Age-specific relationships between immunity and life-history traits in a wild mammal

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Abstract

Age-specific relationships between immunity and life-history traits in a wild mammal

Immunity determines an organism's sensitivity to pathogens and parasites and thus represents a crucial function that affects the fitness of individuals in the wild. However, immune function also entails several energy costs for development and use, and in natural conditions, resources are limited. Organisms consequently face energy allocation trade-offs between immunity and other costly functions such as growth or reproduction. On the long term, these allocations are supposed to have serious consequences on the probability of individuals to reproduce and to survive at each age.

The aim of this thesis was to describe age-related variations of immune phenotype in a wild and long-lived mammal, the roe deer (*Capreolus capreolus*), and to provide a better understanding of energy trade-offs between immune function and other life-history traits. This thesis was conducted in roe deer of both sexes and from two natural populations, which allowed us to test the influence of sex and contrasting environmental conditions on these variations.

We first established that rapid growth does not impair the development of young roe deer immune phenotype (measured as the levels of innate and adaptive traits), neither on the short-term (during growth), nor on the long-term (during adulthood). We also proved that, in this species, immune development of juveniles is not dependent of maternal age, but is strongly influenced by maternal body condition. In adult roe deer, we have described the precise patterns of age-related changes using twelve immune traits reflecting both innate and adaptive immunity. It revealed that roe deer are subjected to profound changes in their immune profile with increasing age, *i.e.* an increase in the production of inflammatory markers (haptoglobin, beta-globulin) and a decrease in the adaptive response (lymphocytes). In the same individuals, the parallel increase with age of parasite load supports the idea that deer are subject to immunosenescence. Finally, we described age-related changes in leukocyte telomere length. We found no clear associations between telomere length and proportions of each leukocyte form. However, we observed that high levels of some inflammatory markers (beta- and alpha1-globulin) tend to be associated with short telomeres in immune cells. These results open many avenues for a better understanding of the physiological mechanisms underlying aging.

Key-words: eco-immunology, senescence, trade-off, telomere dynamics, *Capreolus capreolus*

Résumé

Effet de l'âge sur les relations entre l'immunité et les traits d'histoire de vie chez un mammifère sauvage

Face à la menace des agents pathogènes présents dans l'environnement, l'immunité représente une fonction cruciale pour la valeur sélective des organismes. Cependant, cette fonction a aussi divers coûts de développement et d'utilisation, et le caractère limité des ressources dans l'environnement impose des compromis d'allocation entre différentes fonctions (immunité, croissance, reproduction). Sur le long-terme, ces choix peuvent avoir de lourdes conséquences sur les probabilités de se reproduire et de survivre à chaque âge.

L'objectif de cette thèse a été de décrire les variations avec l'âge du phénotype immunitaire d'un mammifère longévif, le chevreuil (*Capreolus capreolus*) et de mieux comprendre les compromis régissant l'allocation de ressources entre l'immunité et les autres grandes fonctions de l'organisme. Cette thèse a été menée au sein de deux populations naturelles, permettant de tester l'influence de conditions environnementales contrastées sur ces variations.

Nous avons montré qu'une croissance rapide pendant les premiers mois de vie du chevreuil n'impose pas de coût ni en termes de développement du phénotype immunitaire sur la même période (niveaux des traits innés et acquis), ni sur le long-terme. Nous avons aussi montré que le développement de l'immunité des jeunes n'était pas dépendant de l'âge de leur mère, mais était fortement influencé par la condition corporelle de celle-ci. Chez les adultes, nous avons décrit les variations avec l'âge de douze traits reflétant l'immunité innée et adaptative. Cela a permis de mettre en évidence de profondes modifications du profil immunitaire aux âges avancés, *i.e.* une augmentation de la production de marqueurs inflammatoires (haptoglobine, beta-globulines) et une diminution de la réponse adaptative (lymphocytes). L'augmentation parallèle avec l'âge de la charge parasitaire des individus appuie l'idée que le chevreuil est sujet à l'immunosenescence. Enfin, nous avons montré que la longueur des télomères leucocytaires varie avec l'âge. Nous n'avons pas trouvé d'association entre la longueur des télomères et les proportions de chaque forme leucocytaire (neutrophiles, monocytes, lymphocytes). Cependant, nous avons montré que de forts niveaux de certains marqueurs inflammatoires (beta- et alpha1-globulines) semblent être associés à des télomères courts dans les cellules immunitaires. Ces résultats ouvrent de nombreuses pistes pour une meilleure compréhension des mécanismes physiologiques à la base du vieillissement.

Mots-clés : éco-immunologie, senescence, compromis d'allocation, dynamique des télomères, *Capreolus capreolus.*

List of articles included in the main manuscript

CHAPTER 3.

L. Cheynel, F. Douhard, E. Gilot-Fromont, B. Rey, F. Débias, S. Pardonnet, J. Carbillet, H. Verheyden, A.J.M. Hewison, M. Pellerin, J-M. Gaillard & J-F. Lemaître (2018) *Does body growth impair immune functions in a large herbivore?* Oecologia, *in press*.

L. Cheynel, E. Gilot-Fromont, B. Rey, F. Débias, S. Pardonnet, M. Pellerin, J-M. Gaillard & J-F. Lemaître (2018) *Age and body mass of mothers shape offspring condition in a wild mammal*. *In preparation.*

CHAPTER 4.

L. Cheynel, J-F. Lemaître, J-M. Gaillard, B. Rey, G. Bourgoin, H. Ferté, M. Jégo, F. Débias, M. Pellerin, L. Jacob & E. Gilot-Fromont (2017*) Immunosenescence patterns differ between populations but not between sexes in a long-lived mammal.* **Scientific Reports**, 7: 13700.

CHAPTER 5.

R.V. Wilbourn, H. Froy, M.-C. McManus, **L. Cheynel**, J.-M. Gaillard, E. Gilot-Fromont, C. Regis, B. Rey, M. Pellerin, J.-F. Lemaître & D.H. Nussey (2017) *Age-dependent associations between telomere length and environmental conditions in roe deer.* **Biology Letters**, 13(9):20170434.

List of articles included in the appendices

J-F. Lemaître, **L. Cheynel**, F. Douhard, G. Bourgoin, F. Débias, H. Ferté, E. Gilot-Fromont, S. Pardonnet, M. Pellerin, B. Rey, C. Vanpé, M. Hewison & J-M. Gaillard (2018) *The influence of early-life allocation to antlers on male performance during adulthood: evidence from contrasted population of a large herbivore*. **Journal of Animal Ecology**, 87:921–932.

J-F. Lemaître, J. Carbillet, B. Rey, R. Palme, H. Froy, R.V. Wilbourn, **L. Cheynel**, J-M. Gaillard, A.J.M. Hewison, H. Verheyden, F. Débias, J. Duhayer, C. Régis, S. Pardonnet, M. Pellerin, D.H. Nussey & E. Gilot-Fromont. *Glucocorticoids level predicts short-term telomere dynamic in wild roe deer. In preparation.*

List of oral presentations and posters presented at international conferences

❖ Oral communications

L. Cheynel, R. Wilbourn, H. Froy, E. Gilot-Fromont, J.M. Gaillard, B. Rey, J.F. Lemaître & D. Nussey (2017) *Senescence, immune competence and telomere length in a wild mammal.* **Conference of Animal Ecophysiology III**, Strasbourg, France. **Price of oral communication**. Grant for registration fees, travel and accommodation.

L. Cheynel, R. Wilbourn, H. Froy, M.C. McManus, E. Gilot-Fromont, J.M. Gaillard, B. Rey, J.F. Lemaître & D. Nussey (2017) *Age-dependent associations between telomere length and environmental conditions in a wild mammal*. **Conference on Understanding diversity in telomere dynamics**, Edinburgh, UK**.** Grant for registration fees, travel and accommodation.

L. Cheynel, J-F. Lemaître, J-M. Gaillard, B. Rey, G. Bourgoin, M. Pellerin & E. Gilot-Fromont (2017) *Immuno-senescence patterns in two populations of a long-lived mammal*. **Annual meeting of the immuno-ecology working group of the REID** (French network of sustainable interactions ecology), Montpellier, France.

L. Cheynel, E. Gilot-Fromont & J-F. Lemaître (2016) *Patterns of immunosenescence differ between two contrasted populations of a long-lived mammal.* **Conference on Understanding diversity in telomere dynamics**, Edinburgh, UK. Travel and accommodation grant obtained. Grant for registration fees, travel and accommodation

❖ Posters

L. Cheynel, J-F. Lemaître, J-M. Gaillard, B. Rey, G. Bourgoin, M. Pellerin & E. Gilot-Fromont (2017) *Patterns of immunosenescence differ between two contrasted populations of a long-lived mammal.* **Annual meeting of the LABEX ECOFECT**, Lyon, France.

L. Cheynel, J-F. Lemaître, J-M. Gaillard, B. Rey, G. Bourgoin, M. Pellerin & E. Gilot-Fromont (2016) *Patterns of immunosenescence differ between two contrasted populations of a long-lived mammal.* **Annual meeting of the British Ecological Society (BES)**, Liverpool, UK.

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CHAPTER 1

General introduction

1. The study of immunity in the wild

In the wild, animals are facing a great variety of pressures that fluctuate seasonally or unpredictably, such as climatic conditions, the access to nutritional resources or the presence and abundance in parasites and pathogens. The balance costs/benefits shape the evolution of behavioural, anatomical and physiological adaptations of organisms, in order to maximize fitness of individuals, in response to a given environment. The immune system is considered one of the most important physiological functions, particularly for its role in survival (Sorci *et al.*, 2008). Since the early 90's, studying the fitness costs and benefits of different immune defence strategies has become a major field of study called "eco-immunology".

Eco-immunology, the emergence of a new discipline

The emergence of eco-immunological studies date back to the seminal article of Hamilton and Zuk (1982). This founding paper proposed that parasitism could have an important role in the evolution of sexually selected characters and of female mate choice. Briefly, because male secondary sexual characters are costly traits, they would be more developed in "good quality males" able to support their cost while resisting to parasite pressure. Sexual characters would therefore play a role of honest signal of male quality driving mating choice of females. It introduced the idea that relationships between hosts and pathogens are a major part of species life-history and provide a first link between the study of immune function and evolutionary ecology.

This new field of investigation, called *ecological/eco-immunology* (Martin *et al.*, 2011) or *wild immunology* (Pedersen and Babayan, 2011), then fully develops in the early 90's, at the interface of several disciplines (disease ecology, evolutionary biology, ecology, immunology). At this time, most of our knowledge on the immune system derived from studies conducted in humans, laboratory and domesticated animals, so this new approach was in contrast to most previous research. During this period, two other articles became references in the field. The first one of Folstad and Karter (1992) provided a physiological extension of Hamilton and Zuk's theory, *i.e.* testosterone would be the underlying mechanism reducing immuno-competence while stimulating the development and maintenance of secondary sexual characters *via* an energy reallocation process. The second article of Sheldon and Verhulst (1996) also proposed a concept that became central in eco-immunology from this date: in most environments, resources are limited, so trade-offs among competing and costly physiological functions should affect *whether* and *how* hosts defend themselves against infections.

