Research Article

Deer in an arid habitat: dental microwear textures track feeding adaptability

Emilie Berlioz[1,*], Concepción Azorit[2], Cécile Blondel[1], María Sierra Tellado Ruiz[2], Gildas Merceron[1]

[1]iPHEP UMR 7262, CNRS & Université de Poitiers, Bât. B35 - TSA-51106, 6, rue M. Brunet, 86073 Poitiers Cedex 9, France
[2]Área de Zoología, Campus Las Lagunillas, Edificio Ciencias Experimentales y de la Salud (B3), Dependencia: B3-148, Universidad de Jaén, E23071 Jaén, España

Abstract

Teeth constitute a bridge between an organism and its environment. Dental wear is a good proxy for (paleo) ecologists to better comprehend the ecology and habitat of modern and extinct species. In this study, we showed Dental Microwear Texture Analysis to be a useful tool, integrating not only specific plant selection but also dietary quality and digestibility in order to understand resource use. Resource-partitioning, seasonal and sexual variations in the diet of two deer species on a Spanish game estate are explored here through Dental Microwear Texture Analysis. This Mediterranean area is on the fringes of the average European environments in terms of constraints and diet for extant red and fallow deer, resulting in an opportunity to understand their ability to live in harsh conditions and the feeding strategies they developed. These two taxa already experienced harsh living conditions during the Pleistocene. Dental microwear texture shows both deer feeding differently on the herbaceous layer in a context where it is the main resource consumed annually. These differences are linked to body mass. With its smaller incisor arcade, Dama dama is able to be more selective, hence focusing on less fibrous parts of forages. Cervus elaphus is more plastic, with dietary variations corresponding to seasonal plant availability and the physiological requirements of stags and does. In general, C. elaphus consumes a more fibrous and less digestible vegetable material than D. dama.

This study brings light on the feeding behavior of the two game species under constraint conditions. The results of this study are discussed in terms of realized vs potential ecological niches.

Introduction

Deer are widespread in Europe. They have experienced a significant, global increase in abundance and spatial distribution since the fifties because of reintroduction, lack of predators and establishment of hunting regulations (Apollonio et al., 2010; Nugent et al., 2011). Cervids are characterized by a relative physiological plasticity, allowing them to adapt to different types of both natural and anthropic habitats.

The red deer, *Cervus elaphus*, is the most widely distributed cervid in the world (Deinet et al., 2013). In the Northern hemisphere, it occupies a large range of environments, from the cold forests of Norway to the Scottish moorlands and the Mediterranean forests of Tunisia and Algeria (Gebert and Verheyden-Tixier, 2001; Hajji et al., 2007). In the past, this taxon tended to live in more open habitats (Drucker et al., 2003). Conversely, the latitudinal range of the fallow deer *Dama dama* is more restricted, extending from 61° N to 46° S. This taxon is mainly present in the western part of the Palearctic ecozone, where it has been (re-)introduced in many countries. Fallow deer occupy a large range of mosaic habitats, from forests to grasslands and scrublands, with broad-leaved forests being the most usual environment. Indigenous fallow deer populations were described in North Africa, but disappeared there during the 19th century (Chapman and Chapman, 1980). The geographic distributions of *C. elaphus* and *D. dama* in Europe overlap (Fig. 1a; Deinet et al., 2013; Chapman and Chapman, 1980). Both taxa co-occur along the northern margin of the Mediterranean basin, characterized by an arid climate contrasting with their optimal environment (Fig. 1e; Bugalho and Milne, 2003).

Both red and fallow deer are classified as intermediate feeders, feeding opportunistically on mixed diets of grasses and concentrate food items such as browse, forbs and fruits (Hofmann, 1989). The diet of the red deer depends on resource availability throughout the year (Gebert and Verheyden-Tixier, 2001). Whereas in other European areas red deer diet is dominated by browse (Clutton-Brock et al., 1989) with a constant significant proportion of silica-bearing herbaceous monocots (about 30%) all year round (Gebert and Verheyden-Tixier, 2001), the main source of food in the Mediterranean ecosystem is herbaceous monocots (see Table 2 in Azorit et al., 2012).

Herbaceous monocots constitute an important proportion of the fallow deer diet whenever available, regardless of the type of habitat (England: Jackson, 1977; Poland: Obidziński et al., 2013; Spain: Garcia-Gonzalez and Cuartas, 1992). Browse complements this diet, as well as acorns as soon as these food items are available (Nugent, 1990; Bruno and Apollonio, 1991; Azorit et al., 2012). Fallow deer even feed selectively on plants that are often not abundant in their environment (Jackson, 1977; Nugent, 1990).

Throughout an entire year of observation, Azorit et al. (2012) explored the dietary habits of sympatric red and fallow deer from the Mediterranean environment of Lugar Nuevo in southeastern Spain by means of a large dataset of rumen contents. Being a semi-arid environment, Lugar Nuevo offers a unique opportunity to explore the feeding strategies of the two sympatric game populations in the context of a peripheral constraint ecospace.

Teeth are the very first organs of the digestive tract and are the bridge between an organism and its environment. Therefore, paleoecologists use dental wear as a proxy to identify the dietary habits, the type of environment and the ecological niche of fossil species, the niche partitioning between species occurring in sympatry, and to track changes

*Corresponding author

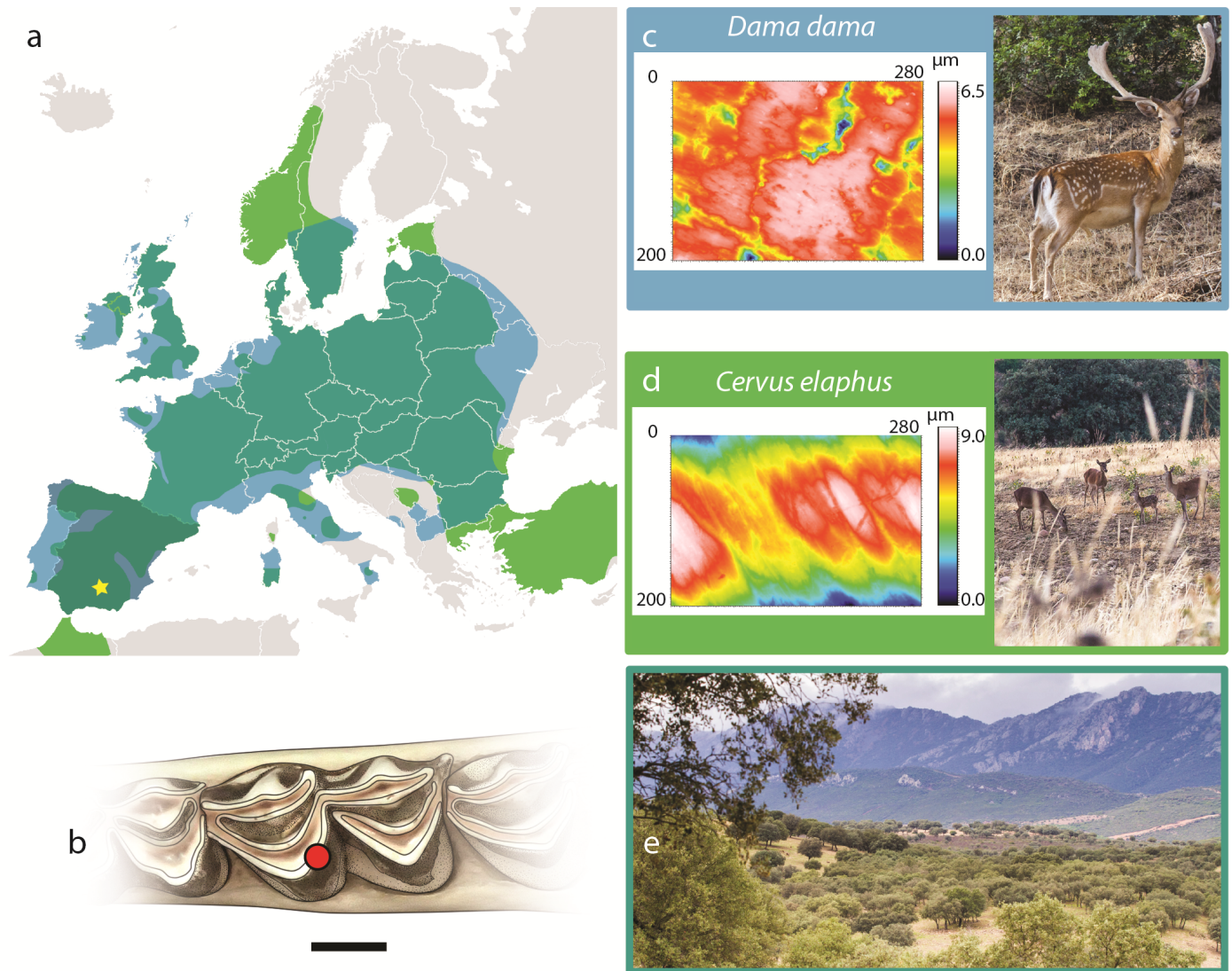*Email address:* emilie.berlioz@univ-poitiers.fr (Emilie Berlioz)

