

Ultrastructural morphological features of the hair in a sexual signal: the dark ventral patch of male red deer

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Abstract

Chemical signals play a decisive role in communication in many mammal species. In red deer, the dark ventral patch has recently been described as a male chemical signal involved in intrasexual competition. Morphological specializations of the hair of this area might contribute to retaining the volatile compounds found here. In this study, we examined differences in the ultrastructure between hair associated with the dark ventral patch and hairs from the dorsolateral region of the body as a control. We obtained a gallery of images from a scanning electron microscope to study the possible variation in the detailed anatomy as well as the surface and pattern of cuticular scales of hairs of the two body regions examined. In addition, we used a 2D-3D microscope to measure hair diameter (thickness) and the shape and size of the cuticular scales. We found that the hairs of the dark ventral patch were narrower than those from the dorsolateral region. We also found a different cuticular scale pattern of the dark ventral patch hairs, suggesting a possible specialization for the retention of compounds associated with this chemical signal. Moreover, some inter-sexual differences in hair morphology also support this idea. This study shows, for the first time, the differentiation of hairs related to the dark ventral patch, which contributes to our better understanding of this chemical communication in red deer.

Key words: Dark ventral patch, Iberian red deer, scale cuticular pattern, hair morphology.

Introduction

Odour signals play an important role in communication in most terrestrial vertebrates (Hurst, 2005; Müller-Schwarze, 2006; Wyatt, 2014). Chemical signals can be deposited in the environment as scent marks, released into the air or spread over the animal's body (Müller-Schwarze, 1977, 2006). In mammals, many olfactory signals are composed of a complex chemical mixture or blend (Wyatt, 2005, 2014), where the variation in the relative abundance of compounds can reveal characteristics of the sender involved in sexual selection, including age or sex (Lawson, Putman & Fielding, 2001), social status (Martín *et al.*, 2007), body condition (Jemiolo *et al.*, 1995; Miller *et al.*, 1998), as well as hormone levels (Galván *et al.*, 2019). In polygynous species, where male competition for mates is strong, communication between rivals is relevant to mediate competitive interactions and to avoid or reduce the risk of injury or death (Andersson, 1994). Thus, signalling works synergistically with odoriferous secretions to optimize communication (Stoddart, 1976; Walther, 1984; Gorman & Trowbridge, 1989).

Previous studies have revealed the importance of the hair structure in mammals as an adaptive specialization to climatic conditions, functioning as a coat (Meyer, Schnapper & Hülmann, 2002), for locomotion (Meyer, Seger & Hülmann, 1995), or for feeding (Howell & Hodgkin, 1976). Hairs with morphological specializations that retain the compounds for chemical signalling have been called 'osmetrichia' (Müller-Schwarze, Volkman & Zemanek., 1977). Some mammalian glandular scent organs are associated with these 'osmetrichia', which tend to be larger in diameter than other hairs of the body and have "pinecone-like" cuticular-scale patterns (Stoddart, 1980).

Specialized hair structures associated with secretory glands have been shown for many mammals (e.g., black tailed deer: Müller-Schwarze *et al.*, 1977; African crested rat: Stoddart, 1979; brown antechinus: Toftegaard and Bradley, 1999; bats: Hickey &

Fenton, 1987; Scully, Fenton & Saleuddin, 2000; shrew: Balakrishnan, 1987; lion: Poddar-Sarkar *et al.*, 2008; porcupines and hedgehogs: Poddar-Sarkar *et al.*, 2011). For example, Toftefaard and Bradley (1999) described a unique cuticular structural modification in hairs of an area associated with the sternal gland, which have a role in reproduction in the dasyurid marsupial *Antenichus stuartii*. Chemical communication is also extensively used in bats, and in *Taphozous melanopogon* there is specialization in the structure and shape of hairs associated with scent glands in both size and cuticular-scale pattern (Scully *et al.*, 2000), although just the former should be considered as ‘osmetrichia’. For ungulates, in black-tailed deer, *Odocoileus hemionus*, the hairs of the tufts that cover the tarsal glands are impregnated with sebaceous and urinary compounds and have rugose scales and combs that form compartments between the scales that can retain lipids of chemical signals (Müller-Schwarze *et al.*, 1977).

The red deer (*Cervus elaphus*) has a polygynous mating system, where males compete to monopolize females. A sexually selected signal in males has recently been described for the Iberian red deer (*Cervus elaphus hispanicus* Hilzheimer, 1909); a large and conspicuous dark ventral patch caused by urine spraying on hair of this ventral body area (de la Peña, unpublished data). The hair’s dark coloration is produced by the oxidation of a urine compound in contact with air (Galván *et al.*, 2019). The hair from the dark patch is impregnated with large amount of lipophilic volatile compounds, many of which also come from the urine, which operate as chemical signals to reveal the dominance rank, age and body condition of male red deer (Martín *et al.*, 2014; de la Peña, Martín & Carranza, 2019).

Because of this signaling function of the compounds contained in the dark ventral patch of male Iberian red deer, we aimed in this study to investigate whether there is also a differential morphology and ultrastructure of the hair from this body area

that could be considered as an adaptation to enhance the retention of compounds. Firstly, we examined hair of males from the dark ventral patch and from the dorsolateral body region as a reference to characterize the diameter of the hairs, the detailed anatomy of cross-sections, and the size and shape of the cuticular scales. Secondly, we also tested for differences between the sexes in the hairs' diameters and cuticular scale parameters from both the ventral and the dorsolateral regions. We predicted that hairs from the dark ventral patch of males should differ in thickness, likely being thinner to increase total surface area, compared to hairs from the dorsolateral region, and compared with to hairs from the ventral area of females (that do not show the dark patch). We also expected a specialized cuticular scale pattern in the dark ventral patch of males to enhance the retention of urinary volatile compounds.