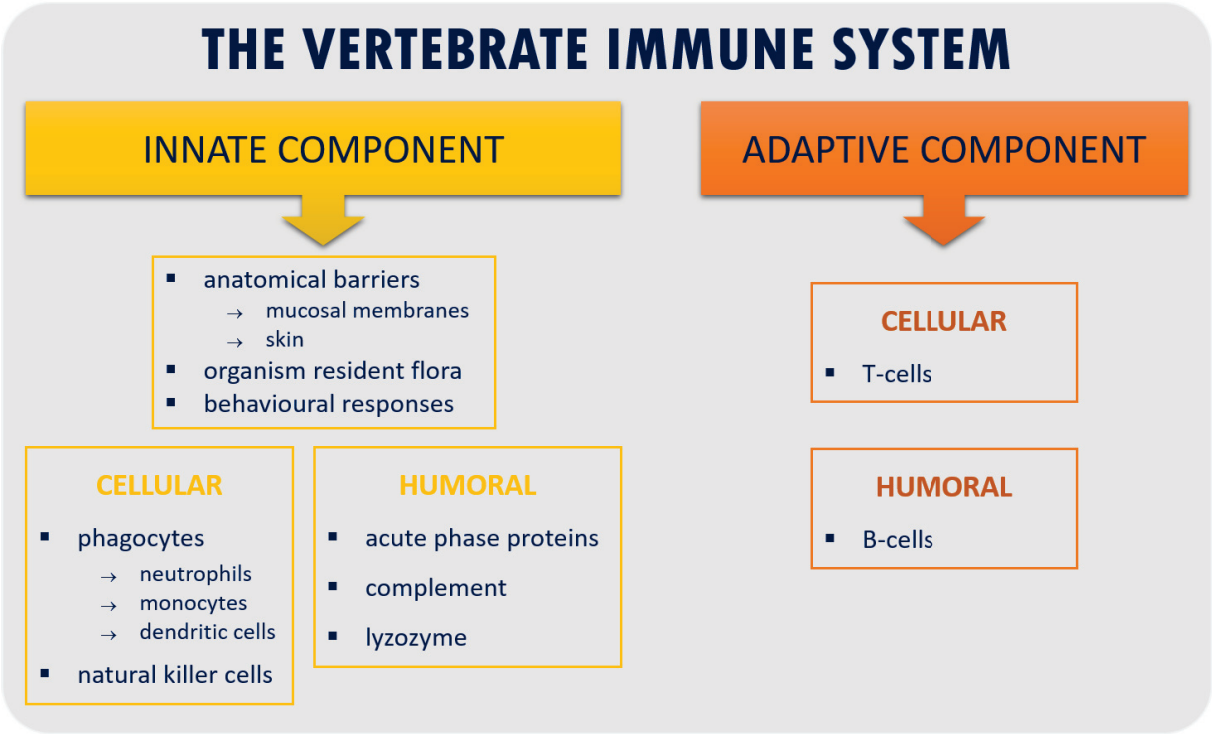
Following the publication of these seminal papers, studying of variations observed in immune function across individuals in natural conditions, became a large field of investigation focussing on the fitness consequences of such variation. This new discipline aims to understand "*the complexity of the interactions among host physiology (e.g. immune function) and disease ecology (e.g. pathogen prevalence) in a wide range of environmentally relevant contexts*" (Demas and Nelson, 2012). These studies have obviously benefited from the wide knowledge acquired through classic immunological studies on laboratory animal models, farm and zoo animals. However, contrary to classic approaches that minimize factors of variation considered as "noise" (Maizels and Nussey, 2013), eco-immunological studies fully consider the natural variation of immunity according to different factors such as sex, seasonality or the presence of multiple infections. Over time, it became clear that immune responses are influenced by genetic variability, host traits (sex, age, previous and current reproductive state) and infectious history (immune memory, current infections), environmental conditions (*e.g.* resources availability, meteorological variables) and co-evolution between host and pathogen (Pedersen and Babayan, 2011).

Ecological immunology is now a fully recognized discipline that tackles a wide array of questions, and promotes debate on numerous topics, from the molecular mechanisms of immune response to the role of immunity in shaping the evolution of life histories (Brock *et al.*, 2014). Recently, it also became obvious that studying immune function in free-living wild animals is an approach that could be more and more needed for human health. Indeed, like wild species, humans experience highly variable environments, display genetic diversity and suffer from numerous infections (Ezenwa *et al.*, 2010; Telfer *et al.*, 2010). Particularly, human population nowadays displays a major increase, which has a strong impact on the environment (*e.g.* fragmentation of habitats, concentration of populations, loss of biodiversity) (Mace *et al.*, 2005). These profound changes are accompanied by a parallel increase of infectious diseases or emerging pathogens affecting humans, but also wildlife and domesticated species (Jones *et al.*, 2008a; Keesing, *et al.*, 2010). Eco-immunological studies are likely to be of high importance for a better understanding of the driving mechanisms that shape outbreaks and threaten human health, species conservation, and ecosystem services.

The challenge of measuring immunity in the wild

Organisms have developed very complex physiological responses to protect themselves from the wide range of pathogens present in their environment (Fig. 1.1 and Box 1.1 for an overview of the vertebrate immune system). The vertebrate immune system relies on numerous specialized cells and factors able to distinguish "self" from "non-self" involved in the elimination of the "non-self" substances that can endanger the host.

Figure 1.1. A typical vertebrate immune system.



Box 1.1. The vertebrate immune system

In vertebrates, the immune system has two different but complementary components: the innate and the adaptive system (Stanley, 2002). Interactions between these two systems are essential for an effective immune response (Muehlenbein and Bribiescas, 2005). These two components are themselves compound by cellular and humoral effectors. Humoral immunity is the response mediated by macromolecules found in extracellular fluids (*e.g.* antibodies or proteins) and cellular immunity is the response based on cells activity (*e.g.* phagocytes).

Innate immunity is the oldest immune response in the evolutionary history of vertebrates. It is a rapid and non-specific response. This defence mechanism is activated immediately, or within hours, and represents the first line of organism defence against invading pathogens. The innate immune component first includes all anatomical barriers (mucosal membranes or skin), resident flora (bacteria that are not pathogenic) and comprises behavioural adaptations intended to avoid infections. It also includes anti-microbial soluble proteins (*e.g.* complement, lysozyme, acute phase proteins), natural killer (NK) cells and phagocytic cells (macrophages, neutrophils, dendritic cells) (Stanley, 2002). All these cells and proteins are constitutively present at low levels in the blood, allowing a rapid defence. The inflammatory response, that involves acute phase proteins and the recruitment of phagocytic cells to the site of injury or infection, is an important aspect of innate immunity. Phagocytic cells ingest pathogens and produce cytokines that recruit additional white blood cells and help organize induced immune responses. NK cells recognize and destroy infected or abnormal host cells. Complement proteins form complexes that lyse pathogens, or tag them for recognition by antibodies and phagocytic cells (Carroll, 1998). Finally, natural antibodies are the most specific part of the innate immune response, and their interaction with the complement is an important link between innate and adaptive immunity.

Contrary to innate response, adaptive (acquired) immunity is based on the ability to recognize and target specific antigens. This mechanism is not very efficient at the first contact with the antigen, is slow to develop but is more rapid and efficient upon secondary exposure. Indeed, it is capable of producing long-lasting immunological protection against particular parasites as a result of immunological memory. This response is mediated by lymphocytes, composed principally of B and T cells (Stanley, 2002). Activated B cells are differentiated into memory cells, with a function of surveillance; and in plasma cells, that secrete antibodies when exposed to an antigen (humoral immunity). Antibodies (also called immunoglobulins or Ig) have many roles: they neutralize pathogens and their products, induce complement activation, enhance phagocytosis and recruit cells to the site of infection (Black, 2002). In mammals, there are five antibody isotypes: IgA (protect mucosal surfaces from infections), IgD (activate basophils and mast cells to secrete antimicrobial factors), IgE (in particular against helminth infections but also during allergic response), IgG (provide the majority of antibody-based immunity, neutralize bacteria and their toxins), and IgM (first Igs produced during the humoral response, neutralize viruses and toxins and activate the complement) (Wallace Taylor, 2002). T cells include three types of cells: cytotoxic T cells (Tc), which recognize and destroy cells with intra-cellular pathogens, helper T cells (Th) which coordinate immune response to infection, and regulatory T cells (Treg) which play a key role in maintaining peripheral immunological tolerance by actively suppressing the immune response (Stanley, 2002). Th cells are differentiated into several subtypes, most importantly Th1 and Th2 depending on their specific production of cytokines (O'Garra, 1998). Th-1 cytokines include mostly IFNg, TNFa, IL-2, IL-3, and IL-12, which activate white blood and T cells. Th-2 cytokines include, among others, IL-4, IL-5, and IL-10, which activate B cells. Th1 cells are mainly involved in response against intracellular pathogens while Th2 cells are most active against extracellular pathogens. Th1 and Th2 cells are reciprocally downregulated, it has been evidenced that organism can favor one over the other, illustrating that trade-off could occur among immune components (Ezenwa *et al*., 2010).

Due to its complexity, eco-immunologists often encounter several methodological limitations when studying immune function in the wild (Martin *et al.*, 2011).

Access to samples

In the wild, age and previous parasite exposure are only available when individuals have been captured from young age or during a long period, thus longitudinal survey are required. Longitudinal data also required to separate within-individual changes in immunity with age from between-individual heterogeneity (Nussey *et al.*, 2008), but a continuous monitoring from birth to death is often challenging in free-living wild animal species. The stress that results from capture and handling can also impact some biological measures. To sample wild species in the field necessitates to be able to appropriately transport and store biological materials. As measure of immune traits sometimes requires intact living cells, it also adds a strong time-related constraint.

Measures on non-model species

Traditional specific assays to measure immune traits have been developed on a few laboratory animal models, but are most of them not suitable for a great variety of species that are now sampled in the wild. There is a lack of specialized (species-specific) assays for studies quantifying immune function in non-model species. Overall, assays measuring simple components and especially the most conserved ones (*e.g.* Ig) can be used on non-model species, but assays are not available to finely discriminate effectors such as cells (*e.g.* lymphocytes). For example, specific markers to discriminate lymphocyte populations necessitate specific development (Nussey *et al.*, 2012).

Interpretation of data

The complexity of the immune function may not be captured by measuring of one or two immune markers (Matson *et al.*, 2006). To provide a good description of an immune phenotype, many assays are needed to capture the multiple components of the immune response (Lee, 2006). However, the use of a large variety of indexes to quantify and qualify the immune system of wild species raises the question of the ecological interpretation of these various measures. Eco-immunologists often face contradictory results, with immune traits that are not correlated with same life history traits, that sometimes co-vary with each other's, or present patterns of covariation that are not always consistent among species or populations over time. The confusing results that are sometimes observed in studies using a large number of markers

highlight the need to link these traits as a part of a single unified system rather than considering them as independent indicators (Matson *et al.*, 2006; Cohen *et al*., 2012). In eco-immunological studies, it is also difficult to interpret the level of an immune trait without a precise knowledge of the immunological antecedents of individuals, never perfectly known in natural conditions.

In conclusion, a large effort dedicated to the development of methodology is still needed in eco-immunology to develop meaningful metrics that can more precisely describe immune profile in a way that is ecologically relevant and physiologically sound (Tieleman, 2018).

2. Allocation in the immune function

As any physiological function, immune system requires energy to properly operate. The costs of immunity are substantial but variable across subsystems of defence (*i.e.* innate or adaptive immune response; cellular or humoral component) and across stages (*i.e.* development, maintenance or activation) (McDade, 2016). Considering the balance between costs and benefits, immune phenotype is shaped by local ecological conditions.

Diverse costs of immune responses

Although immunity is a primary physiological function involved in individual's survival, it is a costly defence mechanism that entails subsequent energy costs. It thus requires sufficient nutrient intake (Sheldon and Verhulst, 1996; Zuk and Stoer, 2002) and may have pathological costs when immune or inflammatory processes are not regulated. Energy and biochemical substrates are necessary for immune function functioning, and adequate energy and nutrient stores determine the profile of immune defences (Lee, 2006; McDade, 2016).

The developmental costs of innate immunity are thought to be quite low (Klasing and Leshchinsky, 1999) while adaptive immunity that requires diversification processes for lymphocyte development appears much energetically costly to develop (Lee, 2006). Adaptive immunity thus involves significantly higher up-front developmental costs. Cellular components of immune responses are also considered costlier to develop than humoral functions (Klasing, 2004). For instance, cell-mediated immune processes are very sensitive to a decrease in macronutrient resources (Gershwin *et al.*, 2000). In contrast, humoral-mediated immune processes (*e.g*. B-lymphocytes) are much less impacted by resources limitation, even in the case of severe malnutrition (Gershwin *et al*., 2000). The costs of maintenance of innate and adaptive

immune defences are considered to be low, in comparison to developmental or activation costs (McDade, 2016). It principally corresponds to the replacement of cells, such as phagocytic cells, and of soluble proteins with limited half-lived such as immunoglobulins (Klasing and Leshchinsky, 1999). Considering other physiological functions as growth, the nutritional cost of maintaining a normal immune system is minimal (Klasing, 1998).