Figure 1 – Locality of Lugar Nuevo, South of Spain. a) Distribution ranges in Europe for *C. elaphus* in green and *D. dama* in blue, modified from http://www.iucnredlist.org. Lugar Nuevo is illustrated by a star. b) Occlusal view of a left red deer lower m2. The lingual facet of the protoconid of the m2 is circled in red. c) Topography of the dental facet and photography of a fallow deer from Lugar Nuevo. d) Topography of the dental facet and photography of red deer from Lugar Nuevo. e) Panoramic view of the dehesa of Lugar Nuevo.

in the feeding ecology of extinct species through time and space (see Calandra and Merceron, 2016; DeSantis, 2016; Ungar, 2015). These changes reflect macroevolutionary diversity patterns affecting the balance between major feeding types in ungulates (Solounias and Semprebon, 2002; Raia et al., 2011).

Dental Microwear Texture Analysis (DMTA) quantifies tooth wear surfaces and reflects the physical properties (hardness, toughness and abrasiveness; Ungar, 2015) and the inner composition of the food items ingested (phytoliths) as well as the way to comminute them. It reflects what a given animal has eaten during the last few weeks or days before death. DMTA not only allows the identification of grazers, browsers, and mixed feeders, but also to distinguish between fruit and leaf browsers, and between variable grazers and obligate grazers (Calandra and Merceron, 2016). Furthermore, DMTA is an efficient tool for deciphering ecological niche partitioning between species, variations in diet at the inter-populational scale, and even subtle variations between sexes and between seasons within a population (Merceron et al., 2010, 2014). In this study, DMTA is coupled with Scale Sensitive Fractal Analysis (SSFA; Scott et al., 2005, 2006). This latter approach has already been successfully applied to analyze the dental microwear signal in several taxa including primates, ungulates and carnivores (Ungar, 2015). More than a proxy for paleoecologists, DMTA can be used as a key tool for biologists as to evaluate the quality and physical properties of the diet of modern species.

This study uses a large dataset of red and fallow deer sampled with rumen content, known sex, date and location of death from Azorit et al. (2012) and Tellado and Azorit (2014). Given that the mandibles were gathered at the same time as the stomach content sampling, the proposal here is to explore the feeding habits and resource partitioning of these two living cervids on the fringe of their present core distributions by using DMTA (Fig. 1). We aimed to investigate: i) the seasonal variations in the diet of the two deer populations and the physical properties of this diet, ii) dietary variations depending on sex, a result of the differing physiological requirements of males and females and then iii) the different feeding behaviors and how the two species share the available vegetal resources throughout the year in the constraint periods of this Mediterranean environment.

Materials and Methods

Materials

Study area

Lugar Nuevo situated in the Sierra de Andujàr Natural Park, in southeastern Spain (38°9′ N, 4°3′ W), is a 10 km² enclosed game estate that displays the typical vegetation of the Iberian Peninsula, called "dehesa" (Fig. 1e). This Mediterranean ecosystem, similar to the Portuguese "montado", is a Spanish traditional agro-silvo-pastoral system made up of scattered tree cover with an understory of open savannah-like grasslands. The vegetation grows in low fertility soil subject to light preci-

pitation, but with Mediterranean herbage characterized by a density of between 120–180 species/1000 m^2 (Marañón, 1991). Such a high density of flowering plant species ranks the pasture grasses in Sierra Morena amongst some of the most diverse plant communities and constitutes one of the most diverse types of vegetation found in the temperate zone (Marañón, 1991; Naveh and Whittaker, 1980; Olea and San Miguel-Ayanz, 2006). The herbaceous communities in this Mediterranean ecosystem are typical of siliceous soil, including monocotyledon species such as Cyperaceae, Iridaceae, and mainly Poaceae but also dicotyledons such as Fabaceae, Asteraceae, Scrophulariaceae, Lamiaceae and more. The tree cover in the dehesa of Lugar Nuevo is composed mainly of cork, gall and holm oaks (*Quercus suber*, *Q. faginea*, *Q. rotundifolia*) as well as conifers (*Pinus* sp.) and wild Olive trees (*Olea europaea* var. *sylvestris*). The main bush species are *Citysus* sp., *Ulex* sp., *Lavandula* sp., *Hilimium* sp., *Erica* sp., *Pistacea* sp., *Phillyrea* sp., *Cistus* sp. and *Rosmarinus officinalis* (Joffre et al., 1988; Diaz et al., 1997; Olea and San Miguel-Ayanz, 2006).

The climate in Lugar Nuevo is wet and mild in winter, with significant precipitations during spring and autumn (annual precipitation varies between 200 and 900 mm) and almost no rain coupled to high temperatures during summer. Year 2008 was characterized by significant rainfall (856.2 mm) separated into two main periods: 40% during spring, and some 30% in autumn (Azorit et al., 2012).

Two cervids: D. dama *and* C. elaphus

We focused on the lower second molars (m2, Fig. 1b) of 48 adult wild *D. dama* and 102 adult wild *C. elaphus hispanicus* shot over a period of 12 months in 2008–2009. Specimens were culled every month to catch the seasonal variation in diet as revealed by the stomach content analysis (Tab. S1). Mandibles, along with life trait history data concerning each specimen were prepared following standard procedures and housed at the Vertebrate Laboratory at the University of Jaen, Spain. During the sampling period, the red deer density in Lugar Nuevo was 23.3 deer per km^2, while the density of fallow deer was 4.0 deer per km^2. Diet was described as the percentage of dry weight composition of grass, fruit and browse in the rumen content (Azorit et al., 2012; Fig. 2 and Tab. S1). Live weights for the red and fallow deer from Lugar Nuevo are not available. However, annual live weight variations of deer have been the focus of numerous scientific articles (Apollonio and Di Vittorio, 2004; Weber and Thompson, 1998; Jopson et al., 1997; Thimonier and Sempere, 1989; Asher et al., 1987; Mitchell et al., 1976; Lincoln, 1971). We considered this bibliography for our interpretations.