Methods

Samples collection and measurements

We obtained samples from six natural populations of Iberian red deer in southwestern Spain (Andalusia and Extremadura regions) from October to December of 2016 and 2017. We collected hairs from the dark ventral patch and from the dorsolateral body region (Supplementary Fig. S1a) of 34 adult males (4 ± 0.3 S.D. years old) harvested during legal hunting activities. We also collected hairs from 8 females (5 ± 0.5 S.D. years old) from the ventral (the dark ventral patch does not appear in females, see Supplementary Fig. S1) and the dorsolateral regions, to examine the differences between the sexes. For detailed information about the hunting regime and the study area see Methods in de la Peña *et al.*, 2019; de la Peña *et al.*, 2020 a,b. No deer were culled specifically in order to carry out this study.

To explore morphological differences between hairs from both regions of the male body, we first took images using a scanning electron microscope located at the support service of the University of Cordoba (SCAI-UCO). For this purpose, hairs were immersed in glutaraldehyde to be fixed in order to preserve their quality and to avoid deterioration until later analyses under the microscope. Samples were dehydrated by ethanol, and then dried using a critical-point drier (BALZERS CPD-030), mounted on carbon-coated studs and covered with a thin layer of platinum in a high vacuum evaporator (LEICA AC-600). Samples were viewed using a scanning electron microscope (JEOL JSM 7800F) at 5 kV and studied at magnifications ranging from 350 to 3000. To explore hair anatomy and the thickness of the cortex, we took a cross-section from the hairs' apical and basal zones from the dark ventral patch and dorsolateral body region. The images from the microscope allowed us to assess which parameters of the hair cuticle scales and their morphology should be measured in posterior analyses.

To make measurements of male and female hair samples, after intense cleaning in ethanol/ether, hairs were viewed with a high-resolution microscope (LEICA DCM8) that allowed the measurement of 2D-3D distances, angles, perimeters and areas. We took measurements of one randomly selected hair from each individual's body region (one hair from the dark ventral patch area and one from the dorsolateral area). We measured the diameter of each hair (μm), in the basal and apical zones of each hair at a 10x magnification (Supplementary Fig. S2a). A total of three randomly selected cuticular scales were measured from each zone (basal and apical) of each analysed hair (Supplementary Fig. S2b). Measurements of the maximum length (μm) and width (μm) of each cuticular scale were made at 50x magnification. Hair measurements were carried out using the software LEICA SCAN 6.5 of the microscope. We also calculated

scale area (μm^2) and scale perimeter (μm), using Adobe Photoshop CS6, and the ratio of scale width to height (Y-/X-feret) following the methods used by Meyer *et al.* (2002).

Statistical analysis

To explore the differences in hair characteristics between the dark ventral patch and the dorsolateral region of the males, we conducted mixed-effects-model analyses with repeated measures, by using the ‘lmer’ function in R. Individuals were treated as a random variable in the model. As repeated measures factors, we included the body region from which hair of each male was collected (dark ventral patch vs. dorsolateral region), the hair zone within each hair (basal vs. apical) and the interaction between them.

First, we ran a linear mixed model, where we tested for potential differences in hair thickness between both body regions using diameter as a response variable. Then, the hair cuticle parameters were calculated (i.e. length, width, area, perimeter and Y-/X-feret of scales) and were used as response variables. Two different models were run for each parameter. The first analysis just included the body region, hair zone and their interaction as repeated measures factors. The second one also took into account the hair diameter as a covariate in all cases, but also cuticular scale length when the scale area and scale perimeter were the dependent variables, in order to control for the size of each hair.

We also performed linear mixed models to test for differences between sexes in hair diameter and cuticular scale parameters (i.e. length, width, area, perimeter and Y-/X-feret of scales), including body region (ventral vs. dorsolateral areas) and hair zone (basal vs. apical) as repeated measures within individuals, and sex (male vs. female) as factors. In the analyses where the parameters of cuticular scales were the dependent

variables, we included hair diameter as a covariable to control for the size of each hair. Owing to the close correlation between area and perimeter of the cuticular scales and scale length, this variable was also added in the model to control for its effect. We included the interaction between body region and sex, to test whether there were differences in thickness and pattern of cuticular scales between the sexes in the ventral region but not in the dorsolateral region.

Additionally, we carried out parametrizations of the models comparing the diameters and parameters of cuticular scales (i.e. length, width, area, perimeter and Y-/X-feret of scales) between the sexes, owing to differences in the sample sizes between the sexes (males: N= 34; females: N= 8). Thus, we used parametric bootstrapping (n = 5000) to obtain 95% confidence intervals using the ‘bootMer’ function.

Supplementary Table S1 shows the detailed structure of each model. Because of the non-normality of data distributions, hair diameter and hair cuticle scale parameters were \log_{10} transformed. The means \pm SE were calculated and the level of statistical significance was $P < 0.05$. All statistical analyses were carried out using the ‘lme4’ package (Bates *et al.*, 2015) in R Studio 2.14.1. The dataset used in this study is available in the following link: <https://doi.org/10.6084/m9.figshare.12179223.v1>.

Results

Morphological description of males' hair

Statements in this section correspond to the authors' direct observations of the ultrastructure and morphology of hairs without statistical analysis to support them. We found morphological visual differences between hairs from the dark ventral patch and the dorsolateral region of males. At low magnification, dark ventral patch hairs seemed

narrower and with a rounded cross-sectional shape in all cases (Fig. 1a), while dorsolateral hairs seemed wider, appearing as flattened hairs and even bent in some cases (Fig. 1b).

When observing the cross-section of hairs, we also appreciated differences in the thickness and shape between hairs from both regions of the body. Hairs from the dark ventral patch seemed with a rounded cross-sectional shape and with a more uniform shape (Fig. 1c) than hairs from the dorsolateral region, which were more elongated (Fig. 1d). However, the cross-sections did not show apparent differences between body regions either in the detailed anatomy of the network of tubular spaces inside the hairs, in the partition wall of the medulla or in the thickness of the cortex (Fig. 1c, d).

