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Haematological parameters do senesce in the wild: evidence from different populations of a long-lived mammal

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Abstract

Increasing evidence of senescence has been reported from long-term studies of wild populations. However, most studies have focused on life-history traits like survival, reproduction or body mass, generally from a single intensively monitored population. However, variation in the intensity of senescence across populations, and to a lesser extent between sexes, is still poorly understood. In addition, the pattern of age-specific changes in haematological parameters remains virtually unknown to date for any population of vertebrate living in the wild. Using repeated blood samples collected from known-aged (2–15 years of age) roe deer (*Capreolus capreolus*) from two populations facing highly different environmental conditions, we filled the gap. In particular, we investigated age-specific changes in haematocrit, albumin and creatinine. We reported clear evidence of senescence in all haematological parameters. Moreover, senescence patterns differed between sexes and populations. The rate of senescence was higher in males than in females for haematocrit with no site difference. On the other hand, the rate of senescence in creatinine was higher at Trois Fontaines than at Chizé with no sex difference. Our findings provide a first demonstration of age-specific declines in haematological parameters in wild populations of large herbivores and show that the process of senescence in vertebrates is not restricted to body mass or fitness components. We also demonstrate that the senescence pattern of haematological parameters is context dependent and varies both between sexes and according to environmental conditions.

Introduction

The process of senescence is commonly defined as a deterioration of cellular and physiological functions with increasing age, progressively leading to a decline in performance-related traits, such as reproduction or survival (Monaghan *et al.*, 2008). A large number of case studies have now documented senescence in wild vertebrates, but these studies remain mostly focused on actuarial (i.e. survival) and reproductive senescence (see Nussey *et al.*, 2013 for a recent review). Therefore, compared to humans or model organisms, very little is known about the potential decline in physiological parameters with increasing age in animals living in the wild that often suffer from harsh climatic conditions and resource limitation. Among physiological parameters, haematological parameters are particularly relevant as they are known to reflect not only body condition (Ezenwa *et al.*, 2012) but also reproductive performance (Ots *et al.*, 1998; Nadolski *et al.*, 2006) and survival (Milner *et al.*, 2003). However, age-specific declines in such traits in wild mammals remain poorly studied, whereas senescence in the level of haemoglobin has already been reported in humans (Eisenstaedt *et al.*, 2006). Until now, the few studies that have investigated age dependence in haematological parameters in wild mammalian species simply compared young

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and adults and often failed to detect an age effect (e.g. Rosef *et al.* (2004) in free-ranging red deer, *Cervus elaphus*; López-Olvera *et al.* (2006) in chamois, *Rupicapra pyrenaica*; Rafaj *et al.* (2011) in captive red deer, *C. elaphus*). To our knowledge, only one study has provided evidence of age-related decline in haematological parameters, which involved a decrease in the mean haematocrit and haemoglobin values of reindeer (*Rangifer tarandus granti*) and caribou (*R. tarandus*) with age (McEwan & Whitehead, 1969). However, this study was performed on captive animals and there is evidence that the care provided in zoos can influence biological performance when compared to wild animals (Lemaître *et al.*, 2013). Therefore, studying senescence patterns of haematological parameters in the wild would allow understanding whether and how age-related declines in physiological parameters underpin the process of reproductive and actuarial senescence commonly reported in free-ranging populations of vertebrates (Nussey *et al.*, 2013).

We aimed here to fill the gap by providing an analysis of full age dependence in haematological parameters in a wild mammal. We used data collected in two populations of roe deer (*Capreolus capreolus*) intensively monitored at the individual level (Chizé and Trois Fontaines in France, see Gaillard *et al.*, 2003) to assess age-specific changes in three haematological parameters. The roe deer is a particularly relevant model because senescence in survival and reproduction has been repeatedly reported in these populations (Gaillard *et al.*, 1993; Loison *et al.*, 1999), but the underlying factors causing these declines in performance remain unidentified. We first measured haematocrit, which is the fraction of whole blood comprised of erythrocytes that provides information about individual nutritional status and reflects an animal's oxygen-carrying capacity in muscle (Del Giudice *et al.*, 1992). Moreover, haematocrit may contribute to individual fitness. Indeed, although the relationship between haematocrit and survival could involve nonlinearities (Bowers *et al.*, 2014), there is evidence that a reduction in haematocrit concentration can cause a decrease of ability to carry oxygen leading to a reduction in cardiac efficiency (Birchard, 1997; Schuler *et al.*, 2010). Second, we investigated albumin, which plays a key role in maintaining osmotic pressure and fluid distribution between blood vessels and tissues. As albumin is the most abundant blood protein, albuminaemia is a reliable indicator of nutritional status in ruminants and hypo-albuminaemia reveals a cachectic state resulting from starvation (Stockham & Scott, 2008). Third, we measured the concentration of creatinine in the plasma. Creatinine is the result of creatine degradation in muscular cells and is eliminated through the glomerular filtration in kidneys (Stockham & Scott, 2008). Variations in the level of creatinine reflect total body muscle mass and are negatively related to the efficiency of the renal filtration (Stockham & Scott, 2008).