Following the procedure of Azorit et al. (2012), deer were grouped into a 6-interval framework (January – February; March – April; May – June; July – August; September – October; November – December; Tab. S2 and S3). This procedure best highlights variations in the feeding behaviour of deer that stem from the vegetal phenology and the species biological cycles. However, two other approaches were proposed (Tab. S5–S9): a 4-season (spring, summer, autumn and winter) analysis, and a 2-period (bimodal) sampling considering the impact of the presence/absence of acorns in the diet and year-round deer requirements. These two alternative approaches and the relative results are presented in the supplementary material.

Methods

Pre-processing procedures

Each lower second molar was carefully cleaned with acetone. Molds were made of the disto-lingual facet of the protoconid (Fig. 1b). For this, a high-resolution polyvinylsiloxane was used (Regular Body President, ref 6015 – ISO 4823, medium consistency, polyvinylsiloxane addition type; Coltene Whaledent). The surface of the silicon molds was scanned using a white light confocal surface profilometer Leica DCM8 with a 100× objective (NA=0.90, working distance=0.9 mm) at the iPHEP lab (CNRS and University of Poitiers, France). For each specimen, a 251×333 µm surface (2584×1945 points) at the center of the dental facet was scanned (Fig. 1, c and d). Four adjacent 100×140 µm sub-surfaces were generated from this surface, following the regular procedure (Scott et al., 2006). Each sub-surface was levelled and mirrored in Z (see Calandra and Merceron, 2016).

Acquisition of the textural parameters

The surfaces were analyzed by a Scale Sensitive Fractal Analysis (SSFA) using ToothFrax and Sfrax software (Surfract, www.surfract.com). For each surface, four parameters were calculated using the SSFA algorithms (Scott et al., 2006): Area-scale fractal complexity (hereafter Asfc), exact proportion Length-scale anisotropy of the relief (epLsar), Textural fill volume (Tfv), and Heterogeneity of the Area-scale fractal complexity (HAsfc hereafter; 81 cells; Calandra and Merceron, 2016; Scott, 2012; Scott et al., 2006).

epLsar Asfc and HAsfc respectively measure the anisotropy, the complexity and the heterogeneity of the complexity of the surface roughness. Finally, Tfv depends on the relief of the thin texture of the dental surface.

The mastication of leaves is supposed to favor a low anisotropy of the surface texture (epLsar) coupled with a medium to high complexity (Asfc; Scott, 2012). In the case of hard items like seeds, Asfc is even higher. Conversely, in the case of the mastication of tough abrasive monocotyledons, the anisotropy of the surface (epLsar) will be high, coupled with low values for complexity (Asfc).

When the diet of an animal is monotypic, such as it is for obligate grazers and leaf browsers, the surface heterogeneity (HAsfc) is expected to be lower than for herbivores feeding on a large spectrum of food categories. The Textural Fill Volume (Tfv) is higher when the microwear features are deep and/or large, for example in the case of pits resulting from seed consumption. Interpretations of the textural parameters are based on the work of Scott (2012).

Statistical analysis

The analyses were performed with R software (R version 3.2.2, The R Foundation for Statistical Computing), using the packages "car", "Rmisc", "ape", "vegan" and "agricolae". We used "plotrix" and "ggplot2" for drawing plots. For each specimen, the median of the four adjacent sub-surfaces was calculated for each parameter (Scott et al., 2006). The mean and standard error of mean were calculated for each of the groups and sub-groups considered in this study. First, a permutational multivariate analysis of variance (MANOVA; Tab. 1) considering the species, sex and period of death of individual specimens was performed in order to test for general dietary differences between and within species.

Table 1 – Permutational Multivariate Analysis of variances (MANOVA). Bold indicates a significant difference explained by the modalities of the variable concerned. The six periods considered are Jan.-Feb.: January-February; Mar.-Apr.: March-April; May-Jun.: May-June; Jul.-Aug.: July-August; Sep.-Oct.: September-October; Nov.-Dec.: November-December.

Taxon	Sex	Period	Variable	df	F	p
			taxon	1	14.0497	**0.001**
			sex	1	0.4020	0.785
Cervus			period	5	1.1916	0.263
+	♀♂	6	taxon × sex	1	1.6585	0.170
Dama			taxon × period	5	1.3382	0.186
			sex × period	5	0.7677	0.699
			taxon × sex × period	4	0.9275	0.500

Then, prior to any further analysis, the values for each parameter were replaced by their rank, in order to run parametric tests on data that usually violate the assumption of normality (Scott, 2012; Merceron et al., 2010; Conover and Iman, 1981). Two-way (species, period of the year) analysis of variance (ANOVA) was performed on the whole dataset in order to identify dental microwear textural differences between the two deer. Two-way ANOVAs (period, sex) were run to identify sub-groups based on species, on sex for each season among *Cervus* and *Dama*. With one-way ANOVAs, the aim was to test for differences between sub-groups identified based on the species, sex, and time of death of specimens (Tab. 2). Finally, Post Hoc Tukey's Honestly Significant Difference tests (HSD tests) and Fisher's Least Significant Difference tests with the adjustment method of Bonferroni (LSD tests) were performed on factors presenting more than two levels (i.e. periods), in order to balance the risks of type I and type II errors (Legendre and Legendre, 2012; Cook, 1996; Sokal and Rohlf, 1998).

In the same way, a permutational MANOVA considering the species, sex and period of death of individuals, followed by a rank-transformation (Tab. S5), two-way ANOVAs (species, period of the year and period, sex) and one-way ANOVAs (sex, period and species) were run for the 4-season analysis and the 2-period sampling considering the impact of the presence/absence of acorns in the diet (Tab. S6–S9). As unavailable for this dataset, body mass was not included as a variable in our analyses.

Results

Differences in the dental microwear pattern of *C. elaphus* and *D. dama*

The results of the permutational MANOVA highlight a difference in diet between species (Tab. 1; $p=0.001$). This inter-specific difference is explained by all textural parameters (Tab. 2; $p<0.01$ for all analyses of variance; Fig. 2). Red deer have higher anisotropy (epLsar; 6.11×10^{-3} compared to 3.22×10^{-3}) and Textural fill volume (Tfv; $7684.8\,\mu m^2$ compared to $2762.5\,\mu m^2$) and lower complexity (Asfc; 1.18 compared to 1.59) and heterogeneity of the complexity (HAsfc; 0.78 compared to 1.16) than fallow deer (Tab. S2 and S3, Fig. 2). When observed by time period (Tab. 2, S2 and S3), *C. elaphus* and *D. dama* from the January-February period significantly differ from each other, *Cervus* having higher Tfv ($10\,102.7\,\mu m^2$ compared to $3744.7\,\mu m^2$; $p<0.001$) and lower heterogeneity of the complexity (HAsfc) than *Dama* (0.75 compared to 0.95, $p=0.025$). In May-June, red deer present a higher anisotropy (epLsar; 5.44×10^{-3} compared to 2.85×10^{-3}; $p=0.031$) and a lower HAsfc than fallow deer (0.74 and 1.12 respectively; $p=0.005$). In July-August, *Cervus* has higher epLsar (5.80×10^{-3} compared to 2.69×10^{-3}; $p<0.001$) but lower Asfc (1.1 compared to 1.98; $p=0.035$) and HAsfc (0.81 compared to 1.30; $p<0.001$) than *Dama*. For the September-October interval, *Cervus* still have higher epLsar (4.98×10^{-3} compared to 2.39×10^{-3}; $p=0.003$) and Tfv ($7469.8\,\mu m^2$ compared to $3270.7\,\mu m^2$; $p=0.011$) and a lower HAsfc (0.89 compared to 1.24, $p=0.009$) than *Dama*. There is no significant difference between the two taxa in March-April, or in November-December ($p>0.05$).

