytime.

Although the strength of the wind had an effect upon the flight distance, it has to be pointed out that the effect of wind direction was not tested because, in order to exclude smell-based vigilance, we did not provoke the deer when the wind was blowing from the observer to the animals (see the Methods).

It can be argued that the roe deer might initially respond to auditory cues and secondarily to visual ones. However, care was taken not to record cases when the wind (which would bring auditory and smell signals) blows from the observer to the roe deer. Besides that there is no reason to assume that there would be a difference in the intensity of auditory signals when wearing differently coloured coats.

The fact that the artiodactyls seem to have problems to distinguish short (blue) from medium (green) wavelength light (Alexander & Stevens 1979, Phillips & Lomas 2001) suggests that the dichromacy in artiodactyls may be more complex than thought on the basis of retinal immunocytochemistry. In any case, the hitherto behavioural findings point out the necessity to extend and deepen the studies of visual performance in artiodactyls, not only with reference to the practical implications but, particularly, in order to get better insight into the evolution of mammalian colour vision. Further simple experiments comparable to that reported here, but with vests of the same colour categories but of different brightness and with vests of different colours but the same brightness are needed to get more insight.

SOUHRN

Na základě tzv. "Nařízení o lovecké oranžové (*hunting regulation orange*)" jsou lovci jelenů v Severní Americe a Skandinávii povinni nosit oranžovou vestu, aby se zvýšila jejich bezpečnost. Tvrdí se, že tato praxe nemá negativní dopad na lovecký úspěch, protože jelení zvěř (údajně) nerozlišuje červenou a zelenou barvu. Tento předpoklad je založen na imunocytochemických studiích fotoreceptorů sítnice, nicméně behaviorální důkazy jsou sporadické a kontroverzní. Studovali jsme reakce srnčí zvěře na blížící se osoby, které měly temně zelený oděv a "maskáče", jak je obvyklé u myslivců ve střední Evropě popřípadě fotografů a pozorovatelů přírody, a oranžovou signální vestu, jak je v některých zemích předepsáno. Významný účinek barvy oděvu jsme zjistili v tom, že srnčí zvěř byla nejcitlivější na oranžovou vestu a reagovala na ni na největší vzdálenost. I když nelze zcela vyloučit schopnost rozlišovat zelené a oranžové barvy, je pravděpodobnější vyšší citlivost na jas (luminiscenci) oranžové vesty než na matně zelenou barvou. Rozšíření a prohloubení studií zrakových schopností u sudokopytníků může mít praktické důsledky (naskýtá se např. otázka vhodného maskování lovců a pozorovatelů volně žijících živočichů), ale může také pomoci získat lepší vhled do evoluce barevného vidění savců.

Animal Ethics

The study was performed in the framework of routine regular patrolling of the gamekeepers in charge through the hunting grounds and the study did not involve any disturbance of the animals beyond this regular activity. In this study no laboratory animals were involved. Neither were anesthesia, euthanasia, or any kind of animal sacrifice. Therefore, no permits were required for the described study, which complied with the animal testing regulations of the country where the study was performed (Czech Republic).

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