After providing clear evidence of senescence in these haematological parameters, we compared senescence patterns in both absolute and relative (to body mass) scales between populations and between sexes. Senescence in body mass has been recently reported to occur in females of wild large herbivores, including roe deer (Nussey *et al.*, 2011). In addition, Gilot-Fromont *et al.* (2012) showed that body mass is associated with haematological parameters among roe deer. Therefore, haematological parameters should decrease with increasing age more strongly when analysed on an absolute scale than relative to body mass. If changes in body mass capture the whole age-specific variation in physiological parameters, we expect haematological parameters to remain constant with age once body mass variation has been accounted for.

Environmental conditions can influence both the timing and the intensity of senescence in survival and reproduction in wild vertebrates (Nussey *et al.*, 2007; Bouwhuis *et al.*, 2010). For instance, in large herbivores, species feeding mostly on grass have higher rates of actuarial senescence in free-ranging conditions than in protected environments (Lemaître *et al.*, 2013). The two roe deer populations we studied live in different environments with a poor habitat quality in the Chizé forest and a highly productive habitat in the Trois Fontaines forest (Pettorelli *et al.*, 2006). We previously reported that haematological parameters and body mass were lower in Chizé than in Trois Fontaines (Gilot-Fromont *et al.*, 2012), suggesting that resource availability does influence haematological parameters. However, the influence of habitat quality on patterns of physiological senescence has remained unexplored. In addition, senescence patterns often differ between sexes in both invertebrates (e.g. Zajitschek *et al.*, 2009) and vertebrates (Loison *et al.*, 1999; Lemaître & Gaillard, 2013), for which a steeper rate of actuarial senescence is commonly observed in males compared to females. The roe deer is not an exception to the rule (Gaillard *et al.*, 1993). However, evolutionary causes of between-sex differences remain unclear and debated (Maklakov & Lummaa, 2013). So far, sex differences in physiological senescence have only been documented in humans with a less pronounced decline in haemoglobin in women compared to males (Nilsson-Ehle *et al.*, 2000). Assessing whether physiological senescence occurs in mammals in the wild would improve our understanding of the sex differences in age-specific survival, because the diversity of environmental conditions in the wild is likely to modify senescence patterns reported from laboratory or captive conditions (Kawasaki *et al.*, 2008; Lemaître *et al.*, 2013).

Overall, we predict that (i) haematological parameters (i.e. haematocrit, albumin and creatinine concentration) should decline with increasing age for both sexes in both populations according to the only study of haematological parameters performed so far in a wild

mammal species in captive conditions (i.e. McEwan & Whitehead, 1969 on reindeer, *R. tarandus*) and according to studies in humans (e.g. Eisenstaedt *et al.*, 2006), (ii) a more intense senescence in haematological parameters in the population with more limited resources (Chizé) than in the population with more favourable environmental conditions (Trois Fontaines), (iii) a steeper rate of senescence in physiological parameters in males than in females and (iv) a steeper decrease of haematological parameters with age when considering an absolute scale than relative to body mass.

Materials and methods

Ethics

All necessary permits were obtained for the described field studies. The protocol of capture and blood sampling of roe deer were performed under the authority of the ONCFS and have been approved by the Director of Food, Agriculture and Forest (Prefectoral order 2009–14 from Paris). This permission is given at the national level. The land manager of both sites, the Office National des Forêts (ONF), authorized the study of the population (Partnership Convention ONCFS-ONF dated 2005-12-23).