The results of the permutational MANOVAs considering four and two periods (Tab. S5) highlight a difference in diet between species ($p=0.001$). There is also a difference between the periods with and without acorns when considering both species together ($p=0.004$).

Influence of the season and the sex of the red deer on their diet

The anisotropy (epLsar) is significantly higher for female red deer than it is for males (6.48×10^{-3} and 5.38×10^{-3} respectively; $p=0.006$; Tab. 1, S2 and S3). These differences occur from January to April ($p=0.005$ and 0.021 respectively). There is also a significant difference of complexity (Asfc) between males and females in November-December (1.48 compared to 0.95, $p=0.012$), males having a higher Asfc than females. There is no sexual differentiation possible from May to October ($p>0.05$).

When considering only males, there is no significant difference of the dental microwear parameters over the year (Tab. 2; $p>0.05$). On the other hand, female red deer differ in anisotropy (epLsar) and complexity (Asfc) from one period to another ($p<0.05$ in both cases). These differences were identified by the post hoc tests (Tab. S4). The complexity (Asfc) values are significantly higher in September-October than in May-June (1.4 and 0.8 respectively; $p<0.05$) and the anisotropy (epLsar) values are significantly higher in March-April than they are from May to October (9.27×10^{-3} compared to 5.30×10^{-3}, 5.23×10^{-3} and 5.60×10^{-3}; $p\geqslant0.004$). While Tukey's HSD test also supports a difference of epLsar between July-August and November-December ($p=0.044$), the difference is not supported by Fisher's LSD test ($p=0.060$; Fig. 2a; Tab. S4) and will therefore not be considered as reliable enough to be interpreted as a real dietary difference between the females of these two periods.

Influence of the season and the sex of the fallow deer on their diet

There is no sexual differentiation among the fallow deer population over the course of one year ($p\geqslant0.126$). When considering seasons, female fallow deer present a higher HAsfc than stags in May-June. (1.38 compared to 0.99; $p=0.042$; Tab S2 and S3; Fig. 2).

In a group of males only and a group of females only, there is no difference in dental microwear parameters from one period to another ($p\geqslant0.209$; Tab. 2).

The small sample size for male and female fallow deer, (N varies between 4 and 5 for females and between 0 and 8 for males from one season to another) may explain the fact that there is no significant differences between seasons.

Discussion

Two deer species with different physiological requirements in a constraining environment

At Lugar Nuevo, three ecologically distinct periods can be identified throughout the year (Azorit et al., 2012; Bugalho and Milne, 2003): (i) an abundance of fruit corresponding to the acorn mast (gall, holm and cork oaks) from September to February; (ii) a cool and moist winter corresponding to the period of plant regrowth, resulting in the availability of green nutritive plant material until June, (iii) a harsh drought summer season in July and August, characterized by the senescence of the herbaceous layer resulting in scarce food resources of poor quality, with fodder, dry and brown pasture, which is rich in fiber.

Photoperiod is a fundamental parameter that determines the annual cycle of sexual activities of deer as well as influences changes in their food intake resulting in annual live weight variations (Weber and Thompson, 1998; Jopson et al., 1997; Mitchell et al., 1976). Red and fallow deer present similar and relatively synchronous annual reproductive cycles with the mating period extending between mid-September and the end of November and births occurring between May and mid-July (gestation duration of around 233 days for the red deer and 229 days for the fallow deer; Thimonier and Sempere, 1989). The decrease of daylight duration since the summer solstice results for the deer in an increased production of melatonine (Thimonier and Sempere, 1989; Asher et al., 1987). This is notably responsible for male hypophagia that starts weeks before the mating period and results in an important weight loss (Apollonio and Di Vittorio, 2004; Jopson et al., 1997; Mitchell et al., 1976; Lincoln, 1971). At the end of the rut, males lose 15–30% of their optimal weight. After the rut season, males start to regain weight. However, they only reach reach their optimal weight concomitantly with the improvement of food quality. Less important live weight variations occur in females. Their optimal physical condition is reached during the reproductive season (Weber and Thompson, 1998). There is a live weight decrease in winter (Mitchell et al., 1976) followed by an increase in spring following the improvement of available resource quality.

Table 2 – Inter- and intra-population analyses of variances (ANOVA). Bold indicates significant differences between taxa, sex or time of year, considering both sexes, only males and only females. Asfc: complexity; epLsar: anisotropy; HAsfc-8l: heterogeneity of the complexity; Tfv: Textural fill volume. Jan.-Fev.: January-February; Mar.-Apr.: March-April; May-Jun.: May-June; Jul.-Aug.: July-August; Sep.-Oct.: September-October; Nov.-Dec.: November-December.