At increased magnification, the patterns of the cuticular scales also differed between body regions, with hairs from the dark ventral patch having narrower and shorter scales (Fig. 1e). By contrast, dorsolateral hairs showed wider scales, whose length even occupied the entire visible surface of the hair (Fig. 1f).

We also noticed differences in the shape and size of cuticular scales between hairs from each body region. The cuticular scales of the dark ventral patch hairs seem to lift up from the hair surface like the scales on a pinecone, giving the appearance of a rough surface and ridges. By contrast, the scales on the dorsolateral hairs seem not to protrude so much and presented a smoother surface (Fig. 1, a-g vs. b-h), although these apparent differences deserve further detailed measurement and statistical comparison.

Measurements of males' hair

The mean hair diameter differed between body regions (Fig. 2); hairs from the dark ventral patch were significantly thinner than those from the dorsolateral region in both the apical and the basal zones of the hair (Table 1).

Considering the first set of analyses for absolute measurements of scales, we found that cuticular scales from hairs from the dark ventral patch were significantly smaller in length, area, and perimeter than those of the dorsolateral hairs (Table 1; Fig. 3). Also, the shape of the scales (length to width ratio; Y-/X-feret) differed between body regions, the cuticular scales of the dark ventral region being wider relative to their length than those in the dorsolateral region (Fig. 3c). Except for significant differences in scale width between apical and basal zones of the hair (Table 1c; Fig. 3b), no other significant differences were found between zones of the same hair nor for their interaction with body region.

We also found morphological differences in some features of the cuticular scale pattern when we corrected the size of the scales for the size of the hair strand (Table 2). Length, width, and area of cuticular scales from the dark ventral patch were relatively smaller than those from the dorsolateral region. However, we did not find significant differences in cuticular scale relative perimeter, nor in the relative ratio of scale length to width between each body area. Also, there were differences in the relative area of cuticular scales between hair-strand zones. The apical scales were relatively smaller in surface area than the basal ones (Table 2). The rest of the comparisons and interactions were not significant.

Measurements of hairs from males and females

The diameter of hair strands differed between sexes, with males having significantly thicker hair than females in all the body regions (Table 3; Fig. 4a). For all the measured parameters of the cuticular scales, we only found significant differences between the sexes in the relative area of the cuticular scales controlling by for and scale size (Table

3e), with males presenting relatively smaller scales than those of females (Fig. 4b). The interaction between sex and body region was significant. Therefore, the relationship between cuticular scale area in ventral and dorsolateral regions differed between males and females. In the dorsolateral region, scales of males were bigger than those of females, while in the ventral region the opposite was found (Fig. 4b). Moreover, the bootstrapping of these raw models supported these intersexual differences in hair thickness and cuticular scale area (Table 4).

Discussion

We have reported differences in the diameter and cuticular-surface structure of the hairs from the dark ventral patch of male Iberian red deer, with hairs of this patch having a smaller diameter and smaller, narrower scales than the hairs from the dorsolateral region of the body. We suggest that these morphological differences may contribute to the retention of urinary volatile compounds, thus enhancing the signalling function of the dark ventral patch.

‘Osmetricchia’, hairs specialized for scent dispersal (Muller-Schwarze *et al.*, 1977), have been reported in other mammal species such as in the Mongolian gerbil (*Meriones unguiculatus*) and crested rat (*Lophiomys imhausi*) (Stoddart, 1980), as well as in mule deer (*Odocoileus hemionus*) (Muller-Schwarze *et al.*, 1977). Osmetricchia are specialized body hairs that differ in size and shape and are associated with glands or structures that hold glandular products (Müller-Schwarze *et al.*, 1977). Our results are the first evidence of differences between the ultrastructural morphology of the dark ventral patch hairs and the hairs from another body region in red deer, which suggests that the signalling role may have influenced the evolution of the structural features of the former. Benedict (1957) already noticed in bats that the gland hairs differed from

other body hairs. However, until the studies of Hickey & Fenton (1987), it was not shown that these differences were due to a specialization of hairs involved in scent dispersal. Contrary to our results, they found that these gland hairs were larger in diameter and have longer scales than those of mid-dorsal body hairs in some bat species. These differences from our findings may be explained because, in the case of male Iberian red deer, the modified hairs of the dark ventral patch are not directly associated with a small gland located in the same area than the hairs, but they occupy a very large area over which the urine is sprayed. For this reason, hair specialization may be focused on increasing the surface available for the retention and dissemination of volatile compounds in urine, thus prolonging and enhancing the chemical signal. Also, the dark ventral patch may not only be a chemical signal but also a visual one (de la Peña *et al.*, 2020 a,b; Carranza *et al.*, 2020). Thus, volatile compound retention and the density of thinner hair might contribute to the darkness of the patch, although this hypothesis deserves further research.

Previous studies have shown in different mammal species differences in hair specializations for efficient storage of compounds involved in chemical signals, encompassed within the term 'osmetrichia'. For example, specialized orientation in the African crested rat (Stoddart, 1979), hairs with ridges as a comb in black-tailed deer: (Müller-Schwarze *et al.*, 1977), thicker and larger scales in brown antechinus (Toftegaard & Bradley, 1999), or thick hairs and modified cuticular pattern in black-bearded tomb bat (Scully *et al.*, 2000). The presence of specialized integumentary structures close to glands facilitates the odour perception of the substances that they produce not only in the case of mammalian hairs, but also in bird feathers, such as the tuft of feathers that surrounds the uropygial gland in species with greater capacity to perceive odours (Galván & Møller, 2013). We suggest that the modified cuticular scale

pattern that we found in hairs of the dark ventral patch of male Iberian red deer may be considered another example of hair specialization associated with a chemical signalling function (Martín *et al.*, 2014; Galván *et al.*, 2019; de la Peña *et al.*, 2019).