Study sites

We used data from two contrasting populations of European roe deer. Both sites are enclosed forests managed by the ONF, in which roe deer populations have been intensively monitored by the Office National de la Chasse et de la Faune Sauvage since more than three decades.

The Réserve Biologique Intégrale of Chizé is a 2614-ha enclosed forest located in western France (46°05′N, 0°25′W). This site has a temperate oceanic climate, with Mediterranean influences, including mild winters and hot dry summers. The forest has a low productivity due to poor quality soils and frequent summer droughts (Pettorelli *et al.*, 2006) and thus offers a poor habitat to roe deer. In contrast, the Territoire d'Etude et d'Expérimentation of Trois Fontaines (1360 ha), located in north-eastern France (48°43′N, 4°55′E), has a continental climate characterized by moderately severe winters, and warm and rainy summers. This site has high-quality soils and offers habitats of high quality to roe deer.

Data collection

Roe deer are small, forest-dwelling cervids with a low sexual size dimorphism. Only males carry antlers. Roe deer can live up to 14 (males) and 17 (females) years (Loison *et al.*, 1999). Survival of roe deer is low and variable from birth to weaning (Plard *et al.*, 2014), increases up to 2 years of age, when it remains quite high and constant for about 6 years (prime-age stage), and then decreases from 8 years of age onwards (senescent stage) (Gaillard *et al.*, 1993). Roe deer start reproducing at 2 years of age, whereas most females give birth (generally to twins) at 2 years of age, most males become territorial and thus mate only from 3 years of age onwards (Andersen *et al.*, 1998).

The study period was December 2009 to March 2013. Every winter (December–March), roe deer were captured using drive netting (see Gaillard *et al.*, 1993 for further details). Captured roe deer were identified, sexed and weighed. We collected 4 mL of blood (EDTA and serum tube) at the jugular vein of each adult known-aged roe deer caught. Blood samples were stored between 4 and 6 degrees, then were received at the laboratory within 40 h and analysed within 4 h after reception. Haematological and biochemical assays were performed at the Biochemical and Endocrinological laboratory, VetAgro-Sup, France. We obtained complete blood counts using an ABC Vet automaton (Horiba Medical, Montpellier, France). Red blood cell counts were measured by impedance technology considering parameters for bovine samples. Haemoglobin concentration was measured following cyan methaemoglobin conversion at 550 nm. Haematocrit (%) was calculated by numeric integration. Biochemical parameters were analysed with a Konelab K30 ISE automatic analyser (Thermo Electron SAS, Clinical Chemistry & Automation Systems, Eragny Parc, BP 249, 95615 Cergy-Pontoise, France). Creatinine, in $\mu mol\ L^{-1}$, was measured by enzymatic photometric determination at 540 nm using the reagents creatinine (enzymatic) 98 18 45 provided by Thermo Electron SAS. The measuring range is 10–10 000 $\mu mol\ L^{-1}$, the detection limit (zero sample + 3 SD) is 2 $\mu mol\ L^{-1}$, and the within-run and between-run errors ranged between 0.3% and 2.1%. Proteins (albumin in $g\ L^{-1}$) were separated and quantified by electrophoresis, performed using an automatic gel electrophoresis processor HYDRASYS (Sebia, Parc Technologique Léonard de Vinci, CP8010 Lises, 91008 Evry Cedex). Total proteins were measured with a refractometer.

Data analysis

To assess age-specific changes in haematological parameters, we included each of these parameters at a time as the response variable, age as a covariate and both year of sampling and individual identity as random effects to avoid pseudo-replication issues (*sensu* Hurlbert, 1984) and thereby associated confounding effects of individual heterogeneity (Van de Pol & Verhulst, 2006) and to account for possible differences in the assays across years.