| Taxon	Sex	Period	H_0	df	Asfc F	Asfc *p*	epLsar F	epLsar *p*	HAsfc-81 F	HAsfc-81 *p*	Tfv F	Tfv *p*
Cervus + Dama	♀♂	**All**		1	7	**0.009**	36.87	**<0.001**	47.3	**<0.001**	10.60	**0.001**
		Jan.-Feb.		1	1.45	0.243	3.63	0.071	5.87	**0.025**	29.50	**<0.001**
		Mar.-Apr.		1	0.38	0.548	1.20	0.296	2.71	0.128	0.27	0.615
		May.-Jun.		1	0.14	0.717	5.61	**0.031**	10.41	**0.005**	0.77	0.394
		Jul.-Aug.	equality between species	1	4.95	**0.035**	17.10	**<0.001**	19.3	**<0.001**	0.41	0.528
		Sep.-Oct.		1	1.37	0.251	10.66	**0.003**	7.68	**0.009**	7.22	**0.011**
		Nov.-Dec.		1	0.33	0.570	1.36	0.255	0.13	0.726	0.62	0.440
Cervus	♀♂	**All**		5	1.42	0.224	5.04	**<0.001**	1.51	0.196	1.54	0.187
	♀	**All**	equality between seasons	5	2.96	**0.019**	6.16	**<0.001**	0.89	0.496	2.31	0.055
	♂	**All**		5	0.51	0.764	2.18	0.084	1.45	0.238	0.39	0.854
	♀♂	**All**		1	3.05	0.084	7.95	**0.006**	2.81	0.097	0.48	0.490
		Jan.-Feb.		1	1.09	0.315	11.21	**0.005**	1.38	0.262	0.01	0.913
		Mar.-Apr.		1	0.38	0.554	8.16	**0.021**	1.78	0.219	0.38	0.554
		May.-Jun.		1	1.76	0.209	0.00	1.00	0.25	0.625	0.06	0.808
		Jul.-Aug.	equality between sexes	1	0.00	0.963	2.95	0.105	1.08	0.315	1.08	0.315
		Sep.-Oct.		1	0.16	0.689	3.96	0.059	0.62	0.440	0.97	0.336
		Nov.-Dec.		1	7.81	**0.012**	1.20	0.287	2.62	0.122	2.33	0.143
	♀♂	**All**	sex × seasons	5	1.59	0.171	3.25	**0.010**	0.55	0.74	0.96	0.448
Dama	♀♂	**All**		5	1.86	0.126	1.53	0.203	1.22	0.319	0.61	0.691
	♀	**All**	equality between seasons	4	1.63	0.209	1.48	0.249	1.52	0.237	0.18	0.948
	♂	**All**		5	1.14	0.373	0.69	0.638	0.94	0.476	0.94	0.477
	♀♂	**All**		1	0.84	0.364	0.04	0.848	0.35	0.558	1.70	0.200
		Jan.-Feb.		1	4.07	0.084	0.51	0.500	0.95	0.361	1.62	0.244
		Mar.-Apr.		1	0.43	0.559	0.43	0.559	3.00	0.182	0.43	0.559
		May.-Jun.		1	0.00	1.000	0.83	0.414	8.73	**0.042**	0.04	0.843
		Jul.-Aug.	equality between sexes	1	0.24	0.634	0.24	0.634	1.04	0.331	2.05	0.183
		Sep.-Oct.		1	0.03	0.874	0.03	0.874	0.24	0.634	0.70	0.422
		Nov.-Dec.		1	–	–	–	–	–	–	–	–
	♀♂	**All**	sex × seasons	4	0.51	0.726	0.16	0.957	1.02	0.409	0.32	0.860
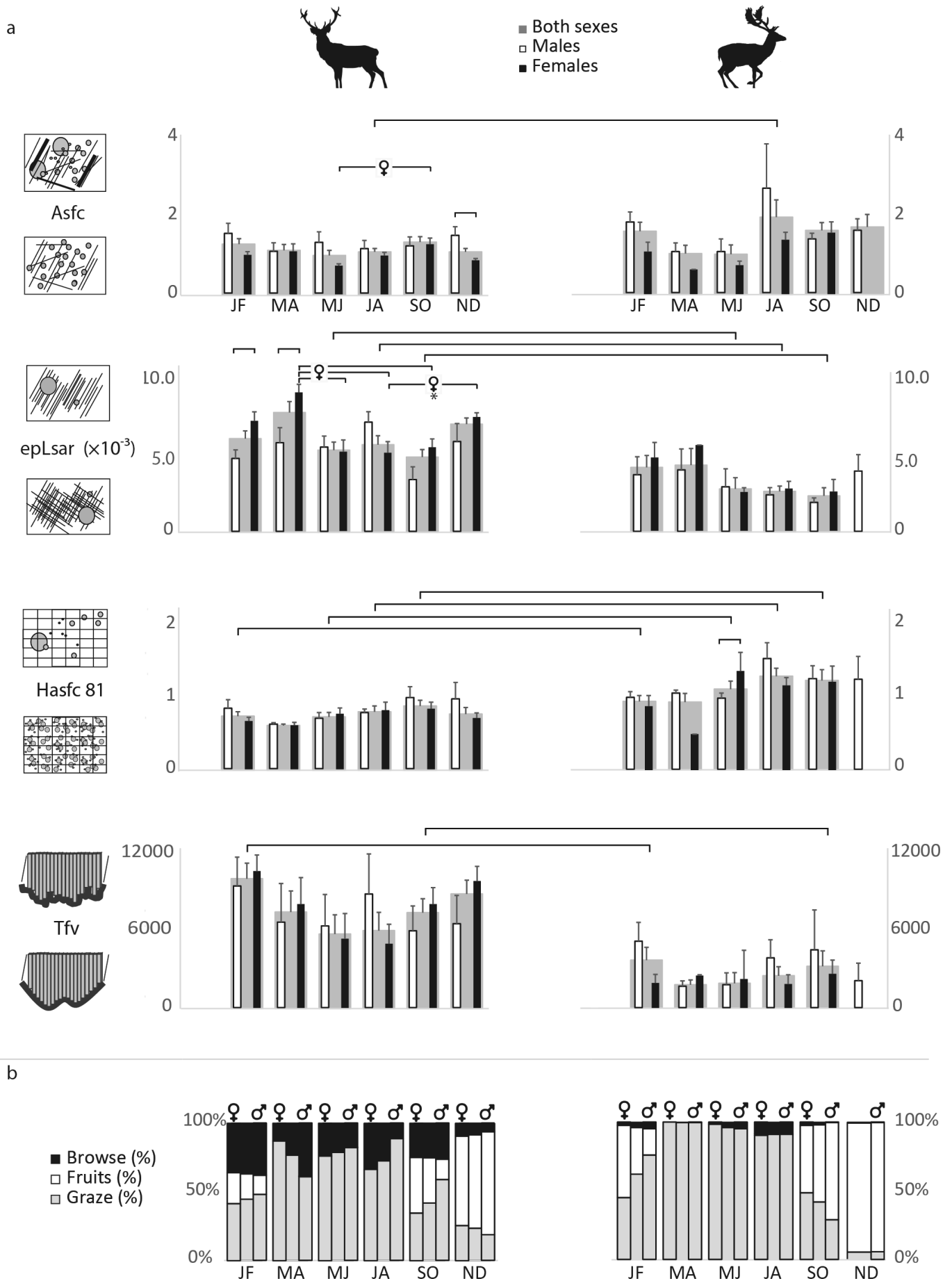
Figure 2 – Inter- and intra-population variations of the dental microwear parameters and percentage of Browse, Fruits and Graze in diet. The division of the year is as follows: JF: January-February; MA: March-April; MJ: May-June; JA: July-August; SO: September-October; ND: November-December. a) Results for the red deer are on the left and fallow deer on the right of the figure. Mean and standard error of mean (s.e.m.) for the global populations are illustrated in grey, mean and s.e.m for the females are in black, and males are in white for each of the six periods of the year. The square brackets represent significant differences between species, seasons and sexes and refer to the results of ANOVAs and Post Hoc tests. All the significant differences shown are supported by both LSD and HSD Post Hoc tests, excepting the difference marked with a star (*). In this case, only the HSD test supports the difference. b) Percentage of Browse, Fruits and Graze in the diet of *C. elaphus* and *D. dama* for the six periods of the year. The consumption of Browse is illustrated in black, Fruits are in white, and Graze in grey and are illustrated for each of the six periods, for the entire population (middle), the females (left) and the males (right).

Overall, there is a latency of several weeks between food intake reduction and the resulting live weight loss (Weber and Thompson, 1998).