Alternatively, these differences in the adaptation for compound retention, but were due to selection for a protective function in the dorsolateral area. The dorsolateral area is more exposed to external impacts, such as exposure to sun and rain, vegetation or insects, and the hairs in this area may be thicker to provide protection from these factors. Woods *et al.* (2011) showed that the guard hairs and underfur of mid-flank red deer present differences in structure to provide protection and insulation, in case of guard hairs, and to enhance thermal insulation during winter in case of under hairs. It is possible that ventral hairs, which are protected by the body, could be thinner, like wool hairs (Mayer, 1952), for insulation and thermoregulation. However, this alternative possibility does not exclude that this hair structure could have acquired a secondary adaptive function to retain the compounds coming from the urine, which provide for a chemical and visual signal. We propose an alternative hypothesis that the smaller diameter of hairs from the dark ventral patch may be an exaptation (Gould & Vrba, 1982), that may have evolved initially by natural selection for another function but that might have later evolved to harbour and retain volatile compounds during the rutting season.

Under our starting hypothesis of hair specialization for male signalling, we expected to also find differences in size and pattern of cuticular scales of ventral hairs between males and females. However, we observed that in both sampled body regions, female hairs are thinner than male hairs. For this result, the most parsimonious explanation may be that, due to the smaller body size of females compared to males, the diameter of their hairs should also be smaller, both for the ventral area and for the

dorsolateral region. Our study has shown that, controlling for hair thickness and scale length, the mean area of the scales of male ventral patch hairs was smaller than that of females. Conversely, these intersexual differences are the opposite in the dorsolateral region, with males having larger scales than females, which is consistent with the greater thickness of the males' hairs. Hence, smaller cuticular scales in coarser hairs implies a greater number of cuticle scales per unit of surface area in the ventral hairs of males than in those of females. This may increase the total length of contact along the edges of the scales, which might facilitate compound transmission along the hairs of males, as well as greater retention of compounds on the contact surface. This result supports the idea that hairs from the dark ventral patch are specialised to favour the retention of substances coming from urine during the rutting season. For the similarities found between the hairs of males and females in some scale parameters, we cannot reject that some morphological traits that evolved due to selection in one sex may be expressed also in the other sex if they do not entail any costs to their expression in the other sex, such as nipples in mammals or femoral pores in lizards (Zahn, 2019).

Irrespective of the evolutionary origin of the modified morphology of the hairs of the dark ventral patch of males, the small diameter of the hairs allows a greater number of hairs per skin surface area. This may increase the area available for holding urine, thereby increasing the chemical signal strength. Similarly, considering the relative size of the hairs, the cuticular scales of the ventral region have a smaller area with respect to the dorsolateral body region. By being smaller, both the number of scales and the number of gaps between them are higher per unit of hair surface, likely facilitating the retention and storage of volatile compounds sprayed by urine over the fur. Future work should investigate the actual hair density per unit of skin surface area in different body regions, to test this interpretation.

Hairs from the African lion's mane have a normal scale structure, but have grooves that retain osmic signalling compounds (Poddar-Sarkar *et al.*, 2008). It was suggested that lion mane hairs are equipped with pockets to retain chemical compounds. However, in our study of red deer, we did not find differences in the network of tubular spaces inside each type of hair. It would be necessary to assess the functionality of the tubular cavities to understand the potential role they may play in compound retention and chemical signal strength.

Our study sheds light on the differences in morphology of the dark ventral patch hairs. Although we have evidence on the use of the dark ventral patch in sexual signalling (Martín *et al.*, 2014; de la Peña *et al.*, 2019; de la Peña *et al.*, 2020 a,b), here we did not test the role of hair structure in communication either in the wild or in the lab. Thus, experimental work is needed to examine the accumulation and retention capacity throughout time of these hairs in relation to other body regions and their effects on chemical and visual features of the signal. Future studies may also be aimed at exploring whether there are differences between the hair ultrastructure of the ventral area in male Iberian red deer and other red deer subspecies where the dark ventral patch is also present, but which have not yet been studied, as well as to study the existence of hairs associated with specific glands potentially located in the dark ventral patch and the chemical and visual role of hair structural differences.

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References

- Anderson, D.R. & Burnham, K.P. (2002). Avoiding pitfalls when using information-theoretic methods. *J. Wildl. Manag.* **66**, 912-918.
- Andersson, M. (1994). *Sexual selection*. Princeton: Princeton University Press.
- Balakrishnan, M. (1987). Sebum-storing flank gland hairs of the musk shrew, *Suncus murinus viridescens*. *J. Zool. Lond.* **213**, 213-219.
- Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1-48.
- Benedict, F.A. (1957). Hair structure as a generic character in bats. *Univ. California Publ. Zool.* **59**, 285-548.
- de la Peña, E., Martín, J. & Carranza, J. (2019). The intensity of male-male competition may affect chemical scent constituents in the dark ventral patch of male Iberian red deer. *PLoS ONE* **14**(9), e0221980.
- de la Peña, E., Martín, J., Barja, I., Pérez-Caballero, R., Acosta, I., Carranza, J. (2020 a). The immune challenge of mating effort: steroid hormone profile, dark ventral patch