To assess senescence, we only included roe deer from 2 years of age onwards, that is having reached the

minimum age generally required to reproduce (Gaillard *et al.*, 1992 on females, Vanpé *et al.*, 2009 on males). All haematological parameters investigated correlated each other on a statistical basis, but the strength of the correlation was consistently weak (Table S1). We calculated repeatability as the ratio of the between-group variance over the sum of between-group variance and within-group variance (Nakagawa & Schielzeth, 2010). Moreover, haematological parameters for fawns were consistently lower than those of adults, although not always on a statistical basis at Trois Fontaines from using Student's *t*-tests (Chizé: mean of haematocrit (in %): 51.48 ± 0.65 for fawns, 53.95 ± 0.39 for adults (t_{215} = 3.22; P = 0.001); mean of albumin (g L^{-1}): 31.78 ± 0.66 for fawns, 34.46 ± 0.35 for adults (t_{215} = 4.02; P = 0.0001); mean of creatinine (μmol L^{-1}): 84.27 ± 2.04 for fawns, 107.94 ± 1.63 for adults (t_{215} = 7.74; P < 0.0001); Trois Fontaines: mean of haematocrit (in %): 53.77 ± 0.60 for fawns, 53.74 ± 0.39 for adults (t_{238} = −0.03; P = 0.970); mean of albumin (g L^{-1}): 35.35 ± 0.49, for fawns, 37.43 ± 0.37 for adults (t_{238} = 3.37; P = 0.0009); mean of creatinine (μmol L^{-1}): 117.70 ± 3.45 for fawns, 119.13 ± 1.69 for adults (t_{238} = 0.37; P = 0.710). To avoid overly complex age-specific models, we excluded haematological parameters of fawns from this analysis of senescence patterns. Although the three haematological parameters were interrelated in a statistically significant way (Table S1), the highest correlation (0.34) was below the threshold above which multicolinearity problems have to be accounted for (Graham, 2003).

To assess age-specific variation in haematological parameters, we fitted models with different onsets of senescence: a linear or quadratic change from 2 years of age (first age to reproduce) onwards and a linear or quadratic change from 8 years of age (onset of actuarial senescence in roe deer, Festa-Bianchet *et al.*, 2003) onwards. Two-way interactions among age, sex and site were included. To assess the specific impact of body mass on haematological parameters, the procedure of model selection was performed both with and without including body mass as a covariate. The model with the lowest AIC was selected. When two competing models had a difference in AIC < 2, we retained the model with the lowest number of parameters to satisfy the parsimony criterion (Burnham & Anderson, 2002).

All analyses were carried out in R, v. 2.15.3 (R Development Core Team, 2013). Results are presented as mean ± standard error.

Results

We collected 47 blood samples in 2010, 91 in 2011, 73 in 2012 and 109 in 2013. We analysed senescence patterns in haematological parameters from 170 blood samples at Trois Fontaines (67 samples from males including one individual captured in three different years, 14 individuals captured in two different years and 36 individuals captured only once; and 103 samples from females including eight individuals captured in three different years, 17 individuals captured in two different years and 45 individuals captured only once) and 150 blood samples at Chizé (54 samples from males including two individuals captured in three different years, six individuals captured in two different years and 36 individuals captured only once; and 96 samples from females including one individual captured in four different years, six individuals captured in three different years, 15 individuals captured in two different years and 44 individuals captured only once). Among haematological parameters, the repeatability was lowest for albumin (haematocrit: 0.43; albumin: 0.17; creatinine: 0.34). There were no detectable differences of sex distribution between sites (χ^2 = 0.395; P = 0.530). The age distribution differed between sites (χ^2 = 24.441; P = 0.002) (Table S2) because of an excess of very young (2 years old) and very old (older than 10 years of age) roe deer at Trois Fontaines compared to Chizé. However, roe deer aged 7–9 were over-represented at Chizé, leading the proportion of old individuals to be similar in both sites.

As expected, we found evidence of senescence in body mass in both sexes and in both populations (Table S3). Patterns of senescence in body mass were similar across males of both populations (Wald test: difference of slope = 0.04; t_{130} = 0.84; P = 0.159, Table S3, Table 1) and between males and females at Chizé (Wald test: difference of slope = 0.05; t_{185} = 1.76; P = 0.08, Table S3, Table 1). Haematological parameters for males tended to be slightly lower than those of females (mean of haematocrit (in %): 52.77 ± 5.43 for males, 54.56 ± 5.17 for females; mean of albumin (g L^{-1}): 34.96 ± 5.20 for males, 37.04 ± 4.81 for females; mean of creatinine (μmol L^{-1}): 109.70 ± 22.51 for males, 114.41 ± 23.16 for females). Senescence patterns varied widely among haematological parameters, but each of the three parameters we studied increased with increasing body mass (Table 2). For all haematological parameters, the model including body mass was selected when

Table 1 Threshold regressions of body mass on age in both sexes and both populations. Displayed is the senescence rate (with standard errors).