Significant differences in dental microwear textures were identified between the two deer (Tab. S2 and S3). The diet of *C. elaphus* from Lugar Nuevo reflects a diet richer in fibers, while *D. dama* displays a trend toward a bimodal diet, poorer in silica-bearing monocots (Fig. 2a). These significant dietary differences reflected by DMTA between the two deer throughout the year are the result of differences in food preferences and metabolic requirements. Indeed, it is well known that larger ruminants tolerate lower quality food than smaller species (Van Soest, 1994; Gordon and Illius, 1988). The fallow deer, with its smaller incisor arcade is able to take more selective bites and feed on better quality, less fibrous parts of forages. *Dama dama* even feeds on the parts of taller herbaceous monocots that are of better nutritive quality, as well as on herbaceous dicotyledon fabaceae that are higher in cellular protein and less fibrous than mature herbaceous monocots. Moreover, due to its lower body mass, the fallow deer presents higher metabolic requirements and is therefore less able to process low quality food (Müller et al., 2013; Illius and Gordon, 1992; Demment and Van Soest, 1985; Geist, 1974). On the other hand, the red deer is able to consume forages with slower digestion rates, richer in fibers.

Resource-partitioning between the two species is expected to be critical in July and August when habitat conditions are harsher. The differences in dental microwear textures clearly depict this resource-partitioning: the red deer grazes on vegetation that is highly abrasive compared to the diet of *D. dama*. Furthermore, the fallow deer dental microwear textures tend to support soft (leaf) browsing. For this time interval, the ingestion of fresh growing herbaceous monocots, poor in biosilica compared to mature plants, is not supported since these plants grow during the wet season in a Mediterranean context (Fig. 2).

The environmental pressure decreases in September-October with the introduction of acorns into the diet as soon as this food resource is available. The acorn consumption results in a tendency towards an increase of the heterogeneity of the complexity (HAsfc) and a decrease of anisotropy (epLsar) as a consequence of the processing of large, hard food elements.

In contrast to the DMTA, stomach content analysis (Azorit et al., 2012) indicates higher grazing habits for the fallow deer than for the red deer (Fig. 2b). The dental abrasion showed by the DMTA of red deer is related to feeding on herbaceous monocots supposed to be more abrasive and fibrous than other components of forages.

The mean percentage of herbaceous monocots consumed by both deer is very high in this area: 57.06%±13.17 in red deer versus 61.25%±10.72 in fallow deer (Tab. 2 in Azorit et al., 2012). These results are strongly contrasting with the regular 30% of herbaceous monocots consumed by the red deer in Europe regardless of sex, habitat and season (Gebert and Verheyden-Tixier, 2001), confirming the peculiar environmental status of the dehesa within the ecological range of the red deer. The characterization of the 3D dental microwear texture allows us to distinguish between deer feeding differently on herbaceous monocots in a context where this food category is the main resource consumed throughout the year, peaking in spring. The diet of the fallow deer is less flexible, as this species feeds selectively on better quality plant species or on more nutritive and softer parts of plants, less rich in fibers than the food ingested by red deer (van der Merwe and Marshal, 2014). The DMTA results are coherent with the results on diet quality and digestibility, with red deer consuming a significantly more indigestible diet than fallow deer all year round (Tellado and Azorit, 2014).

One could be surprised to notice contrasted results between DMTA and stomach content analysis. Actually, DMTA does not record the dietary composition itself but the physical properties of the food items consumed the very last few weeks before death. Besides, stomach content analyses do not picture the whole diet but the dietary bolus of the last few hours. Moreover, this latter method suffers from the sieving procedures and the differential digestibility of the food items.

Seasons, sex and diet in the red deer population from Lugar Nuevo

Scott (2012) studied the inter-specific variation in DMTA of a large set of modern African bovids covering the whole dietary spectrum. The red deer from Lugar Nuevo plot within the variable/obligate grazing ecospace. These results provide a new piece of evidence and confirm the habitat-dependent diet of the red deer (Gebert and Verheyden-Tixier, 2001). Conversely to most of the European populations of red deer, herbaceous monocots represent a much higher proportion of the dietary bolus in Lugar Nuevo (around 60% compared to 30% for most populations) illustrating once again the feeding plasticity of the red deer. Although its diet is largely composed of herbaceous monocots all year round, there are visible seasonal variations and differences between males and females. These differences are common for red deer in their natural range (Roberts et al., 2015; Clutton-Brock et al., 1982).

It is worth to mention that these seasonal variations are only significant for does. The dental microwear textures and especially the anisotropy (epLsar) of these textures suggest that does feed less and less from March to August on herbaceous monocots rich in silica phytoliths and fibers. This might reflect the drop in the nutritive qualities of herbaceous monocots over time (Bugalho and Milne, 2003). As a consequence, does switch to a food of better quality, poorer in herbaceous monocots and richer in herbaceous dicots, in order to reach their nutritional requirements during the crucial period of milk production (Groot Bruinderink and Hazebroek, 1995; Mitchell et al., 1976). For female red deer, spring and summer is also a period of body weight increase to reach an optimal physical condition and weight before the reproductive season (Mitchell et al., 1976). The shift towards a browsing signal in September-October is most likely the result of the introduction of acorns to the diet in autumn, as this fallback food resource constitutes an important, highly digestible source of energy during the rutting period (Poli et al., 1996).

There are differences between stags and does from November to March. These differences first occur during the autumnal mating season when stags maintain harems on a limited geographic area. Stags lose around 15–30% of their body mass during this period because they cease feeding (Mitchell et al., 1976). This is supported by both DMTA and the rumen content analysis. Then, during winter, when males are recovering after the rutting period, stags are known to experience an episode of post-rut hyperphagia (Groot Bruinderink and Hazebroek, 1995) that is however not as fast as to reach the top yearly body weight anytime before the spring plant regrowth period (Lincoln, 1971). Females ingest lower quantities of food as compared to their food intake in spring (Mitchell et al., 1976). This peculiar gender difference in feeding behavior may explain the significant variation in DMTA between sexes during this period. Such gender difference in dental microwear textures has been also reported on roe deer, with male feeding much more on acorns than females during winter (Merceron et al., 2010).

From April to October, when red deer of the two sexes are spatially separated, there is no difference in DMTA. This is true even during the lactation period, when females ingest important quantities of better quality food. In addition, there are no changes in dental microwear textures for the stags during the antlerogenesis (April – September), which is coherent with previous ecological analyses (Groot Bruinderink and Hazebroek, 1995).

Seasons, sex and diet in the fallow deer population from Lugar Nuevo

D. dama present dental microwear textures that contrast with the diet of *C. elaphus* throughout the year and tend to favor a generalist or Browser-Grazer intermediate diet (Scott, 2012). These results are very interesting because in several studies herbaceous monocots have been reported to be predominant in the fallow deer diet (Obidziński et al., 2013; Putman et al., 1993; Garcia-Gonzalez and Cuartas, 1992; Jackson, 1977). Browse is however an important component of the diet of this deer in other study areas (Bruno and Apollonio, 1991; Nugent, 1990). These results highlight the relative flexibility of fallow deer, already underlined by some authors (Obidziński et al., 2013; Feld-

harner et al., 1988; Chapman and Chapman, 1980). In this study, it was found that the fallow deer is able to browse and to select plants of a high quality and with low hardness (i.e. fabaceae) among the herbaceous layer, as well as to select plant parts of better quality as a result of the small size of its bites (Van Soest, 1994).