- and parasite burden in relation to intrasexual competition in male Iberian red deer. *Integr Zool* doi.org/10.1111/1749-4877.12427
- de la Peña, E., Martín, J., Barja, I., Carranza, J. (2020 b). Testosterone and the dark ventral patch of male red deer: the role of the social environment. *Sci. Nat.* **107**, 18.
- Galván, I., Solano, F., Zougagh, M., Andrés, F., Murtada, K., Ríos, A., de la Peña, E. & Carranza, J. (2019). Unprecedented high catecholamine production causing hair pigmentation after urinary excretion in red deer. *Cell. Mol. Life Sci.* **76**, 397-404.
- Gorman, M.L. & Trowbridge, B.J. (1989). The role of odor in the social lives of carnivores. In *Carnivore behavior, ecology, and evolution: 57–88*. Gittleman, J.L. (Ed.). Ithaca, N.Y.: Cornell University Press.
- Gould, S.J. & Vrba, E.S. (1982). Exaptation - a missing term in the science of form. *Paleobiology* **8**, 4–15.
- Hurst, J.I. (2005). Scent marking and social communication. In *Animal communication networks: 219-243*. McGregor, P.K. (Ed.). Cambridge: Cambridge University Press.,
- Hickey, M.B.C. & Fenton, B.M. (1987). Scent-dispersing hairs (*Osmetricchia*) in some *Pteropodidae* and *Molossidae* (*Chiroptera*). *J. Mamm.* **68**, 381–384.
- Howell, D.J. & Hodgkin, N. (1976). Feeding adaptations in the hairs and tongues of nectar-feeding bats. *J. Morphol.* **148**, 329-339.
- Jemiolo, B., Miller, K.V., Wiesler, D., Jelinek, I., Novotny, M. & Marchinton, R.L. (1995). Putative chemical signals from white-tailed deer (*Odocoileus virginianus*). Urinary and vaginal mucus volatiles excreted by females during breeding season. *J. Chem. Ecol.* **21**, 869–879.
- Lawson, R.E., Putman, R.J. & Fielding, A.H. (2001). Chemical communication in Eurasian deer (*Cervidae*): do individual odours also code for attributes? *J. Zool. Lond.* **253**, 91–99.

- Martín, J., Moreira, P. & López, P. (2007). Status- signalling chemical badges in male Iberian rock lizards. *Funct. Ecol.* **21**, 568-576.
- Martín, J., Carranza, J., López, P., Alarcos, S. & Pérez-González, J. (2014). A new sexual signal in rutting male red deer: age related chemical scent constituents in the belly black spot. *Mamm. Biol.* **79**, 362–368.
- Mayer, W.V. (1952). The hair of California mammals with keys to the dorsal guard hairs of California mammals. *Am. Nat.*, **48**, 480.
- Meyer, W., Seger, H. & Hülmann, G. (1995). Remarks on specific adaptive scale structure of the hair cuticula in some European bats. *Eur. J. Morph.* **33**, 509-513.
- Meyer, W., Schnapper, A. & Hülmann, G. (2002). The hair cuticle of mammals and its relationship to functions of the hair coat. *J. Zool. Lond.* **256**, 489-494.
- Miller, K.V., Jemiolo, B., Gassett, J.W., Jelinek, I., Wiesler, D. & Novotny, M., (1998). Putative chemical signals from white-tailed deer (*Odocoileus virginianus*): social and seasonal effects on urinary volatile excretion in males. *J. Chem. Ecol.* **24**, 673–683.
- Müller-Schwarze, D., Volkman, N.J. & Zemanek, K.F. (1977). Osmetricchia: specialized scent hair in black-tailed deer. *J. Ultrastr. Res.* **59**, 223-230.
- Müller-Schwarze, D. (2006). *Chemical ecology of vertebrates*. Cambridge: Cambridge University Press.
- Poddar-Sarkar, M., Chakroborty, A., Bhar, R. & Brahmachary, R.L. (2008). Putative pheromones of Lion mane and its ultrastructure. In *Chemical signals in vertebrates* 11: 61–67. Hurst J., Beynon, R.J., Roberts, S.C., & Wyatt, T.D., (Eds.) New York: Springer.
- Poddar-Sarkar, M., Raha, P., Bhar, R., Chakroborty, A. & Brahmachary, R.L. (2011). Ultrastructure and lipid chemistry of specialized epidermal structure of Indian procupines and hedgehog. *Acta Zool.* **92**, 134–140

- Toftegaard, C.L. & Bradley, A.J. (1999). Structure of specialized *osmetrichia* in the brown antechinus *Antechinus stuartii* (Marsupialia: *Dasyuridae*). *J. Zool. Lond.* **248**, 27-30.
- Scully, M.R.W., Fenton, M.B. & Saleuddin, A.S.M. (2000). A histological examination of the holding sacs and glandular scent organs of some bat species (*Emballonuridae*, *Hipposideridae*, *Phyllostomidae*, *Vespertilionidae*, and *Molossidae*). *Can. J. Zool.* **78**, 613-923.
- Stoddart, D.M. (1976). *Mammalian odours and pheromones*. London: Edward Arnold Ltd.
- Stoddart, D.M. (1979). A specialized scent-releasing hair in the Crested rat *Lophiomys imhausi*. *J. Zool. Lond.* **189**, 551-553.
- Stoddart, D.M. (1980). *The ecology of vertebrate olfaction*. New York: Chapman and Hall.
- Walther, F.R. (1984). *Communication and expression in hoofed mammals*. Bloomington: Indiana University Press.
- Wyatt, T.D. (2005). Pheromones: Convergence and contrasts in insects and vertebrates. In *Chemical Signals in Vertebrates 10*: 7–19. Mason, R.T., LeMaster, M.P., & Müller-Schwarze, D. (Eds.). Boston, MA: Springer.
- Wyatt, T.D., (2014). *Pheromones and animal behaviour: chemical signals and signatures*. Cambridge: Cambridge University Press.
- Zahn, L.M. (2019). The genetics of sexual dimorphism. *Science* **365**, 245-247.