On the contrary, when organisms face an immune challenge, the use of the immune system entails several types of costs (Viney *et al.*, 2005): the energetic costs of immune defences activation and the immunopathology costs of activated defences, added to the direct costs of the infection/pathogen presence (*direct pathology*). Mounting an immune response elevates energy expenditure (Lochmiller and Deerenberg, 2000). Some responses of the immune system are particularly energetically expensive. It is for instance the case of fever, which is a crucial mechanism of the acute-phase reaction to an immune challenge. Depending on the species, fever involves for each superior Celsius degree in body temperature an increase in caloric energy production, comprised between 7 and 15% (Nilsson, 2003). Overall, these studies demonstrate that considerable nutritional costs (are associated with up-regulation of the immune system. While innate immunity represents low developmental costs, it represents very high operating costs for the activation of acute phase proteins, cytokines or the proliferation of leukocytes (McDade, 2016). On the contrary, the activation costs of acquired immunity, once developed, are low (McDade, 2016).

In addition, during an infection, organisms have to support the direct cost of the presence of the pathogen and the side effects of immune responses, whether it is normally regulated (metabolic cost) or not (immunopathological cost). It represents for instance damages of tissues and/or the disruption of the ordinary physiological processes, *e.g.* the nutrient intake, digestion and absorption (Lochmiller and Deerenberg, 2000). The synthesis and degradation of proteins, the metabolism of fatty acids or carbohydrates and the treatment of amino acids, electrolytes or vitamins are deteriorated by an infectious state (Beisel, 1977), exacerbating the costs supported by the infected host. In few days, an infection could result in a protein malnutrition that would take several weeks to develop during a simple starvation. Finally, when considering the activation costs of immunity, it is important to consider the immunopathology costs of activated immune defences (Metcalf *et al.*, 2017). Leukocytes or others phagocytes activated during inflammatory response release pro-oxidant compounds (e.g. Ames *et al.*, 1993). These oxidants have cytotoxic effects that are used to counteract pathogens, yet they also cause oxidative damages impairing DNA, cells and tissues (Monaghan *et al*., 2009). A study on wild kestrel

(*Falco tinnunculus*) suggested for instance that a T-cell mediated immune response causes an increased oxidative stress through an increased production of reactive oxygen metabolites (Costantini and Dell'Omo, 2006). Innate immune response, because of their non-specificity, produces higher "collateral" damages than adaptive immunity (Klasing and Leshchinsky, 1999).

Environmental conditions mediate the allocation to the different components of the immune system

Immunologists have long known that "immunologically, more is not necessarily better" (Viney *et al*., 2005). Considering the substantial costs entails by the immune function (including the immunopathology costs) and the negative cross-regulations among immune components (see Box 1.1), the various components of immunity do not operate all the time at maximum (Martin *et al*., 2008). It supposes the existence of a balance between the important and diverse costs entailed by highly responsive immune system and the fitness benefits of preventing or neutralizing diseases or pathogens, in response to local environmental conditions.

Resources availability, but also local variation in pathogen pressure are key determinants of allocation in various component of immunity. The "antigen-exposure" hypothesis states that overall investment in immune function will be favoured (over other physiological functions) in a highly pathogenic environment (McDade, 2016). Conversely, a strong allocation to the immune system might not be necessary in an environment with a low parasite pressure. More precisely, if the costs of increasing allocation in all aspects of immune function are too high in a highly pathogenic environment, this theory predicts that allocation in acquired immunity will be prioritized over innate immunity, because of its low cost of use (McDade, 2016). This investment would be particularly favoured in long-lived species, that are likely to encounter the same pathogens or parasites repeatedly in their environment and would strongly beneficiate from an adaptive response (Lee, 2006). A study conducted on several populations of small ground finches (*Geospiza fuliginosa*) from Galapagos islands illustrates this pattern (Lindström *et al*., 2004): on larger islands, where birds experience higher prevalence of parasites, they display higher levels of adaptive immunity. On the contrary, on small islands where the parasites pressure is lower, they show greater levels of innate immune response. Access of nutritional resources is also supposed to be a strong determinant of immune phenotype. A study in tree swallow (*Tachycineta bicolor*) nestlings assessed multiple markers of innate and acquired immunity across habitats with low and high food quality and found some evidences

for trade-offs between innate and acquired immune measures in poor quality habitats (Pigeon *et al.*, 2013).

Sex-differences in immune responses

The existence of sex-differences in immunity have been described for many years, in a large range of species and among different taxa (*i.e.* reptiles, birds and mammals) (Kelly *et al*., 2018). Generally in adults mammal and birds, females display stronger immune responses (outside the gestation period) than males do (*The sicker sex*, Zuk, 2009). Consistent with this observation, in many species males exhibit higher incidence of parasitism than females (Poulin, 1996; Zuk and McKean, 1996; Moore and Wilson, 2002). The greater immune ability of females allows a faster clearance of pathogens and provides a better protection against infections. However, in human, this stronger immune activity results for instance in a higher susceptibility to inflammatory and autoimmune diseases of women compared to men (*e.g*. for scleroderma or autoimmune thyroid disease, see Whitacre, 2001). Differences between males and females in the allocation to the immune system can however be age-specific. In humans, pro-inflammatory responses are higher in men at puberty, while inflammatory responses are consistently higher in women at adult age (Klein and Flanagan, 2016). On the contrary, other differences are constant from birth to old age, for example the higher number of CD4+ T cells and CD4/CD8 T cell ratios in women (Klein and Flanagan, 2016).

On a proximate level, differences in immune responses have been thought to be the result of both genetic (Markle and Fish, 2014) and hormonal (Wedekind and Folstad, 1994) influences. Sexually dimorphic patterns of parasite infection could also be due to differences in behaviour between sexes (*e.g.* mating behaviour, foraging) that influence exposure to parasites (Poulin, 1996; Skorping and Jensen, 2004). From an evolutionary perspective, sex-differences in the allocation to immune function could result from the sexual dimorphism in males and females life-history strategies (Rolff, 2002). In many species, females gain fitness through an increased longevity, while males gain fitness by increasing mating rates (Bateman, 1948; Trivers, 1972; Clutton-Brock, 1988). To increase their survival probability, females would thus invest more heavily in immune function – which is however costly (see above) – than males do. This theory provides an explanation to the higher immune responses displayed by females, consistent with their higher resistance to infection (Rolff, 2002). Because of these major sex differences in immune function, it is therefore crucial to take into account the factor of sex in immunological studies.

3. Immunity, a function mediating trade-offs

As laboratory studies have repeatedly revealed that the immune response is resource dependent (Soler *et al*., 2003; Brommer, 2004) and energetically demanding (Lochmiller and Deerenberg, 2000), immunity is considered to a relevant mediator of life history trade-offs (Sheldon and Verhulst, 1996; Zuk and Stoer, 2002; Lee, 2006) (Box 1.2). Accumulated evidences proves that the costs entailed by immune function may compete with other costly functions such as growth or reproduction (Sheldon and Verhulst, 1996; Lochmiller and Deerenberg, 2000).

Box 1.2. Life-history trade-offs

The concept of trade-off is a fundamental aspect of the life-history theory (Stearns, 1992). Trade-offs are generally considered in terms of resource allocation. As resources are most of the time limited in natural conditions, they are insufficient to cover the entire costs for two life-history traits (that are sharing a common resource pool). Consequently, from the *principle of allocation* (Cody, 1966; Williams, 1996), a trade-off occur: an increase in the allocation of resources to one trait necessitates a decrease in the allocation of resources to another trait, and thus negative consequences on this trait (van Noordwijk and de Jong, 1986; Zera and Harshman, 2001). However, trade-offs also occur when the performance of one activity generating negative impacts on other traits (Monaghan *et al*., 2009). The proximate mechanisms underlying these trade-offs are much less understood. Several physiological mechanisms are supposed to mediate the resources allocation strategies and thus drive life-history trade-offs (Finch and Rose, 1995) as the immune response (Sheldon and Verhulst, 1996), the oxidative stress (Monaghan *et al*., 2009) or the telomere dynamics (Monaghan and Haussmann, 2006).

Trade-off between reproductive effort and immunity

Eco-immunologists early described a positive association between reproductive effort and incidence of parasitic infections in wild species (Festa-Bianchet, 1989; Gustafsson *et al*., 1994; Norris *et al*., 1994; Apanius *et al*., 1994). While females typically harbor lower parasite loads than males, Festa-Bianchet (1989) observed indeed that lactating bighorn ewes (*Ovis Canadensis*) show high prevalence and intensity of parasitic infections, superior to non-lactating ones, suggesting that allocation to reproductive effort weakens the immune system (see also Turner *et al*., 2012; East *et al*., 2015). Experimental studies on birds then confirmed that females investing heavily in reproduction may experience depressed immune function and

consequently higher parasite loads (Deerenberg *et al*., 1997; Nordling *et al*., 1998; Moreno *et al*., 1999). However, most of these studies have focused on females, probably because it is easier to measure reproductive effort in females than in males (Lemaître *et al*., 2015). Quantifying male reproductive allocation (*e.g.* sperm quality, secondary sexual traits or reproductive success) is indeed extremely difficult in wild populations of vertebrates.

Several hypotheses have been proposed to explain the negative relationship between reproductive effort and immune-competence, and the positive association with parasitic burden. Firstly, reproductive behaviour increases the likelihood of exposure to disease, and increases the risk of contracting a sexually transmitted disease (Nunn *et al*., 2000). Reduced immune responses during reproduction might be also an adaptation to avoid immunopathological damages naturally produced by strong immune responses (Råberg *et al*., 1998) - that may create damages to sperm (Hillgarth *et al*., 1997) or could induce spontaneous abortion of the foetus (Wegmann *et al*., 1993). In males, weakened immune responses during reproductive state have been also attributed to elevated levels of sex steroid hormones. Reproductive male behaviour is indeed tightly linked with testosterone production, but this hormone is also known to modulate immune responses in vertebrates (Balenger and Zuk, 2014). In mouse lemurs (*Microcebus murinus*) for instance, peaks of testosterone during male roaming behaviour coincides with an elevated level of parasitism in males, suggesting weakened immune responses (Hämäläinen *et al*., 2015).

The reduced immunocompetence often associated with reproduction may also result from an energy trade-off. In mammals, the reproductive effort is costly for individuals and demands maximal nutritional resources (Speakman, 2008). Female mammals bear particularly high costs of reproduction, both during pregnancy and postparturition through postnatal care (*e.g*. lactation, Oftedal, 1985; Clutton-Brock *et al*., 1989). Consequently, to maximize reproductive success in a context of limited resources, females may reallocate resources away from parasite defence to the benefit of reproduction (Sheldon and Verhulst, 1996). This strategy of allocation may result in a reduced resistance and ability to fight diseases, leading to an increased risk of invasion by parasites and pathogens. On the long-term, the weakened immune-competence associated with high reproductive investment would lead to a lower body condition of individuals, and in a long-term to a much steeper decline in fitness (*i.e.* reproductive success, survival, or both) with a decreased survival (Sheldon and Verhulst, 1996).

Partial understanding of the relationship between immunity and growth

Growth is a key life-history trait that entails substantial energy and nutrient requirements (Wieser, 1994). It is particularly crucial for young individuals to develop a large body size rapidly, to buffer the effects of fluctuating environmental conditions (Dmitriew, 2011) and to spend less time at the critical early-life stage, when juveniles are particularly vulnerable to predators (Metcalfe and Monaghan, 2003; Ronget *et al*., 2018). In the wild, the survival of juveniles is thus strongly influenced by growth, but also by the early development of efficient immune responses, to be protected from the constant threat of infections and diseases (Sheldon and Verhulst, 1996; Klasing and Leshchinsky, 1999; Schmid-Hempel, 2003).

Evidences for a trade-off between immune function and growth firstly came from laboratory and poultry studies. In the latter case, researchers have taken advantage of poultry lines that have been selected for either growth (body mass) or an aspect of immune function (Van der Most *et al*., 2011). These studies showed that selection for high growth has a negative influence on immune function. On the contrary, selection for high investment in baseline immune function did not consistently affect growth, whatever the immune components observed (Van der Most *et al*., 2011). This limited impact of immune manipulation found on juvenile growth suggests that the amount of energy required for the immune response is much lower that required for tissue growth (Klasing, 1998).

While high investment in baseline immune function do not impact growth (see above), experiments based on parasite challenge or immune stimulation in young birds revealed on the contrary that inducing immune challenge can have a negative influence on nestling growth (Alonso-Alvarez and Tella, 2001; Whitaker and Fair, 2002; Nilsson, 2003; Soler *et al*., 2003; Brommer, 2004; but see Hõrak *et al*., 2000). Immune challenge in juveniles can also have long-term effects on adulthood, with *e.g*. a negative impact on body condition (Alonso-Alvarez and Tella 2001; Sanz *et al*., 2004) or an increase in metabolic rate (Ots *et al*., 2001). In wild vertebrates, the vast majority of studies assessing the existence of such trade-off have been however conducted on birds (Soler *et al*., 2003; Brommer, 2004; Mauck *et al*., 2005). Finally, whether differential allocation to growth could entail costs influencing immune development have been studied in farm animals (see above) but rarely in wild species.