Parameter	Sex		Trois Fontaines β ± SE	Chizé β ± SE
Body mass	Males	Intercept	20.00 ± 0.95	16.65 ± 0.80
		Age	1.58 ± 0.36	1.41 ± 0.29
		Age²	−0.11 ± 0.02*	−0.07 ± 0.02
	Females	Intercept	20.70 ± 0.70	15.25 ± 0.78
		Age	0.62 ± 0.24	1.72 ± 0.27
		Age²	−0.03 ± 0.01*	−0.12 ± 0.02*

*Statistically significant Wald tests when comparing models.

Table 2 Effect of age alone or in interaction with sex or site on haematological parameters with parameter estimates (β), associated standard errors (SE) and statistical significance of the effect sizes (p-values of Wald tests).

Haematological parameter	Variable	$\beta \pm$ SE	P
Haematocrit	Intercept	45.60 ± 2.63	< 0.001
	Sex (Males)	−1.02 ± 0.79	0.197
	Age (Years)	0.68 ± 0.37	0.066
	Age²	−0.07 ± 0.03	0.020
	Body mass	0.37 ± 0.11	0.018
	Sex (Males) : age²	−0.03 ± 0.01	0.003
Albumin	Intercept	33.81 ± 2.63	< 0.001
	Site (Chizé)	−2.08 ± 0.54	< 0.001
	Sex (Males)	−2.60 ± 0.44	< 0.001
	Age (Years)	0.38 ± 0.31	0.220
	Age²	−0.07 ± 0.02	< 0.001
	Body mass	0.19 ± 0.10	0.057
Creatinine	Intercept	86.31 ± 13.00	< 0.001
	Site (Chizé)	−14.01 ± 5.20	0.007
	Sex (Males)	−7.51 ± 2.43	0.002
	Age (Years)	−3.74 ± 0.49	< 0.001
	Body mass	2.31 ± 0.52	< 0.001
	Site (Chizé) : age	2.20 ± 0.81	0.007

compared to the equivalent model without body mass (for haematocrit: ΔAIC = 4.405, for albumin: ΔAIC = 4.995, for creatinine: ΔAIC = 12.831, Table S4).

Haematocrit

For haematocrit, we selected the model including body mass and a quadratic effect of age with interactive effects between the quadratic term and sex. Haematocrit increased with body mass (slope of 0.30 ± 0.12, Table 2) and was higher in males than in females (difference of 0.03 ± 0.01; Table 2, Fig. 1a). Senescence in haematocrit started at around 7 years of age for roe deer females and at around 6 years for roe deer males. Senescence rates in haematocrit did not differ between sites.

Albumin

For albumin, we selected the model including body mass, site and a quadratic effect of age. Albumin