Table 1. Results from linear mixed models, including individual as random effect (males: N= 34), taking into account hair diameter (μm) and hair cuticle parameters (i.e. length, width Y-/X-feret, area and perimeter of scales) as dependent variables, comparing body region (dark ventral patch vs. dorsolateral area), hair zone (apical vs basal) and the interrelation between them in male Iberian red deer (*Cervus elaphus hispanicus*). *Dark ventral patch* (DVP) was the reference value of Body region; *Apical* was the reference value of Hair zone. Significant results are indicated in bold.

| | Body region (DVP) | | Hair zone (Apical) | | Body region x Hair zone | |
|-----------------------------------|----------------------|----------------|-----------------------|--------------|-------------------------------|-------|
| | $F_{1,31}$ | P | $F_{1,31}$ | P | $F_{1,31}$ | P |
| Hair diameter (μm) | 65.81 | < 0.001 | 0.57 | 0.453 | 0.59 | 0.441 |
| Scale length (μm) | 48.67 | < 0.001 | 0.05 | 0.819 | 0.25 | 0.619 |
| Scale width (μm) | 38.05 | < 0.001 | 4.25 | 0.041 | 0.11 | 0.745 |
| Y-/X-feret | 4.74 | 0.032 | 2.18 | 0.143 | 0.39 | 0.535 |
| Scale area (μm^2) | 77.14 | < 0.001 | 0.01 | 0.934 | 0.06 | 0.807 |
| Scale perimeter (μm) | 48.858 | < 0.001 | 0.009 | 0.925 | 0.128 | 0.721 |

Table 2. Results from linear mixed models, including individual as random effect (males: N= 34), taking into account hair cuticle parameters (i.e. length, width Y-/X-feret, area and perimeter of scales) as dependent variables, comparing body region (dark ventral patch vs. dorsolateral area), hair zone (apical vs. basal) and the interrelation between them, and controlling for hair size (diameter and scale length as covariates) when appropriate in male Iberian red deer (*Cervus elaphus hispanicus*). *Dark ventral patch* (DVP) was the reference value of Body region; *Apical* was the reference value of Hair zone. Significant results are indicated in bold.

| | Scale length | | Hair diameter | | Body region (DVP) | | Hair zone (Apical) | | Body region x Hair zone | |
|-----------------------------------|--------------|------------------|---------------|------------------|-------------------|------------------|--------------------|------------------|-------------------------|-------|
| | $F_{1,30}$ | P | $F_{1,30}$ | P | $F_{1,30}$ | P | $F_{1,30}$ | P | $F_{1,30}$ | P |
| Scale length (μm) | 32.55 | <0.001 | 8.05 | 0.005 | 0.54 | 0.817 | 0.78 | 0.379 | | |
| Scale width (μm) | 5.02 | 0.027 | 13.20 | <0.001 | 3.48 | 0.065 | 0.04 | 0.850 | | |
| Y-/X-feret | 10.66 | 0.001 | 0.02 | 0.889 | 3.33 | 0.071 | 0.72 | 0.397 | | |
| | $F_{1,29}$ | P | $F_{1,29}$ | P | $F_{1,29}$ | P | $F_{1,29}$ | P | $F_{1,29}$ | P |
| Scale area (μm^2) | 223.76 | <0.001 | 0.03 | 0.860 | 14.28 | <0.001 | 0.22 | <0.001 | 0.10 | 0.750 |
| Scale perimeter (μm) | 858.76 | <0.001 | 0.74 | 0.390 | 0.77 | 0.381 | 0.67 | 0.416 | 0.06 | 0.811 |

Table 4. Results from bootstrapping analyses from the linear mixed models where the dependent variables were a) diameter and b) scale cuticle area of female and male Iberian red deer (*Cervus elaphus hispanicus*). β indicates the direction and magnitude of effect; S.E. is the standard error; 95% C.I. = 95% Confidence interval from parametric bootstrapping (n = 5000); t-value = t-Student value with associated *p*-value. *Ventral* was the reference value of Body region; *Apical* was the reference value of Hair zone; *Male* was the reference value of Sex. Significant results are indicated in bold.

| Hair diameter (μm) (A) | | | | | |
|--|---------|--------|-----------------|-----------------|------------------|
| <i>Fixed factors</i> | β | S.E. | 95% CI | <i>t</i> -value | <i>P</i> |
| Intercept | 4.945 | 0.123 | 4.705 to 5.197 | 40.071 | <0.001 |
| Body region (Dorsolateral) | 0.760 | 0.138 | 0.479 to 1.042 | 5.498 | <0.001 |
| Hair zone (Basal) | 0.029 | 0.060 | -0.089 to 0.141 | 0.492 | 0.624 |
| Sex (Male) | 0.267 | 0.133 | 0.002 to 0.524 | 2.014 | 0.047 |
| Body region x Sex | -0.212 | -0.154 | -0.517 to 0.091 | -1.373 | 0.172 |

| Scale area (μm^2) (B) | | | | | |
|---|---------|--------|------------------|-----------------|------------------|
| <i>Fixed factors</i> | β | S.E. | 95% CI | <i>t</i> -value | <i>P</i> |
| Intercept | 5.525 | 0.069 | 5.390 to 5.666 | 79.042 | <0.001 |
| Body region (Dorsolateral) | 0.004 | 0.056 | -0.106 to 0.115 | 0.063 | 0.949 |
| Hair diameter | <0.001 | <0.001 | -0.002 to 0.003 | 0.350 | 0.726 |
| Scale length | 0.016 | 0.001 | 0.0142 to 0.018 | 16.875 | <0.001 |
| Hair zone (Basal) | -0.019 | 0.023 | -0.064 to 0.024 | -0.879 | 0.381 |
| Sex (Male) | -0.115 | 0.056 | -0.229 to -0.005 | -2.072 | 0.041 |
| Body region x Sex | 0.120 | 0.057 | 0.004 to 0.234 | 2.081 | 0.039 |

Figure legends:

Figure 1: Scanning electron micrographs showing hairs of male Iberian red deer from (a) the dark ventral patch (scale bar= 10 μm ; 750x) and (b) the dorsolateral region (scale bar= 10 μm ; 700x). Cross-sections showing the detailed internal anatomy of hairs from (c) the dark ventral patch and (d) dorsolateral region (scale bars = 100 μm ; 270x and 180x, respectively). Differences in the slope of the cuticular scales and the scale pattern are shown in hairs from (e, g) the dark ventral patch (scale bars = 10 μm ; 450x and 1800x, respectively). and (f, h) the dorsolateral region (scale bars = 10 μm ; 450x and 1500x respectively).