4. Age-related variation in immune response

Wild vertebrates show profound age-related variation in their immune function over their lifespan (Cichoń *et al*., 2003; Palacios *et al*., 2007; Nussey *et al*., 2012). From early life to adulthood, animals have to develop mechanisms to resist and fight infectious challenges, but with increasing age, immune function seems to be progressively compromised by dysregulations. Young and old individuals thus display higher sensitivity to parasites than middle-aged adults (Body *et al*., 2011).

The development of immunity

In the wild, young individuals display higher parasite intensity than adults, as proved in Soay sheep (*Ovis aries*) lambs and yearlings (Wilson *et al*., 2004; Craig *et al*. 2006) and in roe deer fawns (Body *et al*., 2011). More precisely, Soay sheep lambs exhibit much higher levels of nematodes in their gastro-intestinal tract than adults (+183%, Craig *et al.*, 2006). Similarly, the prevalence of gastro-intestinal strongyles is much higher in fawns (100%) than in adult roe deer (*Capreolus capreolus*) (~60%) (Body *et al*., 2011). Indeed, the immune system of young vertebrates is not fully developed and operational at birth; some immune responses develop progressively with age (*e.g.* progressive increase of antibody responses, Watson *et al*., 1994).

Immune-competence requires the development and education of many interacting cell types (Klasing and Leshchinsky, 1999). The innate part of immunity is functional from early embryogenesis. At birth, newborns that come from a protected environment (*i.e*. egg or maternal uterus) are suddenly exposed to a wide range of microorganisms. The innate immunity thus play a key role in the initial resistance to infectious diseases and pathogens (Klasing and Leshchinsky, 1999). As they have limited ability to synthesize antibodies endogenously, a temporary help is provided by a passive maternal transfer of immunity of antibodies and T-cells, which represent a major determinant of immune capacities in young vertebrates (Grindstaff *et al*., 2003). In birds, maternal transmission is through the deposition of antibodies in eggs (Brambell, 1970). In mammals, maternal immunoglobulins are direct transferred across the placenta (*e.g.* in primates) and/or by ingestion by the neonate of the immunoglobulin-rich colostrum immediately after birth. Maternal milk also provides a constant supply of immunoglobulin (IgA in most species) that helps to protect the newborn. The adaptive immunity, which is a slow and resource-demanding process, requires several weeks/months in birds or mammals to reach full maturity (Apanius, 1998). Indeed, juveniles are able to develop adaptive immune responses after a few weeks, but an immuno-competence equivalent to that

of an adult requires several months of development. Typically, maturation of adaptive immune response requires the differentiation and selection of lymphocytes – driven by environmental antigens encountered, to recognize non-self antigens without errors that would cause autoimmunity (Klasing and Leshchinsky, 1999).

Patterns of immune development and profile (*i.e.* balance innate/adaptive immune system) of organisms is shaped by life-history traits and life stage (Norris and Evans, 2000; Sandland and Minchella, 2003; Lee, 2006). Adaptive immunity is costly to develop but is strongly beneficial against repeated infections, because its efficiency is enhanced markedly after a second exposure to a pathogen. Consequently, development of adaptive responses are more likely to be favoured in long-lived species which invest massively in their offspring (Klasing and Leshchinsky, 1999). On the contrary, species with a fast development, short lifespan, infrequent or unrelated pathogen challenges and predictable food resources favour strong and persistent innate immune responses (Klasing and Leshchinsky, 1999). However, due to the very rapid evolution of pathogens and the frequent new immune challenges that organisms face all along their life, non-specific innate responses are nevertheless maintained at baseline levels throughout life, despite their high costs of activation.

Senescence of the immune system

With increasing age, global and gradual dysfunctions of the immune system abilities occur in organisms, a process called "immunosenescence". In humans, it has been associated with an increased susceptibility to infectious diseases, but also contributes to many degenerative diseases (especially neurodegeneration, cancer, cardiovascular and autoimmune diseases, Pawelec, 1999; Castelo-Branco and Soveral, 2014). This increased sensitivity to infections and diseases is expected to impact reproductive success and/or survival under natural conditions (Sheldon and Verhulst, 1996; Schmid-Hempel, 2003) and thus to have a deleterious effect on fitness (see Box 1.3).

Box 1.3. Senescence and evolutionary theories

With increasing age, most organisms experience senescence, a process characterised by progressive and irreversible decline in age-specific reproductive success (*i.e.* reproductive senescence) and survival (*i.e*. actuarial senescence) (Partridge and Barton, 1993). Reproductive and actuarial senescence have been repeatedly documented in laboratory animal models (Bonsall, 2006), captive (Lemaître *et al*., 2013) and wild (Nussey *et al*., 2013) populations, and senescence appears to be the rule rather than the exception in the living world (Nussey *et al*., 2013; Jones *et al*., 2014).

Senescence processes represent however a paradox in biology, at the light of natural selection: indeed, mechanisms of cell repair also exists (Vilchez *et al*., 2014) and senescence have a negative effect on Darwinian fitness (Bouwhuis *et al*., 2012), so why organisms senesce? Several evolutionary theories of ageing have been proposed, I will present here the three main theories.

- The mutation accumulation theory (Medawar, 1952). In a given cohort, a progressive decline in the proportion of individuals alive is observed with age (because of accident, disease, predation…). At older age, less and less individuals of this cohort are still reproducing and contribute to the next generation: the reproductive value declines with age (Fisher, 1930). Medawar suggests that senescence would be a consequence of the decline in the strength of natural selection with age: the accumulation of mutations with deleterious effects late in life occurs across generations, because most individuals have reproduced before the mutations take effect, and mutations are thus not eliminated by natural selection.
- The antagonistic pleiotropy theory (Williams, 1957) proposed that senescence may be the consequence of the active selection of *antagonistically pleiotropic genes,* favoured by natural selection because of their beneficial effects on fitness in early-life, despite their deleterious effects late in life. This theory, as the mutation accumulation theory, involve mutations as the cause of senescence and these two theories are not mutually exclusive (e.g., Rodriguez *et al.*, 2017). The theory of Williams also introduces a major notion of genetic trade-off between early-life performance and late-life costs. In his article, Williams proposed a set of nine "testable deductions" from his theory (*e.g*. prediction 2 "low adult death rates should be associated with low rates of senescence, and high adult death rates with high rates of senescence"). A recent review from Gaillard and Lemaître (2017) present the pros and cons of each prediction based on studies performed in the laboratory and in the wild, and highlights the importance and the relevance of this theory.
- The disposable soma theory (Kirkwood, 1977) is a special case of antagonistic pleiotropy but in a physiological ecology approach. This theory is based on the idea that organisms have to share a limited pool of energy between growth, reproduction and cellular maintenance (Kirkwood and Rose, 1991). The increase of energy allocation in one function would thus be at the expense of another function (Stearns, 1992). Consequently, a high energy allocation to growth or reproduction in early life would lead to a lower investment into somatic maintenance, an accumulation of damages, resulting on the long-term in faster decrease of reproduction or survival. The disposable soma and the antagonistic pleiotropy theories are not mutually exclusive, and proposed similar predictions in terms of trade-off between reproduction and senescence, despite they are based on different mechanisms (Robins and Conneely, 2014). A recent review performed on 26 studies of free-ranging vertebrate populations brings overall support for the existence of a trade-off between performance in early and late life (Lemaître *et al*., 2015).

It is now admitted that the many components of immune function are all subjected to age-related changes, more or less profoundly (as explained in the paragraph below), leading to the general erosion of the immune capacities. Studies that have investigated immunosenescence in humans or mice have first described an age-related decline in the adaptive immune response (Linton and Dorshkind, 2004) and a low-grade chronic inflammatory status in the elderly (Franceschi *et al*., 2000). During many years, innate immunity was considered as well preserved with ageing, but more recently, cumulative evidences proved that cellular components of immunity are also subject to profound modifications with increasing age (Gomez *et al.,* 2005; Larbi *et al.,* 2008).

Studies on humans and laboratory animal model proved that several immune components are affected by the immunosenescence process (as reviewed in Castelo-Branco and Soveral, 2014). Firstly, at older ages, the adaptive components of the immune response show profound dysregulations. It is firstly characterised by a strong decline in the proliferation capacity of the hematopoietic stem cells of the bone marrow, that are responsible for the constant input of progenitors at the origin of all blood cells (*i.e.* of myeloid and lymphoid lineages). These cells are thus required for both innate and adaptive immunity. It is also described a decline in the number of naïve B cells and in the ability of memory B cells clonal expansion, as well as the functional impairment of antibodies. In the same way, the absolute number of T-cells, particularly the naïve ones, decreases with age, and mature T-cells (especially the CD8+) show functional defects. The decline in naïve T-cells is mostly caused by the well described thymic atrophy that occurs naturally with age (Aspinall and Andrew, 2000). The decline of adaptive immune response is associated with an increase of the inflammatory markers production, leading to a chronic and low-grade inflammatory state in old individuals, called "inflamm-aging" (Franceschi *et al.*, 2000). This inflammatory state is particularly characterized by increased levels of circulating cytokines and pro-inflammatory markers, especially pro-inflammatory cytokines TNF-α, IL1 and IL6 (Franceschi *et al*., 2000). This increase in the production of pro-inflammatory markers can however be counteracted by anti-inflammatory cytokines (*i.e*. IL-10), as found in healthy elderly (Lio *et al.*, 2004). The innate component of immunity is also affected by several age-related changes: an impairment of anatomical barriers, an increase of dysfunctional natural killer cells, a decline in phagocytic function of neutrophils (associated with a reduction of the intracellular respiratory burst necessary to kill bacteria), a decline in the ability of dendritic cells to recognize pathogens, and a decline in cytokine production/ T-cells activation by macrophages (Castelo-Branco and Soveral, 2014).

Although immunosenescence has been well studied in laboratory conditions, much less is known in the wild (see a review in Table 1.1). The study of immunosenescence in the wild have been firstly conducted in birds, and to a lesser extent in reptiles (since 2003, see Table 1.1). Studies on wild mammals are even more recent, starting with Nussey *et al.* (2012). For now, 12 studies have investigated possible age-specific changes in wild birds, 6 in mammals and 5 on reptiles (Table 1.1). Overall, the cellular part of adaptive response, *i.e.* T-cell function, does show senescence in all studies in mammals and birds (but not in the only study on reptiles). On the contrary, the humoral part of the innate response does not show marked evidence of senescence, either in mammals, or in birds or reptiles. Finally, evidence of senescence remains contradictory for cellular part of the innate response and humoral part of the adaptive response (Table 1.1).

For the time being, these observations suggest that patterns of age-specific changes in the immune response could be quite similar in wild and laboratory conditions. It particularly highlights that more studies are needed to better understand age-related changes in immune response in natural populations. It is also to note that almost all the studies in this field are cross-sectional. However, as explained in Nussey *et al.* (2008), patterns from cross-sectional studies may be confounded by the selective disappearance of particular phenotypes with age and therefore, there is a real need for longitudinal data to investigate senescence. Finally, studies often described a few numbers of immunological traits (especially in birds or reptiles). As explained above, immunity is an extremely complex function and a precise description of immune profile of individuals would require measures of several traits encompassing the various components of immunity.

Table 1.1. Studies assessing immuno-senescence in wild animals. "Possible" indicated contradictory results (between different methods, or between the several traits). M/F indicates sex of individuals (M: males, F: females). Ab: antibody, Ag: antigenic, NAbs: Natural antibodies, HAP: haptoglobin.