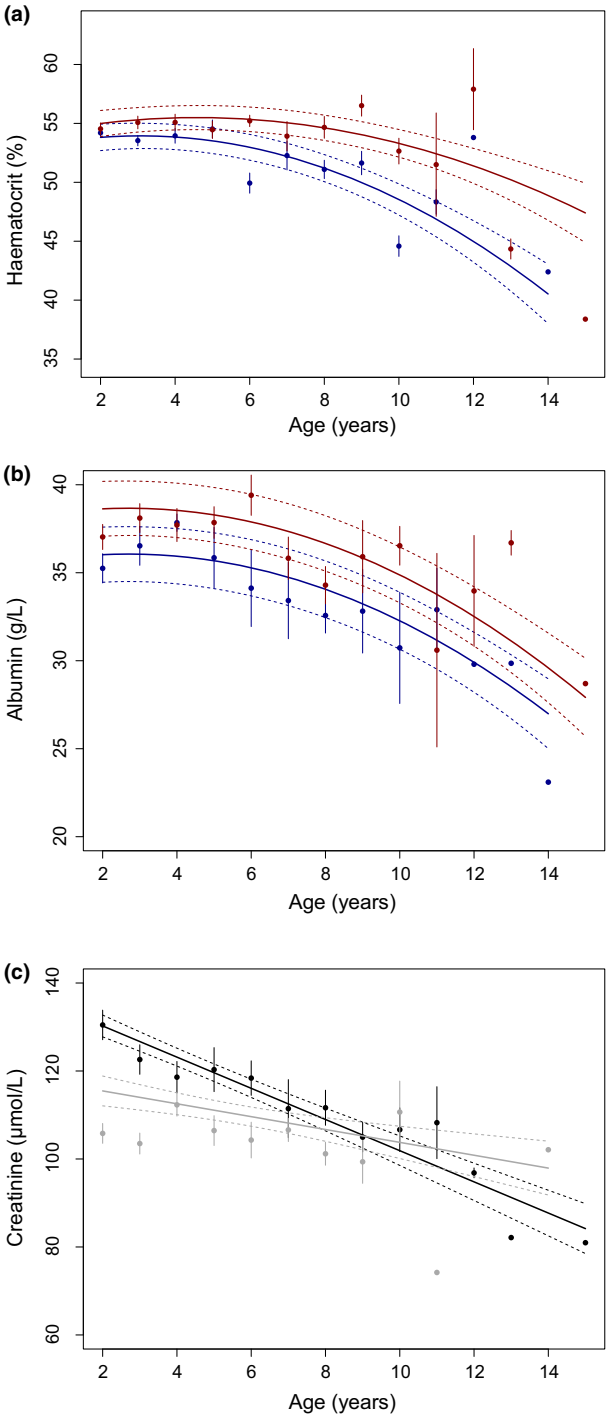

Fig. 1 Age-related decline in haematological parameters. Senescence patterns of haematocrit (%) and albumin (g L^{-1}) in male and female roe deer [respectively, (a) and (b)] with predictions based on the model selected by AIC (blue points corresponding to the average value per age for males, blue line corresponding to the predicted values for males and dotted blue lines corresponding to standard errors for males; red points corresponding to the average value per age for females, red line corresponding to the predicted values for females and dotted red lines corresponding to standard errors for females). Senescence patterns of creatinine (µmol L^{-1}) in roe deer at Trois Fontaines and Chizé (c) with predictions based on the model selected by AIC (black points corresponding to the average value per age at Trois Fontaines, black line corresponding to the predicted values at Trois Fontaines and dotted black lines corresponding to standard errors at Trois Fontaines; grey points corresponding to the average value per age at Chizé, grey line corresponding to the predicted values at Chizé and dotted grey lines corresponding to standard errors at Chizé).

increased with body mass (slope of 0.19 ± 0.10, Table 2) and was higher at Trois Fontaines than at Chizé (difference of 2.08 ± 0.54). The rate of senescence did not differ between sexes or sites (Table 2, Fig. 1b). Senescence in albumin started at around 4 years of age for roe deer females and males.

Creatinine

For creatinine, we selected the model including body mass, sex and a linear effect of age with interactive effects between the linear term and site. Creatinine increased with body mass (slope of 2.12 ± 0.55, Table 2) and was lower in males than in females (difference of -7.07 ± 2.52). The senescence rate was higher in Trois Fontaines (-3.54 ± 0.52) than in Chizé (-1.46 ± 1.37; Table 2, Fig. 1c). Moreover, no interaction between sex and age occurred.

Discussion

Our study reveals clear evidence that haematological parameters do show senescence in mammalian populations in the wild and adds to the compelling evidence that ageing can impact a large range of phenotypic traits in wild populations, including reproductive performance (e.g. Gaillard *et al.*, 2003), immunological performance (e.g. Nussey *et al.*, 2012), body mass (e.g. Nussey *et al.*, 2011) or secondary sexual traits (e.g. Vanpé *et al.*, 2007).

Our results obtained in two different populations and over a large range of ages (from 2 to 15 years) show that in roe deer, haematological parameters are decreasing from prime to old ages with different intensity depending on the focal parameter, sexes, populations and on the scale at which haematological parameters are considered (absolute or relative to body mass). In accordance with our first prediction, all haematological parameters investigated declined with increasing age. In roe deer, there is also evidence of senescence in survival from 7 to 8 years of age onwards in both sexes at Chizé and Trois Fontaines (Gaillard *et al.*, 1993, 2004; Loison *et al.*, 1999) and evidence of senescence in fecundity from 8 years onwards for females (Hewison & Gaillard, 2001). Senescence in body mass starts at around 9 years of age for roe deer females at Trois Fontaines and then accelerates at 13 years of age (Nussey *et al.*, 2011). With our restricted data set, senescence in body mass occurred at around 7 years of age for males and 9 years of age for females at Trois Fontaines, but started at around 7 years for females and 9 years for males at Chizé (Fig. S1). Therefore, age-related declines in haematocrit we reported for males and females in both populations closely match age-specific declines in body mass of female roe deer, but albumin and creatinine concentrations started to decrease at an earlier age than body mass (Fig. 1b,c).