Figure 2: Mean (\pm 95% confidence interval) diameter (μm) of the hair from the dark ventral patch and the dorsolateral region of male Iberian red deer shown separately for apical (black dots) and basal (grey dots) zones of the hairs. Statistical significance according to the analysis in Table 1: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant.

Figure 3: Mean (\pm 95% confidence interval) (a) length (μm), (b) width (μm), (c) Y-/X-feret, (d) area (μm^2) and (e) perimeter (μm) of cuticular scales of hairs from the dark ventral patch and the dorsolateral region of male Iberian red deer shown separately for apical (black dots) and basal (grey dots) zones of the hairs. Statistical significance according to the analysis in Table 2: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant.

Figure 4: Mean (\pm 95% confidence interval) (a) diameter (μm) and (b) scale cuticular area (μm^2) of the hairs of the ventral (black dots) and the dorsolateral regions (grey dots) of male and female Iberian red deer. Statistical significance according to the analysis in Table 4: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns = not significant.

Fig. 1

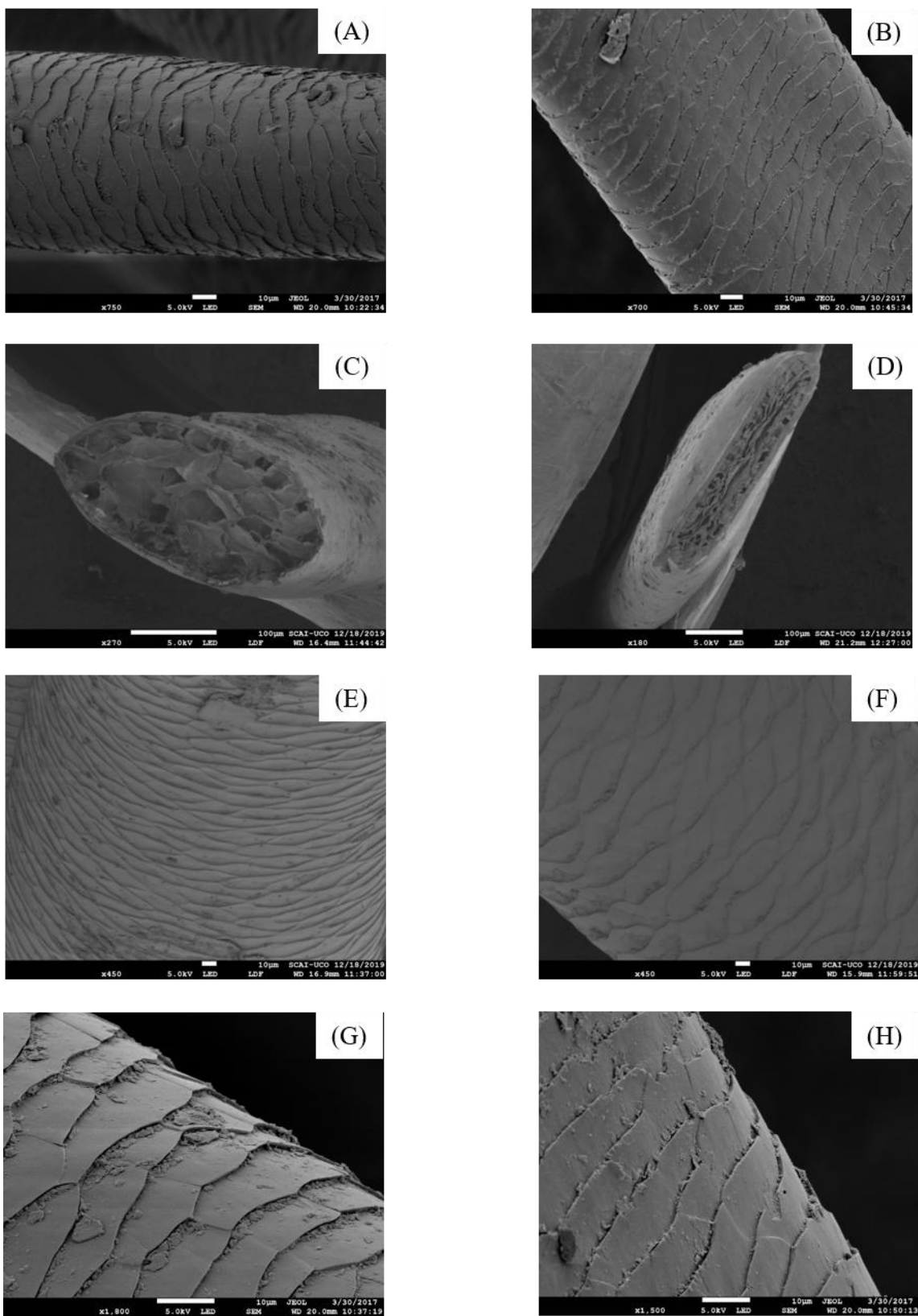


Fig. 2

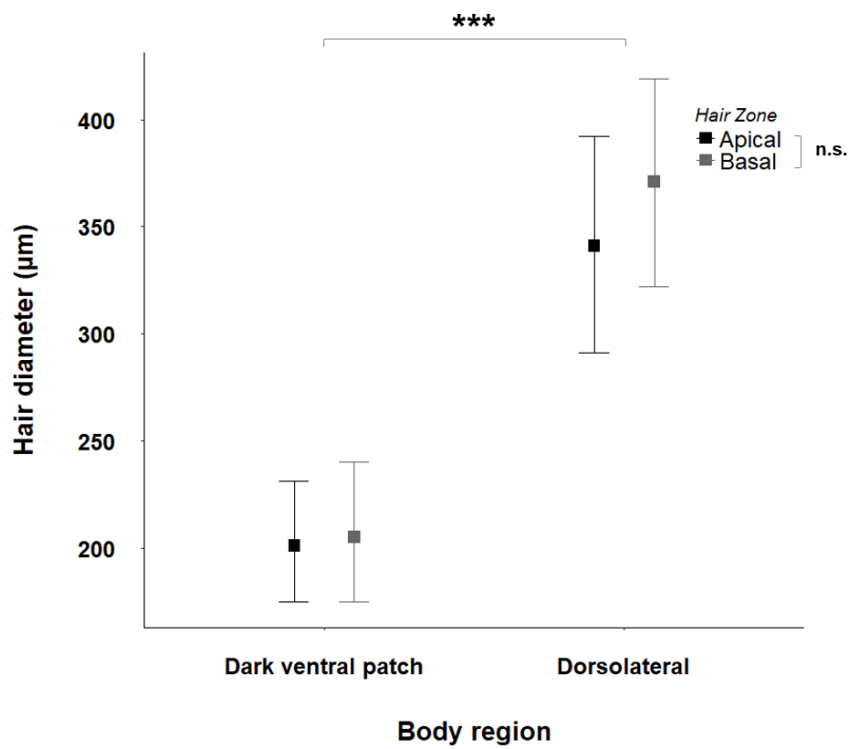


Fig. 3

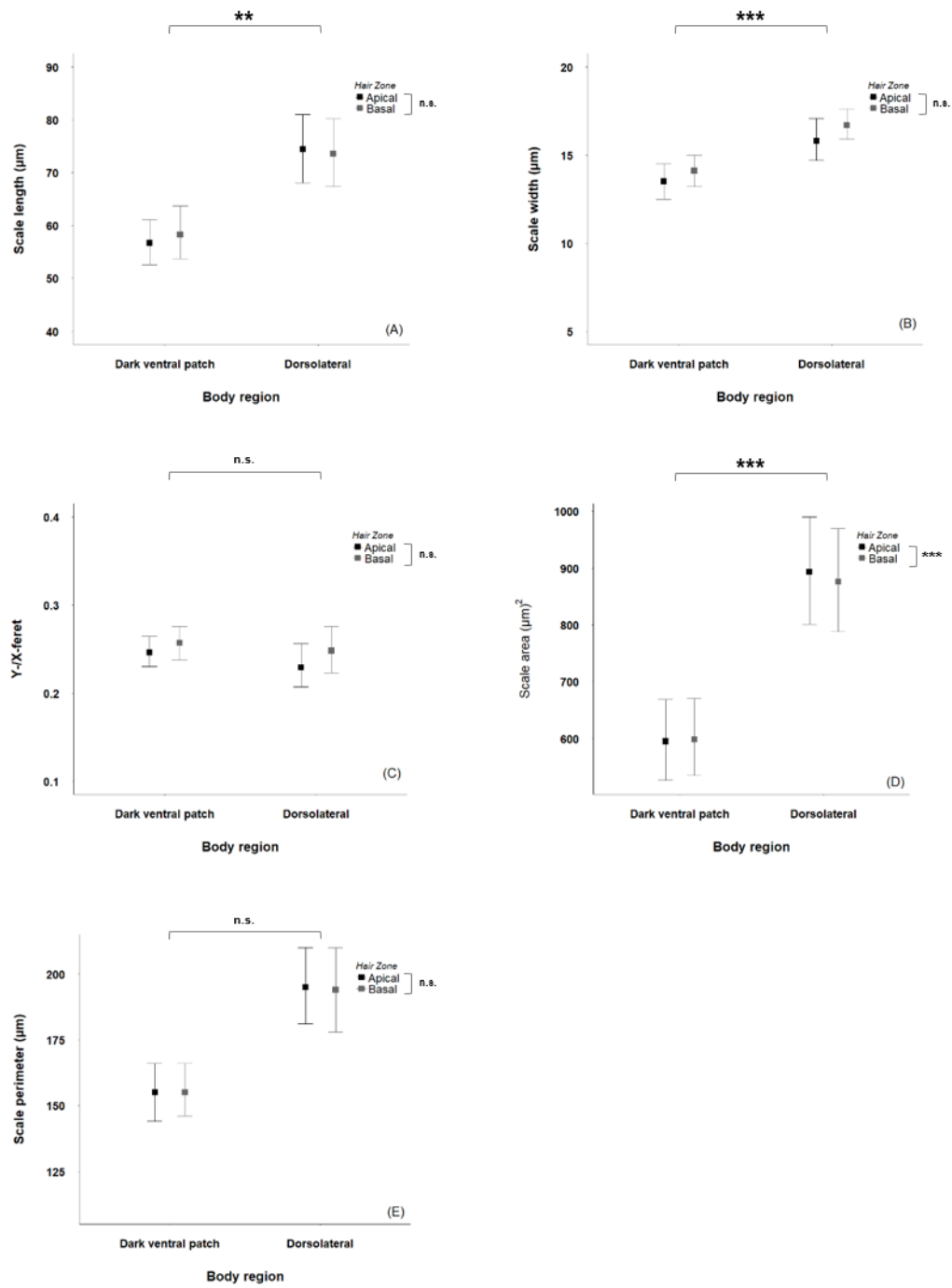


Fig. 4