Evidence of senescence?		M/F	A. Innate cellular	B. Innate humoral	C. Adaptive cellular	D. Adaptive humoral	Number of traits	Detail of immune traits (A/B/C/D)	References
BIRDS	Common tern	M/F	-	-	-	no	1	[D. B-cell function (levels of IgG in plasma)]	Apanius & Nisbet, 2003
	Collared flycatchers	F	-	-	-	yes	1	[D. B-cell function (antibody production via antigenic challenge)]	Cichoń *et al.*, 2003
	Ruff	M/F	-	-	yes	-	1	[C. T-cell function (PHA)]	Lozano & Lank, 2003
	Barn swallow	M/F	-	-	-	yes	1	[D. B-cell function (antibody production via antigenic challenge)]	Saino *et al.*, 2003
		M/F	-	possible	-	-	2	[B. yes: NAbs plasma/no: lysis]	Møller & Haussy, 2007
	Zebra finch	M/F	-	-	yes	-	1	[C. T-cell function (PHA)]	Haussmann *et al.*, 2005
	Tree swallow	M/F	-	-	yes	-	1	[C. T-cell function (PHA)]	Haussmann *et al.*, 2005
	Leach's storm petrel	M/F	-	-	yes	-	1	[C. T-cell function (PHA)]	Haussmann *et al.*, 2005
	Tree swallows	F	-	no	yes	no	4	[B. NAbs plasma, lysis] [C. T-cell (PHA, ConA)] [D. B-cell (LPS)]	Palacios *et al.*, 2007
		F	-	no	possible	yes	4	[B. Plasma antibacterial activity, lyzozyme plasma] [C. T-cell function (PHA)] [D. B-cell function (Ab production via antigenic challenge)]	Palacios *et al.*, 2011
	Wandering albatross	M/F	-	no	-	-	4	[B. HAP, plasma antibacterial activity, Nabs plasma, lysis]	Lecomte *et al.*, 2010
	Swan	M/F	-	-	-	no	1	[D. B-cell function (antibody production via antigenic challenge)]	Hill *et al.*, 2016
MAMMALS	Soay sheep	F	-	possible	possible	-	10	[B. yes: HAP, serum Amyloïd A/no: IL-6, IL-10] [C. T-cell function, yes: CD45RA+, CD4+FoxP3, CD4+FoxP3+CD45RA+, γδ/no: CD4+, CD8+]	Nussey *et al.*, 2012
		M/F	no	-	possible	no	11	[A. eosinophil, NLR] [C. T cell, yes: CD4+ naïve, CD8+ naïve, γδ+ Tcr, Treg/no: CD4+, CD8+] [D. B-cell (levels of IgG, IgA, IgE)]	Watson *et al.*, 2016
	Greater sac-winged bat	M/F	yes	possible	-	possible	3	[A. Total WBC count] [B. yes&no: plasma antibacterial activity] [D. yes&no: B-cell function (levels of IgG in plasma)]	Schneeberger *et al.*, 2014
	European badgers	M/F	-	-	-	yes	1	pro-inflammatory cyctokine production (IFNγ)	Beirne *et al.*, 2016
	Roe deer	M/F	no	possible	yes	no	12	[A/C.Leukocyte forms][B.Nabs plasma,lysis][D. γ-globulins]	Cheynel *et al.*, 2017
	House mouse	M/F	yes	-	yes	no	10	[NKp46+ cells, Ly6G+ neutrophils, CD11c+ DCs, F4/80+ macrophages] [T-cell, CD4+/CD8+/CD19+] [B-cell, levels of IgG, IgE, IgA]	Abolins *et al.*, 2018
	Ring-tailed lemur	M/F	no	-	yes	-	5	[A.neutrophils, monocytes, eosinophils, basophiles][C. lymphocyte]	Singleton *et al.*, 2018
REPTILES	Water pythons	M/F	-	-	-	yes	1	[D. B-cell function (antibody production via antigenic challenge)]	Ujvari & Madsen, 2006
		M/F	-	no	-	yes	2	[B. NAbs plasma] [D. B-cell function (Ab production via Ag challenge)]	Ujvari & Madsen, 2011
	Garter snake	M/F	-	no	-	-	3	[B. NAbs plasma, lysis, plasma antibacterial activity]	Sparkman & Palacios, 2009
	Common lizard	F	-	-	no	-	1	[C. T-cell function (PHA)]	Massot *et al.*, 2011
	Philippine crocodile	M/F	-	no	-	-	2	[B. NAbs plasma, lysis]	Groffen *et al.*, 2013

Underlying mechanisms of immunosenescence

While the description of immunosenescence is increasing in wild animals, the exact underlying physiological mechanisms remains poorly understood.

The decline in the average telomere length of immune cells have been proposed as a potential mechanism of immune senescence. Telomeres are repetitive and non-coding sequences at the end of eukaryotic chromosomes. These complexes maintain genomic integrity by capping the ends of eukaryotic chromosomes and forming complexes with proteins (Armanios and Blackburn, 2012). During mitosis, in the absence of restoration and repair processes, telomeres are shortened because the 5' end of DNA strands is not fully replicated (Blackburn, 1991). The decline in telomere length over time is also largely due to oxidative damage (Monaghan and Haussmann, 2006). *In vitro*, critically short telomeres trigger cellular senescence (Aubert and Lansdorp, 2008). Interestingly, telomere lengths vary among species, as well as the rate of telomere shortening varies among chromosomes, tissues, individuals and according to age (Monaghan and Haussmann, 2006). In humans, the shortening of telomeres with age have been proposed as a biomarker of ageing and age-related diseases (Fossel, 2012). Telomere shortening has particularly been described in a number of chronic inflammatory diseases. In wild species (principally in birds), telomere dynamics would predict survival (*e.g.* Bize *et al*., 2009).

Immunity is a function that requires a constant self-renewal of immune cells, consquently based on multiple divisions and therefore very dependent on an efficient telomere maintenance (Goronzy *et al*., 2006). The hematopoietic stem cells (the "original" that produce all immune cells through divisions) present indeed a very high activity of telomerase, an enzyme that can restore telomeres (Goronzy *et al*., 2006). Despite this efficient repair mechanism, the hematopoietic stem cells would still be subject to telomere shortening with increasing age (Elwood, 2004). Such telomere shortening, even moderate, is likely to have a negative influence on the renewal potential of hematopoietic lineages and immune cells production, and thus negatively affect immune-competence with increasing age (Goronzy *et al*., 2006). For now, the idea that telomere shortening in hematopoietic stem cells is an underlying mechanism of immunosenescence remains an hypothesis, because the dynamics of telomere shortening in hematopoietic stem cells can not been measured directly (Goronzy *et al*., 2006). The dynamics of telomere in stem cells can however been approached through the study of neutrophil telomere dynamics, which are immune cells that undergo few divisions

during the differentiation from hematopoietic stem cells. In humans, neutrophil telomere length declines with age (Robertson *et al.*, 2000), which suggested that telomere length of hematopoietic stem cells is expected to decline with age. Secondly, immune responses are dependent on the rapid proliferation of clonal T- and B-cell populations and on their differentiation into long-lived memory cells, in response to invading pathogens (Goronzy *et al*., 2006). As consequence, memory T-cells generally have shorter telomere lengths (Weng *et al*., 1995) and senescent T-cells repetitively stimulated *in vivo* are characterized by particularly short telomeres (Monteiro *et al.*, 1996). To summarize, age-related telomere shortening in immune cells can reduce immuno-competence in old individuals, by compromising the functions of haematopoietic stem cells and by reducing the potential of lymphocyte proliferation (Weng, 2012).

Finally, immune cell telomere length could be influenced by disease and exposure to pathogens. It has been described in wild-derived house mice (*Mus musculus musculus*) that exposure to an infectious agent can cause a decline in immune cell telomere length (Ilmonen *et al*., 2008). Another study in wild badgers (*Meles meles)* found suggestive associations between immune cell telomere lengths and disease status of individuals (Beirne *et al.*, 2014). A very interesting study in great reed warblers (*Acrocephalus arundinaceus*) also described that birds subjected to chronic malaria infection have shorter telomeres, show reduced lifespan and produced chicks with shortened telomeres (Ashghar *et al.*, 2015). We can hypothesize that this aspect is of a major importance and strongly participated to immunosenescence processes in wild animals very often subjected to pathogens or naturally infection with disease.

To conclude, the decline in immune cells telomere length represents a strong candidate that could underlie immunosenescence. A better understanding of the age-related telomere dynamic - *e.g*. the differences in the rate of telomere attrition between the different types of immune cells - is however still needed. It is also to note that other age-related dysregulations (*not detailed here*) - as the decline in hormonal secretion at old age and the parallel reduction in the sensitivity of tissues to their action - are likely to have additional detrimental effects on immune function with age (Jones and Boelaert, 2015).

5. Structure of the thesis

The aim of this thesis is to provide a better understanding of age-related variation in immune profile, and to decipher the relationships between the allocation to immunity and other life-history traits, in two contrasted populations of a wild mammal, the roe deer (*Capreolus capreolus*). At the crossroad of physiology and evolutionary biology, eco-immunology requires more knowledge about the immune function in wild species, especially in mammals, and how life-history trade-offs are mediated by the allocation to the immune function. This thesis aims to fill this knowledge gap.

The roe deer is a suitable model to study age-related variation in immune response. It is a long-lived mammal: in the wild, male and female roe deer respectively live up to 14 and 18 years of age. The roe deer is one of the very few species in the world where males and females from two different populations are followed individually by capture-mark-recapture, since the 70's. These two populations experience marked differences in their environment that give a unique opportunity to test how relationship between immunity and life-history traits are influence by contrasted conditions. This individual follow-up provides a wide range of data on condition (body mass, parasitism) and life-history traits (reproductive status in females, antler size in male, survival) for known-aged roe deer of both sexes. Since 2010, annual blood sampling in the two roe deer populations allowed us to measure a large set of immune and physiological markers, opening a wide field of investigations and initiating eco-immunology studies in these two populations.

Following the general introduction above, the second chapter of this manuscript presents the *Material and methods* I used to perform this thesis. I present in more details the roe deer as a model species, the characteristics of the two sites of study and finally all the measures of physiological parameters used for this work (immunity, body condition, telomere length).

In the third chapter, I present two studies in which we focused on the immune profile of juvenile roe deer aged 8-months. In a first study, we assessed the existence of a trade-off between immunity and another costly function in early life, *i.e.* growth. We investigated whether a rapid growth could impair immunity roe deer on the short-term (*i.e.* during growth) but also on the long-term (during adulthood), with a comparison between sexes. We hypothesized that a negative association should occur between high investment in growth and immune traits over the short-term (*i.e.* during the growing period) in young roe deer, in particular for adaptive immunity that develops more progressively compared to innate

immunity. We also predicted that a cost of fast growth could impair immune traits over the long-term (*i.e.* during adulthood). In the latter analysis, we separated two stages of growth in young roe deer (*early* and *late* growth) that does not involve similar costs. As the compensatory process of *late* growth entails particularly high costs (Douhard *et al.*, 2017), we predicted that immunity costs of *late* growth should be higher than those of *early* growth. In a second study, we investigated the influence of maternal age and body mass on physiological condition of recently emancipated young roe deer. As senescence of maternal effects is thought to have strong influences on the offspring conditions (Beamonte-Barrientos *et al.*, 2010), we expected lower levels of physiological markers (immunity and body condition) of fawns born to old females compared to prime-aged ones. In addition, as adult body mass is a reliable proxy of individual quality in female roe deer (Plard *et al.*, 2015), we also expected that the deleterious effect of mother age on offspring condition should be more pronounced for fawns born from the lightest females, because of their lower capacities to transfer resources to their offspring. Finally, in the two studies of this chapter, we also tested the importance of environmental conditions in young roe deer immune profile, thanks to the monitoring of two contrasted populations.

In the fourth chapter, we focused on the description of age-related changes in immune function in males and females roe deer of our two populations of roe deer. In roe deer, both parasite prevalence and load have been described as higher in old age classes than in young age classes (Body *et al*., 2011), suggesting the existence of some immunosenescence. However, the exact age-specific changes in parasite burden and immune profile remain to be identified. We measured a large set of immune markers, encompassing both innate and adaptive components of the immune response, to obtain a comprehensive picture of the age-related changes in the immune system of known-aged roe deer. We also assessed age-specific patterns in the parasite burden of the same individuals. We hypothesized, in accordance with previous studies conducted on laboratory and in the wild, a progressive decline of the adaptive immune component, an increased inflammatory markers and an overall maintaining of the innate component in old individuals. Based on our current knowledge we also predicted an earlier and more pronounced immunosenescence in males than in females (Tidière *et al*., 2015). Finally, we investigated whether roe deer living in the population with poor and limited food resources display an earlier and sharper immunosenescence than in the population with rich and abundant food resources.