While the annual biological cycles of male and female fallow deer are similar to the cycles in red deer (Schaal et al., 1991; Teillaud et al., 1991), there is no significant variation in the dental microwear textures of fallow deer throughout the year, two major periods can be nonetheless identified (Fig. 2). During summer, the fallow deer does not show the grazing signal seen in the red deer. The results show a diet with lower abrasive properties, more likely to be the result of soft browse items and young, high quality herbaceous monocots. Conversely, during autumn it is worth noting that although non-significant, the dental microwear textures marginally reflect a tendency to a highest intake of acorn during the mast season. Such a bimodality of the diet of *D. dama* has been tested (see Tab. S8 and S9), but ANOVAs performed on the 2-periode sampling (with and without acorns) showed no significant differences, indicating further data and analysis are probably worth pursuing.

We found no significant annual difference either in the dental microwear textures of males, even when linked to their post-rut nutritive requirements, or in the dental microwear signal of females, even after the parturition in June.

In May-June however, DMTA reflects a significantly different food intake between males and females. The results of the DMTA indicate that the diet of does is more diversified during this period than the diet of stags, with males consuming a more abrasive grazing diet while females eat better quality food at a time when the herbaceous layer has already started its senescence (Bugalho and Milne, 2003). Interestingly, it corresponds to the moment of the parturition and the weeks that precede and follow it, including the beginning of lactation.

Conclusions

This Dental Microwear Texture Analysis is the first comprehensive exploration of resource-partitioning between two sympatric large populations of herbivores in a peri-optimum environment, taking into consideration the physiological requirements of both sexes depending on the season. The dataset of Lugar Nuevo, with available stomach contents, was a unique opportunity to explore in details the differential feeding adaptability of the two deer.

If the two deer populations from Lugar Nuevo are able to survive in a constraint environment thanks to their ability to adapt to a large range of habitats, they do not develop the same subsistence strategies. *C. elaphus* is more involved in grazing food items that are rich in fibers while the smaller size *D. dama* is more selective, feeding on plants and plant parts that are of a better digestibility and nutritive quality. Furthermore these differences are amplified during the summer period, when food resources from the herbaceous layer are low in biomass and quality.

C. elaphus do not adapt to the vegetal phenology by changing their dietary composition, but rather pass through the drought season by foraging on lower quality food items, as reflected by the dental microwear textures. The difference in the diet of male and female red deer links to their annual biological cycle. The analysis highlights autumnal differences between red deer stags and does that were not observable with the stomach content analysis (Azorit et al., 2012).

Among male and among female *D. dama* there is no significant variations in dental microwear texture depending on the season. Both males and females rather focus all year round on plants with high nutritive qualities and a low fiber composition. The parturition is the only period when males and females differ.

Tracking the way different ungulate species share the vegetal resources is an opportunity to assess the health of the populations and to propose appropriate conservation management policies. Lugar Nuevo constitutes an ideal model to better understand the ability of the two deer species to face constraint situations. This study is a way to assess the adaptability and the ecological niche flexibility through dental wear analysis in order to propose a proxy to explore the ecology of extinct cervids in a context of climatic and environmental change. ✍

References

Apollonio M., Di Vittorio I., 2004. Feeding and reproductive behaviour in fallow bucks (*Dama dama*). Naturwissenschaften 91: 579–584.

Apollonio M., Andersen R., Putman R., 2010. European ungulates and their management in the 21st century. Cambridge University Press, Cambridge, UK.

Asher G.W., Day A.M., Barrell G.K., 1987. Annual cycle of liveweight and reproductive changes of farmed male fallow deer (*Dama dama*) and the effect of daily oral administration of melatonin in summer on the attainment of seasonal fertility. J. Reprod. Fertil. 79: 353–362.

Azorit C., Tellado S., Oya A., Moro J., 2012. Seasonal and specific diet variations in sympatric red and fallow deer of southern Spain: a preliminary approach to feeding behaviour. Anim. Prod. Sci. 52: 720–727.

Bruno E., Apollonio M., 1991. Seasonal variations in the diet of adult male fallow deer in a submediterranean coastal area. Rev. Ecol. (Terre Vie) 46: 349–362.

Bugalho M.N., Milne J.A., 2003. The composition of the diet of red deer (*Cervus elaphus*) in a Mediterranean environment: a case of summer nutritional constraint? For. Ecol. Manag. 181: 23–29.

Calandra I., Merceron G., 2016. Dental microwear texture analysis in mammalian ecology. Mammal Rev. 46(3): 215–228.

Chapman N.G., Chapman D.I., 1980. The distribution of fallow deer: a worldwide review. Mammal Rev. 10: 61–138.

Clutton-Brock T.H., Guinness F.E., Albon S.D., 1982. Red deer: behavior and ecology of two sexes. University of Chicago Press, Chicago.

Clutton-Brock T.H., Albon S.D., Guinness F.E., 1989. Fitness costs of gestation and lactation in wild mammals. Nature 337: 260–262.

Conover W.J., Iman R.L., 1981. Rank transformations as a bridge between parametric and nonparametric statistics. Am. Stat. 35: 124–129.

Cook R.J., 1996. Coupled error spending functions for parallel bivariate sequential tests. Biometrics: 52(2): 442–450.

Deinet S., Ieronymidou C., McRae L., Burfield I.J., Foppen R.P., Collen B., Böhm M., 2013. Wildlife comeback in Europe: The recovery of selected mammal and bird species. The Zoological Society of London, London.

Demment M.W., Van Soest P.J., 1985. A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. Am. Nat. 25(5): 641–672.

DeSantis L.R., 2016. Dental microwear textures: reconstructing diets of fossil mammals. Surface Topography: Metrology and Properties 4(2): 023002.

Diaz M., Campos P., Pulido F.J., 1997. The Spanish dehesas: a diversity in land-use and wildlife. In: Pain D., Pienkowski M., (Eds.). Farming Birds in Europe: The Common Agricultural Policy and its implications for bird conservation. Academic Press, London. 178–209.

Drucker D., Bocherens H., Bridault A., Billiou D., 2003. Carbon and nitrogen isotopic composition of red deer (*Cervus elaphus*) collagen as a tool for tracking palaeoenvironmental change during the Late-Glacial and Early Holocene in the northern Jura (France). Palaeogeography, Palaeoclimatology, Palaeoecology, 195: 375–388.

Feldharner G.A., Farris-Renner K.C., Barker C.M., Deer F., 1988. *Dama dama*. Mammalian Species 317: 1–8.

Garcia-Gonzalez R., Cuartas P., 1992. Food habits of *Capra pyrenaica*, *Cervus elaphus* and *Dama dama* in the Cazorla Sierra (Spain). Mammalia 56: 195–202.

Gebert C., Verheyden-Tixier H., 2001. Variations of diet composition of red deer (*Cervus elaphus* L.) in Europe. Mammal Rev. 31: 189–201.

Geist V., 1974. On the relationship of social evolution and ecology in ungulates. Am. Zool. 14: 205–220.

Gordon I.J., Illius A.W., 1988. Incisor arcade structure and diet selection in ruminants. Functional Ecology 2(1): 15–22.

Groot Bruinderink G., Hazebroek E., 1995. Ingestion and diet composition of red deer (*Cervus elaphus* L.) in the Netherlands from 1954 till 1992. Mammalia 59: 187–196.

Hajji G.M., Zachos F.E., Charfi-Cheikrouha F., Hartl G.B., 2007. Conservation genetics of the imperilled Barbary red deer in Tunisia. Anim. Conserv. 10: 229–235.

Hofmann R.R., 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia 78: 443–457.

Illius A.W., Gordon I.J., 1992. Modelling the nutritional ecology of ungulate herbivores: evolution of body size and competitive interactions. Oecologia 89: 428–434.