In large herbivores, increasing tooth wear with age causes a decrease in food intake (Kojola *et al.*, 1998), which leads to a decrease in the amount of nutrients available for the metabolism (Sams *et al.*, 1998) and probably a decrease in physiological parameters. Both nutrient deficiencies and chronic parasitism were thus probably acting in the studied populations. Moreover, these two causes should interact, as food restriction is likely to cause a low body condition, associated to low values of neutrophil/lymphocyte ratio, haptoglobin, alpha-2 globulin and eosinophils in the studied populations (Gilot-Fromont *et al.*, 2012). The decrease in immune functions related to the lack of energy should in turn increase parasitism, giving rise to a vicious circle (Beldomenico & Begon, 2010). We still found evidence of senescence in haematological parameters after having accounted for body mass variation with age although a positive covariation occurred between body mass and physiological parameters (Gilot-Fromont *et al.*, 2012). Nussey *et al.* (2011) suggested that a decline in body mass could involve the loss of muscle mass with age. McArdle *et al.* (2002) showed that creatinine is a by-product of muscle metabolism and evidence of a decline in muscles with age has recently been reported in mammals (Hindle *et al.*, 2009 on shrews, *Blarina brevicauda* and *Sorex palustris*; Hindle *et al.*, 2010 on shrews, *B. brevicauda*). However, the senescence pattern of creatinine we reported for roe deer does not appear to be closely linked with body mass and consequently with muscle loss. Senescence in haematological parameters and senescence in body mass could thus involve different mechanisms. Indeed, the decline in haematological parameters could involve the accumulation of deterioration of cellular damage, whereas the decline in body mass in wild populations could involve a loss of skeletal mass, tooth wear or foraging behaviour (Nussey *et al.*, 2011).

On the contrary to our second prediction, senescence in haematocrit and albumin was not more intense in Chizé than in Trois Fontaines. Moreover, for creatinine concentration, the rate of senescence was higher in Trois Fontaines than in Chizé. Creatinine concentrations in roe deer were lower at Chizé than at Trois Fontaines during prime-age adulthood, but the pattern was reversed at old ages. We suggest that roe deer at Chizé are more often starving than at Trois Fontaines. Indeed, a same creatinine concentration (approximately 117 $\mu mol\ L^{-1}$) for a roe deer is observed at 3 years of age at Chizé and at 7 years of age at Trois Fontaines.

Sex difference in senescence patterns occurred for haematocrit. Haematocrit was lower in males than in females, and the senescence rate was higher in males than in females. Evidence that males show more rapid actuarial senescence than females is now well documented in several populations of large herbivores in the wild (Nussey *et al.*, 2013). Specifically for roe deer, there is clear evidence that annual adult survival is lower in

males than in females (Gaillard *et al*., 1993). This could explain our finding of a higher senescence rate in males compared to females and of lower values for all haematological parameters in males. Toïgo & Gaillard (2003) found that sex differences in adult survival are more likely to occur when resources are scarce because males are more sensitive to starvation than females.

Although the occurrence of senescence in survival and reproduction seems to be the rule in birds and mammals (Nussey *et al*., 2013), our findings provide a first clear evidence of senescence in haematological parameters in a wild mammal since until now empirical evidence of decline in haematological parameters with age were limited to laboratory models such as rats (Wu *et al*., 2008) or house mice (Garratt *et al*., 2011). However, whether the pervasiveness of senescence might be generalized to all physiological parameters in wild species will require further investigations.

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Supporting information

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

Figure S1 Age-related differences in body mass depending on population and sex.

Table S1 Correlation matrix among haematological parameters.

Table S2 Distribution of sex and age of roe deer in the populations of Trois Fontaines and Chizé between 2009 and 2013.

Table S3 Model selection for the effects of age on body mass of both sexes in both populations.

Table S4 Model selection for the effect of age on hematocrit (a), albumin (b) and creatinine (c).

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