In the fifth chapter, we investigated one of the underlying mechanisms of immune competence trough the measure of immune cell telomere length. During my PhD work, we have indeed developed the measure of immune cell telomere length in roe deer, through a collaborative work with Prof. Daniel Nussey (*Institute of Evolutionary Biology, University of Edinburgh*). In a first study, we assessed whether roe deer immune cell are subject to age-related variations in telomere length. We also tested how broad differences in environmental conditions influence telomere dynamics in roe deer by comparing patterns of variation in leukocyte telomere length between our two contrasted populations. Based on the current knowledge on humans and birds telomere dynamics, we predicted that persistent experience of a poor environment would result in shorter telomere length at any given age, owing to lower initial telomere length in early life and a faster rate of telomere length shortening over an animal's lifetime. In a second part, we investigated potential association between the immune cell telomere length, relative proportion of each leukocyte and the inflammatory status of adult roe deer. We aimed at assessing whether leukocyte telomere length could vary in association with proportions of the different leukocyte cell types, which are known to differ in telomere length. Studies in humans also described that inflammation could have a negative impact on telomere length. To our knowledge, this association have not been investigated in the wild. We predicted a shorter telomere length in individuals with high levels of inflammatory markers. This second part of the fifth chapter have been started recently, thus only preliminary results are provided.

The sixth chapter proposes a summary of the findings reported in previous chapters and perspectives that this work open.

Finally, to reflect the overall work I produced during this PhD, papers to which I have contributed are provided in appendices (Appendix 1, Appendix 2). During my PhD, I also passed a university degree in *Evolutionary Biology and Medicine* at the University Claude Bernard, Lyon (France), with the writing of a thesis on "Allergic diseases and the Hygiene Hypothesis" (see Appendix 3, *in french*).

CHAPTER 2

Material and methods

1. The roe deer as a model species

A long-lived mammal

The roe deer (*Capreolus capreolus*) is an ungulate belonging to the Cervidae family (order Artiodactyla). It is a small deer, which reaches 60-75 cm (at the shoulder) and weighs between 18-30 kg. Males are on average larger than females and about 3 kg heavier, but roe deer present overall relatively low dimorphism (Andersen *et al*., 1998; see Fig. 2.1). Males carry antlers as sexual secondary traits, which grow from early December and are casted in next autumn.

Figure 2.1. To adjust their thermal resistance, the pelage of male and female roe deer differs according to the season: thick and grey in winter (at left), thiner and brown-orange in summer (at right). Photography: F. Ribeaudeau.

The roe deer is considered as a long-lived mammal: in the wild, male roe deer can live until 14 years and females until 18 years (Loison *et al*., 1999). The survival of fawn is low and variable from birth to weaning (Plard *et al*., 2014), increases up to 2 years of age, then, survival remains quite high and constant for about 6 years (prime-age stage), before diminishing markedly from 8 years of age onwards (senescent stage) (Gaillard *et al*., 1993 and see Fig. 2.2).

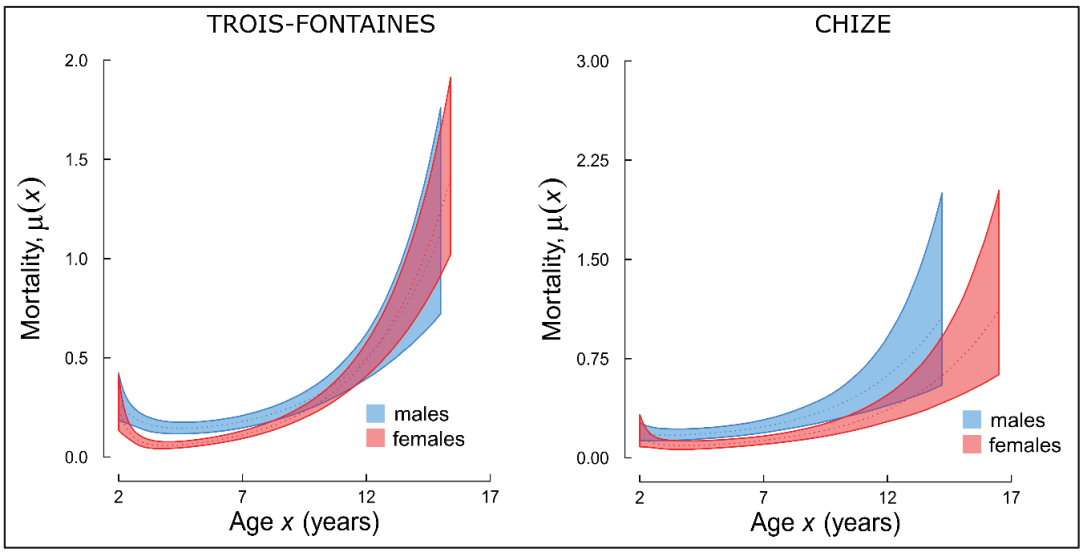


Figure. 2.2. Mortality curves modelled in both sexes of two populations of roe deer in France (Chizé and Trois-Fontaines), from the onset of reproductive age, *i.e.* 2 years of age - based on field data from 1990 to 2010 (V. Ronget, *pers. comm.*).

Variated natural habitats

Roe deer occupy a wide range of natural habitats: all types of forests - hardwood, conifer or shrublands - but also moorlands and marshes (Andersen *et al*., 1998). Their distribution area is however limited by the treelines and they do not live in open alpine habitats. Indeed, the major requirement of roe deer is to have the possibility to be cover to escape from predators and men (Andersen *et al.,* 1998). Roe deer tolerate human activities and landscape fragmentation by occupying small patches of woodland or shrubs. Roe deer also tolerate a wide range of climatic conditions, including climatic extremes. Its great faculty to adapt explain their large distribution in Europe (Figure 2.3).

Roe deer are generalist herbivores, but their small body size and their very low levels of stored energy reserves (Hewison *et al*., 1996) constraints them to be highly selective in their feeding strategy. Although they have also developed behavioural and digestive adaptations to be in capacity to support poor quality food (Andersen *et al*., 1998), they remains very sensitive to variations in the availability of resources, especially the fawns. Birth of fawns so take place to match spring green-up (Andersen *et al*., 2000). Since they do not store large fat reserves, adult body mass varies very little over the years (Andersen *et al*., 2000). Roe deer are considered as “income breeder” (sensu Jönsson, 1997): their survival and reproductive effort only rely on

energy intake (Andersen *et al*., 2000). Roe deer strongly react to nutritional conditions and show marked adaptations to their local environment, which make roe deer an excellent model to study the influence of environmental conditions on biological traits.

Figure 2.3. Distribution of *Capreolus capreolus* in Europe (in orange).

Reproduction and social organisation

Roe deer present high absolute allocation to reproduction, with its quite rapid turn-over for a long-lived animal (generation time of about 5 years). Female roe deer start reproducing at 2 years of age, and generally give birth every year to two fawns (Gaillard *et al*., 1992). Reproductive senescence is observed at around 11 and 12 years of age (Andersen *et al*., 1998). Reproductive cycle of female roe deer is unique in cervids. As female they are monoestrous (*i.e*. they can be fertilised during one single oestrus that last 36 hours, Sempéré *et al*., 1998), mating takes place during an very short period in late summer, and after fertilization, the implantation of the blastocyst is delayed by 5 months, a phenomenon called "embryonic diapause". The embryo is reactivated and starts its development in December-January, a process stimulated by changes in the photoperiod. The gestation thus lasts 300 days including the embryonic diapause, but real gestation lasts 130 days. Birth of fawns occurs between May and early June. During the first ten days of life, neonates remain hidden in the grass and they maintain a very low level of activity, which limits the probability of detection by predators. Then fawns stay with their mother during the 11 first months of life. Lactation is essential for survival during the period of early summer, and offspring orphaned in early August are able to

survive (D. Delorme, *pers. comm.*). Lactation can however last until September or October (Sempéré *et al*., 1988).

Male roe deer father their first offspring during their fourth year of life (Vanpé *et al*., 2009). From one year of age, males develop antlers every year (adult antler size around 200-250 mm). During their first year of life, males only develop a small "button" (*i.e*. a very short antler with a round top instead of a spike) on top of their pedicle. Antlers grow from the end of November until early March (cycle of antler growth illustrated in Fig. 2.4) in a highly vascularized tissue (known as velvet) that covers them. When antlers are cleaned of their velvet, *i.e.* at the end of the winter/early spring, males become territorial. Territoriality is associated with aggressiveness, marking and patrolling behaviour, until after the rut (*i.e.* at the end of August). This behaviour has been related with variations in circulating testosterone concentration (Andersen *et al*., 1998). Males show stability in the choice of territory between years (Andersen *et al*., 1998). Finally, they cast their antlers at the end of October/early November (Sempéré and Boissin, 1981).

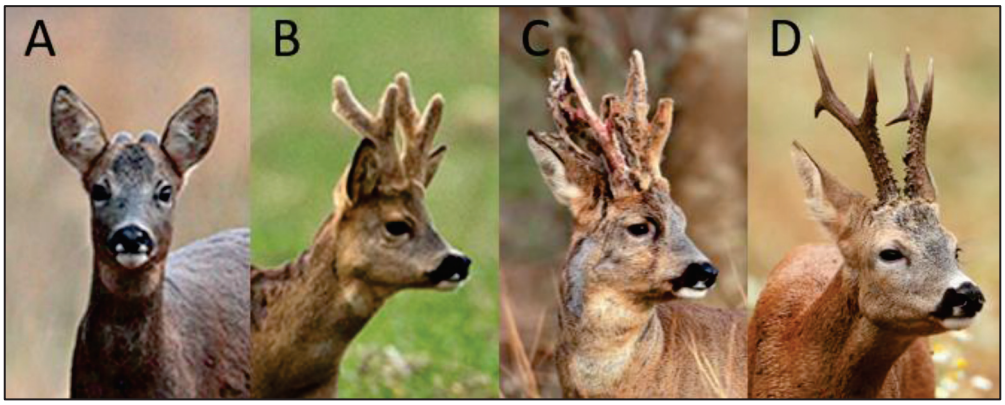


Figure 2.4. Cycle of antlers growth in male roe deer,
with (A) start of growth from a "button", (B) growth in velvet,
(C) loss of the velvet and (D) hard-antlers. Photography: F. Ribeaudeau.

Depending on the habitat, roe deer can modify its social organization (Andersen *et al*., 1998). During summer, females and males are separated: females raise their fawns and males defend mating territories. On the contrary, at the end of autumn and in winter, males and females cohabits in fluid groups. The size of groups can increase during winter in situation of increased population density and/or habitat openness, from few individuals to groups of more than 50 roe deer (Andersen *et al*., 1998).

2. Study sites and population monitoring

The two study sites: Trois-Fontaines and Chizé

In France, two populations of roe deer are followed by programs of Capture-Mark-Recapture (CMR) organized by the French Office National de la Chasse et de la Faune Sauvage (ONCFS) (Fig. 2.5). As detailed below, these two sites display strong differences that influence several characteristics of the roe deer populations (Table 2.1).

Figure 2.5. The two study sites of roe deer in France (Trois-Fontaines and Chizé) managed by the French *Office National de la Chasse et de la Faune Sauvage* (ONCFS).

The first site, Trois-Fontaines, is a nature reserve and a *Territoire d'Etude et d'Expérimentation*, where hunting is permitted at some periods of the year. It is an enclosed forest of 1,360 ha, located in north-eastern France (48°43'N, 4°55'E, Fig. 2.5). This site presents a continental climate characterized by cold winters and warm rainy summers. In terms of habitat, this forest is homogenous and composed of oak (*Quercus sp.*) and beech (*Fagus sylvatica*). Based on wood production, this site is considered as very productive and it is exploited for silviculture by the French *Office National des Forêts*. This site provides high habitat quality for roe deer (Pettorelli *et al*., 2006), as illustrated by the high annual survival rate (0.92) of females roe deer in this site (Douhard *et al*., 2014).

The second site, at Chizé, is an integral nature reserve without hunting or sylviculture. It is an enclosed forest of 2,614 ha, located in western France (46°50'N, 0°25'W, Fig. 2.4). This site presents a temperate oceanic climate with mediterranean influences, frequent summer droughts combined with poor soils, resulting in limited forest productivity (Pettorelli *et al*., 2006). Biomass production is about twice lower in Chizé than in Trois Fontaines. This forest

presents different types of habitats according to the timber stand and the nature of the vegetation (Pettorelli *et al*., 2003). The north of the reserve is principally composed by is oak (*Quercus sp.*), on the contrary of the South of the Reserve is dominated by beech (*Fagus sylvatica*). This site provides a more contrasted environment, which results in spatial variability in annual survival rate of females (from 0.70 to 0.95 ; Douhard *et al*., 2014).