Jackson J., 1977. The annual diet of the fallow deer (*Dama dama*) in the New Forest, Hampshire, as determined by rumen content analysis. J. Zool. 181: 465–473.

Joffre R., Vacher J., de los Llanos C., Long G., 1988. The dehesa: an agrosilvopastoral system of the Mediterranean region with special reference to the Sierra Morena area of Spain. Agrofor. Syst. 6: 71–96.

Jopson N.B., Thompson J.M., Fennessy P.F., 1997. Tissue mobilization rates in male fallow deer (*Dama dama*) as determined by computed tomography: the effects of natural and enforced food restriction. Anim. Sci. 65: 311–320.

Legendre P., Legendre L.F., 2012. Numerical ecology. Elsevier, Amsterdam.

Lincoln G.A., 1971. The seasonal reproductive changes in the red deer stag (*Cervus elaphus*). J. Zool. 163: 105–123.

McNaughton S.J., Tarrants J.L., McNaughton M.M., Davis R.D., 1985. Silica as a defense against herbivory and a growth promotor in African grasses. Ecology 66(2): 528–535.

Marañón T., 1991. Diversidad en comunidades de pasto mediterraneo: modelos y mecanismos de coexistencia. Ecología 5: 149–157. [in Spanish]

Merceron G., Escarguel G., Angibault J.-M., Verheyden-Tixier H., 2010. Can dental microwear textures record inter-individual dietary variations? PLOS One 5: e9542. doi:10.1371/journal.pone.0009542

Merceron G., Hofman-Kamińska E., Kowalczyk R., 2014. 3D dental microwear texture analysis of feeding habits of sympatric ruminants in the Białowieża Primeval Forest, Poland. For. Ecol. Manag. 328: 262–269.

van der Merwe J., Marshal J.P., 2014. Grass attributes and seasonal changes in foraging by a preferentially grazing savanna mixed feeder. Journal of Arid Environments, 102: 34–39.

Mitchell B., McCowan D., Nicholson I.A., 1976. Annual cycles of body weight and condition in Scottish red deer, *Cervus elaphus*. J. Zool. 180: 107–127.

Müller D.W., Codron D., Meloro C., Munn A., Schwarm A., Hummel J., Clauss M., 2013. Assessing the Jarman–Bell principle: scaling of intake, digestibility, retention time and gut fill with body mass in mammalian herbivores. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 164(1): 129–140.

Naveh Z., Whittaker R.H., 1980. Measurements and relationships of plant species diversity in Mediterranean shrublands and woodlands. In: Grassle F., Patil G.P., Smith W., Taillee C. (Eds.), Ecological Diversity in Theory and Practice, Statistical Ecology Volume S6. Internat. Coop. Publ. House,Fairland, Md. 219–239.

Nugent G., 1990. Forage availability and the diet of fallow deer (*Dama dama*) in the Blue Mountains, Otago. New Zealand. J. Ecol. 13: 83–95.

Nugent G., McShea W.J., Parkes J., Woodley S., Waithaka J., Moro J., Gutierrez R., Azorit C., Mendez Guerrero F., Flueck W.T., Smith-Flueck J.M., 2011. Policies and management of overabundant deer (native or exotic) in protected areas. Animal Production Science, 51(4): 384–389.

Obidziński A., Kiełtyk P., Borkowski J., Bolibok L., Remuszko K., 2013. Autumn-winter diet overlap of fallow, red, and roe deer in forest ecosystems, Southern Poland. Open Life Sci. 8: 8–17.

Olea L., San Miguel-Ayanz A., 2006. The Spanish dehesa. A traditional Mediterranean silvopastoral system linking production and nature conservation. Grassl. Sci. Eur. 11: 3–13.

Poli B.M., Focardi S., Tinelli A., 1996. Composition and metabolizable energy of feed used by fallow deer (*Dama dama*) in a coastal Mediterranean ecosystem. Small Rumin. Res. 22: 103–109.

Putman R.J., Culpin S., Thirgood S.J., 1993. Dietary differences between male and female fallow deer in sympatry and in allopatry. J. Zool. 229: 267–275.

Raia P., Carotenuto F., Eronen J.T., Fortelius M., 2011. Longer in the tooth, shorter in the record? The evolutionary correlates of hypsodonty in Neogene ruminants. Proc. R. Soc. B 278(1724): 3474–3481.

Roberts C., Westbrooke M., Florentine S., Cook S., 2015. Winter diet of introduced red deer (*Cervus elaphus*) in woodland vegetation in Grampians National Park, western Victoria. Australian Mammalogy 37: 107–112.

Schaal A., Teillaud P., Campan R., Bon R., 1991. Le daim. In: Campan R., Bon R., Barre V. (Eds.). Les Ongulés Sauvages de France. Bilan des recherches récentes. Revue d'Écologie: 219–232. [in French]

Scott J.R., 2012. Dental microwear texture analysis of extant African Bovidae. Mammalia 76: 157–174.

Scott R.S., Ungar P.S., Bergstrom T.S., Brown C.A., Grine F.E., Teaford M.F., Walker A., 2005. Dental microwear texture analysis shows within-species diet variability in fossil hominins. Nature 436: 693–695.

Scott R.S., Ungar P.S., Bergstrom T.S., Brown C.A., Childs B.E., Teaford M.F., Walker A., 2006. Dental microwear texture analysis: technical considerations. J. Hum. Evol. 51: 339–349.

Sokal R.R., Rohlf F.J., 1998. Biometry: The principles and practice of statistics in biological research. Freeman & Co., New York.

Solounias N., Semprebon G., 2002. Advances in the reconstruction of ungulate ecomorphology with application to early fossil equids. American Museum Novitates 3366: 1–49.

Teillaud P., Bon R., Gonzalez G., Schaal A., Ballon P., Campan R., 1991. Le cerf. Revue d'Ecologie 46(Sup. 6): 185–217. [in French]

Tellado S., Azorit C., 2014. An integrative assay to quantify the nutritional quality of the selected diet of two Mediterranean free-living deer by faecal-FT-NIRS. Animal Production Science 55: 11–16.

Thimonier J., Sempere A., 1989. La reproduction chez les cervidés. INRA Prod. Anim. 2: 5–21. [in French]

Ungar P.S., 2015. Mammalian dental function and wear: A review. Biosurface Biotribology 1: 25–41.

Van Soest P.J., 1994. Nutritional ecology of the ruminant. Cornell University Press, Ithaca

Weber M.L., Thompson J.M., 1998. Seasonal patterns in food intake, live mass, and body composition of mature female fallow deer (*Dama dama*). Can. J. Zool. 76: 1141–1152.

Associate Editor: P. Raia

Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Table S1 Raw data with reference, genus, sex, date of death, percentage of Fruits, Browse and Graze in the rumen contents and values for dental microwear texture parameters.

Table S2 Descriptive statistics for *Cervus elaphus*.

Table S3 Descriptive statistics for *Dama dama*.

Table S4 Results of Fisher's LSD and Tukey's HSD Post Hoc tests.

Table S5 Seasonal analysis: descriptive statistics.

Table S6 Seasonal analysis: inter- and intra-population analyses of variance.

Table S7 Bimodal analysis: descriptive statistics.

Table S8 Bimodal analysis: inter- and intra-population analyses of variance.