Table 2.1. Comparison of the characteristics of the two roe deer populations monitored.

	CHIZE	TROIS-FONTAINES
Location	southwest of France	northeast of France
Population density	~ 14 ind.100ha^{-1}	~ 20 ind.100ha^{-1}
Population growth rate (since 2006)	r = 0	r = 0.15
Study site since	1975	1977
Plant biomass productivity	3.77m^3.ha^{-1}.year^{-1}	5.92m^3.ha^{-1}.year^{-1}
Study area	2610 ha	1360 ha
Number of marked individuals monitored	> 200	> 250
Generation time	7 years	5 years
Fawn survival probability ±SE *(from birth to 8 months)*	(♂) 0.629 ± 0.103 (♀) 0.597 ± 0.095	(♂) 0.692 ± 0.053 (♀) 0.652 ± 0.056
Average adult body mass	12-18 kg	15-20 kg
Mean male antler size ±SE *(standardized, on february 14)*	195.6 ± 1.4 mm	199.6 ± 2.7 mm

Captures of roe deer and ethics

Every year, about one half to two third of the area at Chizé and the whole area at Trois-Fontaines are sampled for roe deer captures (Fig. 2.6). It represents 10-12 days of capture organized every year between December and March, and it results in the capture of 200-300 roe deer in each site. The annual recapture rate of roe deer is about 0.5 (Gaillard *et al*., 1993). In both populations, a high proportion (>70%) of roe deer are known-aged because marked with a unique collar, ear-tags and more recently with a transponder microchip injected subcutaneously in the neck for permanent individual recognition. As roe deer females give birth in spring, systematic searches for newborn fawns are conducted between April and June. Fawns are individually marked, and the filiation with the mother is assessed from field observations. Young roe deer that were not captured at the fawn stage but at 8 months-old at the time of winter captures are identified using tooth eruption patterns (Hewison *et al*., 1999) and marked.

During my PhD, winter captures were managed by two ONCFS agents: Claude Warnant at Trois-Fontaines and Gilles Capron at Chizé, under the supervision of Eric Marboutin (head of the unit *Wild Ungulates* at the ONCFS). Between 150 and 350 volunteers are required for each day of capture. The two sites are divided in sections, and sections of the capture day are choosen randomly. Each day of capture, 25 km of nets are set both morning and afternoon. Roe deer are driven into the nets by beaters and handled immediately after capture by experienced people placed close to the nets Fig. 2.6). Captured animals are transferred into wood boxes and they are moved in a specific room to be manipulated.

Figure 2.6. Captures of roe deer at Trois-Fontaines.

Manipulation lasts 15 minutes at maximum for each individual. During this time, body mass is recorded (to the nearest 50g) and a basic clinical examination is performed (Fig. 2.7). If it is the first capture of a young roe deer of the year, ear-tags are laid which allows to collect a small piece of cartilage for DNA analyses (used e.g. for paternity analyses). Hind foot (to the nearest mm, from the heel to the tip of the hoof) and antlers length are measured. Feces are taken directly into the rectum and conserved at 4°C for parasitic and diet analyses (quantity 5-10 g, around 20 feces/individual). The presence of external parasites (louses, ticks) is recorded. A tuft of hair is taken for DNA measures. The behaviour during manipulation and at release is also recorded (score between 0 and 5, with detailed criteria, to evaluate the level of acute stress of the animal, see the fieldwork form in Appendix 2.1). For known-aged animals, blood samples are collected from the jugular vein (up to 20 mL for a 20 kg roe deer) - with a 20 mL syringe (TERUMO® SST20ES) and a 1.0 mm diameter/30 mm length needle (TERUMO® NN-1030R).

Figure 2.7. Manipulation of roe deer and blood samples.

The blood is distributed into different tubes according to future use. Whole blood is preserved on EDTA at 4°C for white blood cell counts, performed within 52 hours of sampling. The serum extracted by centrifugation from dry tubes immediately upon blood collection and conserved at -20°C for functional measures of activity and assays. Finally, buffy coat containing leukocytes is extracted from heparin tubes after centrifugation and conserved at -80°C for telomere length measures (protocol detailed below in Telomere length measures). For each animal captured, information is recorded on a form (see the fieldwork form in Appendix 2.1). After manipulation, roe deer are released as soon as possible in their section of capture (Fig. 2.8).

© OMM photographie

Figure 2.8. Manipulation and release of roe deer.

In 2016 and 2017, I participated in all winter captures at Trois-Fontaines and some at Chizé. I realized morphological measures, blood and feces samples collection, leukocyte extraction. To be allowed to manipulate animals, I obtained the French diploma *Animal experimentation and Ethics* at the National Scientific Research Agency (CNRS) in Marseille (France) - which represents 50 hours of classes.

Box 2.1. Authorisation & ethics.

The protocol of capture of roe deer under the authority of the Office National de la Chasse et de la Faune Sauvage (ONCFS) was approved by the Director of Food, Agriculture and Forest (Prefectoral order 2009–14 from Paris). The land manager of both sites, the Office National des Forêts (ONF), permitted the study of the populations (Partnership Convention ONCFS-ONF dated 2005-12-23). The protocol of manipulation, measurement and sampling received the approval of the Ethics Committee of Lyon 1 University (project DR2014-09, June 5, 2014).

3. Measure of physiological variables

Blood sampled performed in the field allow to measure several physiological traits in roe deer of the two populations. These measures aims at describing immune phenotype of individuals and at measuring body conditions markers, to study their associations with life-history traits. During this PhD, the development of telomere length measurement and oxidative damages marker opened up a field of investigations beyond eco-immunology.

Determination of immune traits

For each individuals, descriptive and functional measures of immunity are performed, to depict both the innate and the adaptive responses, through the measure of their humoral and cell-mediated components.

First, **innate cellular immunity** is assessed by counting total white blood cells (WBC, in 10^3 cells/mL). WBC count is considered as a proxy of the allocation to immunity. This complete blood count is measured by impedance technology, considering parameters for bovine samples since the size of blood cells is comparable between the two species (Ursache *et al*., 1980) using an ABC Vet automaton (Horiba Medical, Montpellier, France).

The composition of the WBC population (five morphologically distinct cell types, *i.e*. neutrophils, monocytes, lymphocytes, eosinophils and basophils) is also assessed. Neutrophils represent the majority of white blood cells (between 60 and 80% of the total WBC). With monocytes, they constitute the majority of phagocytes involved in the innate response. Basophils, which are quite rare, play a key role against macroparasites such as ticks (Karasuyama *et al*., 2011). Eosinophils are associated with defence against internal parasites and inflammatory response. Finally, as lymphocytes are used to represent the cellular adaptive

immunity (see below). The proportion of the various leukocytes forms is estimated by counting the 100 first cells on blood smears with a microscope under 200x magnification (in %) as represented in Fig. 2.9. Blood smears are stained with May-Grünwald's (#T863.2, Carl Roth GmbH) and Giemsa (#T862.1, Carl Roth GmbH) solutions, a method that has been used before in studies on mammalian immunology (Durbin *et al*., 2009). With both the proportion of each leukocyte forms (in %) and the total white blood cells measurement (10^3 cells/mL), the count of each cell type (10^3 cells/mL) can be obtained.

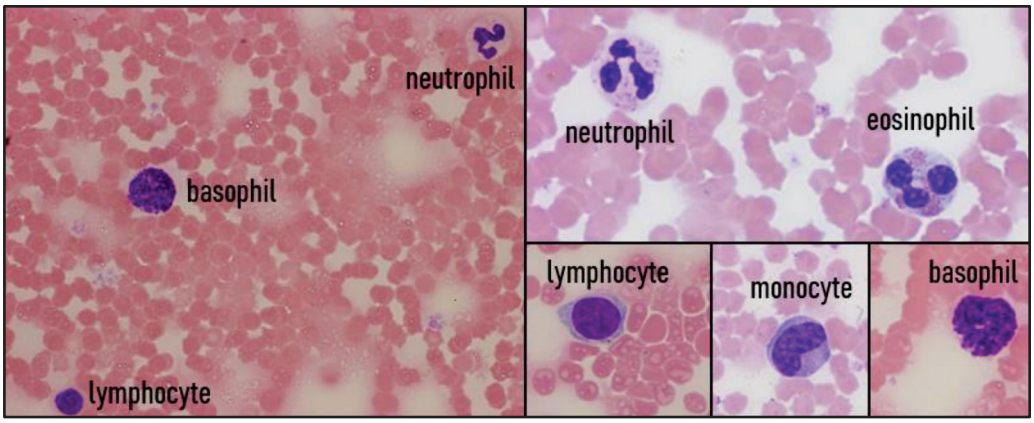


Figure. 2.9. Identification of the different cell types in roe deer blood smears by microscopy. Photography: C. Régis.

Secondly, **innate humoral immunity** is assessed by measuring the circulating levels of natural antibodies (NAbs) and the complement-mediated cell lysis activity, following the hemagglutination-hemolysis (HAHL) assay. This method developed by Matson *et al*. (2005) has been adapted for roe deer, using chicken red blood cells as target cells (Gilot-Fromont *et al*., 2012). In this assay, the HA score (titer) measures the ability of NAbs to agglutinate exogenous cells and provides a proxy of the NAbs concentration, and HL score measures the ability of the complement system to cause hemolysis (see examples in Fig. 2.10 and Fig. 2.11).

Box 2.2. Detailed protocol of HAHL test.

(adapted from Matson et al., 2005)

HAHL test is performed on serum samples extracted in the field and stored at -20°C. The assay is carried out in 96-well plate (12 columns). In the plate, twenty-five microliters of ten plasma samples are pipetted into the first two wells of columns 2 to 11 (columns 1 and 12 are used for positive controls), and 25 µl of 0.01 M phosphate buffered saline (PBS; Sigma #P3813, St Louis, MO) are added to lines 2-8. Using a multi-channel pipetter the contents of the line 1 wells are serially diluted (1:2) through line 7. This results in dilutions ranging from 1 to 1/64 and 25 µl in every well. The 25 microliters of PBS only in line 8 serves as a negative control. For the assay itself, 25 microliters of a 1% chicken blood cell suspension is added to all wells, effectively halving all plasma dilutions. Each plate is then sealed with Parafilm (Pechiney Plastic Packaging, Neenah, WI), and gently vortexed for 10s prior to incubation during which they are floated in a 37°C water bath for 90 min. Upon completion of the incubation, the long axis of each plate is tilted to a 45° angle for 20 min at room temperature in order to enhance visualization of agglutination. Plates are then scanned (full size image at 300 dpi) using the positive transparency (top-lit) setting of a flatbed scanner. Afterward, plates are kept at room temperature for an additional 70 min, and scanned a second time to record maximum lytic activity. From the digitized images, lysis and agglutination are scored for each sample (see examples in Fig. 2.10 and Fig. 2.11). Lysis reflects the interaction of complement and NAbs, whereas agglutination results from NAbs only.

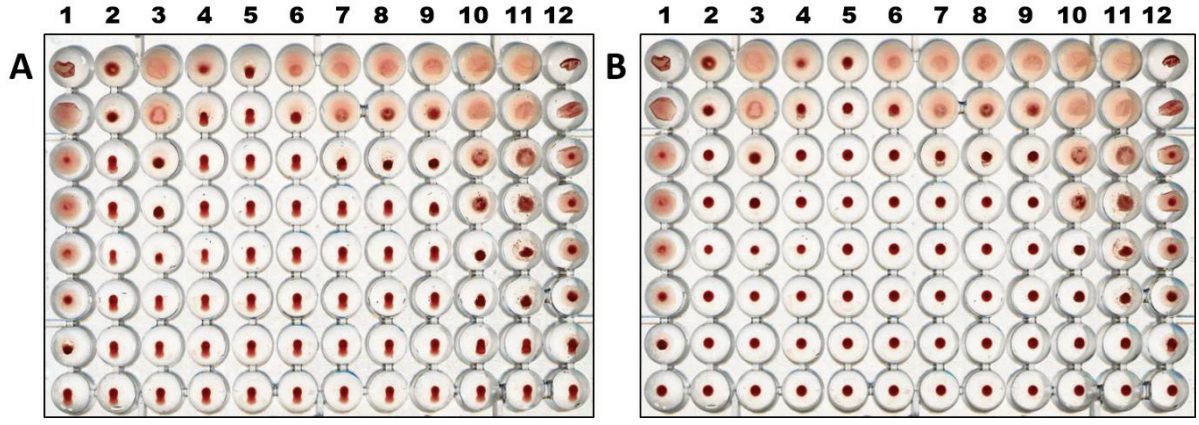


Figure 2.10. Examples of scanned HAHL test plates performed on 10 samples of roe deer serums (columns 2 to 11) and 2 controls (columns 1 and 12) for determination of (**A**) haemagglutination score and (**B**) hemolysis score.

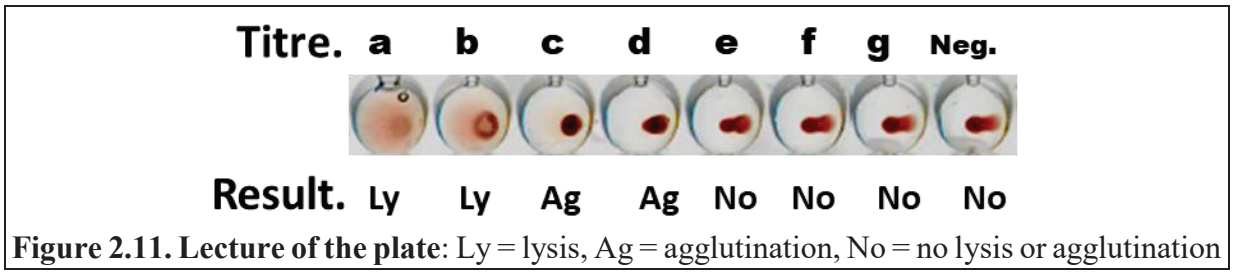


Figure 2.11. Lecture of the plate: Ly = lysis, Ag = agglutination, No = no lysis or agglutination

Innate humoral immunity is also assessed using the levels of alpha1-,alpha-2, beta- and gamma-globulins (mg/mL). Alpha1-, alpha2- and beta-globulins are fractions including several acute phase proteins (APPs), a group of proteins which concentration changes following external or internal challenges such as trauma, inflammation or infection (Cray *et al*., 2009). Total protein content (in mg/mL) is first assessed by refractometry followed by automatic agarose gel electrophoresis (HYDRASYS, Sebia, Evry, France) that separates albumin and the 4 fractions of globulins (α1, α2, β, and γ; see Fig. 2.12).

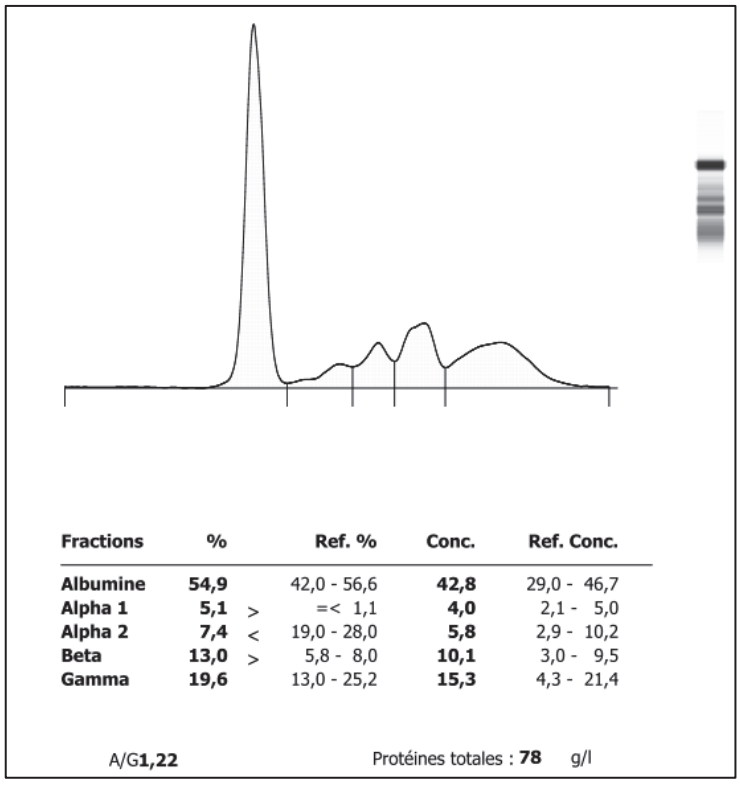


Figure 2.12. Example of proteins quantification by electrophoresis for a roe deer (albumin, alpha1-/alpha2-/beta-/gamma-globulins, total proteins).

The specific level of haptoglobin (in mg/mL) is also measured. Haptoglobin is a protein that belongs to alpha2-globulin fraction, synthesized in case of chronic infection or inflammation.

Haptoglobin analyses is performed on a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France) using phase Haptoglobin assay (Tridelta Development LTD, County Kildare, Ireland) chromogenic kit.

Thirdly, the **humoral component of the adaptive immunity** is assessed by measuring the concentration of gamma-globulins (see above for details about the electrophoresis protocol and Fig. 2.11), or immunoglobulins, which represent the majority of circulating antibodies.

Finally, the **cellular component of adaptive immunity** is assessed by lymphocyte counts (in the white blood cells determination described above). Lymphocyte count includes both T and B cells, B cells being particularly involved in the production of antibodies.

During my PhD, HAHL tests and leukocyte formula have been performed at the LBBE/UMR 5558, Lyon (France) - by Emmanuelle Gilot-Fromont, Corinne Régis, Benjamin Rey, François Débias and myself. Total white blood cells count, globulins and haptoglobin measures have been performed at the laboratory of VetAgro-sup, Marcy l'Etoile (France) - by Céline Dussart, Elodie Moissonier and Cyrille Debard, under the supervision of Benoit Rannou and Emmanuelle Gilot-Fromont.

Determination of body condition

In this study, body condition of roe deer is assessed through three haematological traits that reflect energetic and protein reserves: albumin, hematocrit and fructosamine measure. Albumin (mg/mL) is the most abundant protein in the blood and is separated from other proteins and quantified by refractometry followed by electrophoresis using an automatic agarose gel electrophoresis (HYDRASYS, Sebia, Evry, France) as represented in Fig. 2.12. Haematocrit (%) is the fraction of whole blood comprised of erythrocytes and is calculated by numeric integration on a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France). Fructosamine levels (µmol/L) represent glycated proteins and indicate glycemia during the two-three weeks preceding sampling. This marker gives information on the level of carbohydrates reserves (Stockham and Scott, 2008). Fructosamine is measured using Thermo scientific reagents and ABX Pentrafructosamine reagents respectively, on a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France).

During my PhD, these analyses have been performed in the laboratory of VetAgro-sup, Marcy l'Etoile (France) - by Céline Dussart, Elodie Moissonier and Cyrille Debard, under the supervision of Benoit Rannou and Emmanuelle Gilot-Fromont.

Determination of parasitic burden

Feces are send after sampling in a parasitology laboratory for the parasitic identification and counting. Fecal propagule counts of parasites frequently occurring in roe deer are investigated: nematodes parasite from the lung (protostrongylids), from the digestive tract (gastro-intestinal strongyles and *Trichuris* sp.), and coccidia (*Eimeria* sp., Protozoa). Evidences that egg counts in faeces allow a reliable estimate of the number of parasites in roe deer from the studied populations during the capture period have previously been provided (Body *et al*., 2011).

The McMaster protocol (Raynaud, 1970) is used for the count of gastro-intestinal nematode, in eggs per gram (EPG) and coccidian oocysts per gram (OPG). This protocol uses a counting chamber, which enables a known volume of faecal suspension (2 x 0.15 ml) to be examined microscopically and eggs to be counted.

The Baermann fecal technique (Baermann, 1917) is used for the count of first stage larvae of pulmonary nematodes (protostrongylids, in larvae per gram, LPG). Feces are placed in a special funnel-shaped collection device, covered with warm water and left to stand for 1-2 hours or longer. During this time, larvae actively move out of the fecal material and sink to the bottom of the funnel where they are collected and identified.

Until 2013, feces analyses was performed at the EA4688 VECPAR from the University of Reims (France) - by Hubert Ferté. Since 2014, they are performed in the parasitology laboratory of VetAgro-sup, Marcy l'Etoile (France) - by Marie-Thérèse Poirel and Slimania Benabed, under the supervision of Gilles Bourgoin.

Determination of leukocyte telomere length

Sample preparation

Within 30 min of sampling in the field, whole blood is spun at approximately 2500 g for 10 min and the plasma is drawn off and replaced by the same quantity of 0.9% w/v NaCl solution and is spun again. The intermediate buffy coat layer, comprising mainly leukocytes

(white blood cells) is transferred into a 1.5-mL Eppendorf tube are stored at −80 °C until further use.

DNA extraction

Genomic DNA is extracted from white blood cells using Macherey-Nagel NucleoSpin® Blood QuickPure kit (Catalogue number 740569). DNA yield and purity is quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington DE, USA) and DNA integrity is assessed by running 200 ng total DNA on a 0.5% agarose gel and DNA bands are scored on a scale of 1-5 by visual examination. Samples are passed quality control with a DNA yield of ≥20 ng/µl, an acceptable purity absorption range of 1.7 - 2.0 for the 260/280 nm ratio and > 1.8 for the 260/230 nm ratio, and a DNA integrity score of either 1 or 2 (Seeker *et al.,* 2016).

Box 2.3. Detailed protocol of DNA extraction.

1. Lyse blood samples: pipette 25 µL Proteinase K and up to 150 µL blood (leukocytes) + 50 µL PBS into 1.5ML microcentrifuge tubes. Incubate at room temperature for 1 min. Add 200 µL Lysis Buffer BQ1 to the samples and vortex the mixture vigorously (10-20s). Incubate samples at 70°C for 10 minutes.
2. Adjust DNA binding conditions: vortex again the mixture and centrifuge few seconds at 11,000 x g. Add 200µL ethanol (96-100%) to each samples, vortex again, centrifuge again few seconds at 11, 000 x g.
3. Bind DNA: add the samples to the NucleoSpin Blood QuickPure Columns placed in a collection tubes and centrifuge 1 min at 11,000 x g. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (up to 15,000 x g). Discard collection tube with flow-through.
4. Wash and dry silica membrane: place the Nucleospin blood quickpure column into a new collection tube (2mL) and add 350 µL Buffer BQ2. Centrifuge 3 min at 11,000 x g. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (up to 15,000 x g). Discard collection tube with flow-through.
5. Elute highly pure DNA: place the NucleoSpin blood quickpure column in a 1.5 mL microcentrifuge tube and add 50 µL prewarmed Buffer BE (70°C). Dispense buffer directly onto the silicq membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.

Telomere length measurement

We measure relative leukocyte telomere length (RTL) using a real-time quantitative PCR method (qPCR; Cawthon, 2002) which has previously been optimised and validated in sheep and cattle (Seeker *et al*., 2016). This method measures the total amount of telomeric sequence present in a DNA sample, relative to the amount of a non-variable copy number reference gene (beta-2-microglobulin (B2M)). For telomere reactions we use the following HPLC purified primers, Tel 1b (5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3') and Tel 2b (5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3') (from Epel *et al*., 2004). For B2M reactions, primers are supplied by Primer Design (Catalogue number: HK-SY-Sh-900, Southampton, UK).

Using an automated liquid handling robot (Freedom Evo-2 150; Tecan) we are able to load both the DNA samples and qPCR master mix in 384 well plates; allowing us to run both telomeric and B2M reactions in separate wells but on a single plate. A separate master mix for each primer set is prepared containing 5 µl LightCycler 480SYBR Green I Master Mix (Cat # 04887352001, Roche, West Sussex, UK), 0.5 µl B2M (300 nm) primer or 0.6 µl each tel primer (900 nm), and 2 ng of sample DNA. DNA is amplified in 10 µl reactions. Each plate include a non-treated control (water; NTC), a calibrator sample (2ng) on each row to account for plate to plate variation and robot pipetting error, as well as a 1:4 serial dilution starting at 10ng/µl to visually inspect the qPCR curves. The calibrator sample is DNA that has been extracted from a large quantity of blood obtained from a single wild roe deer. In this case, the calibrator is extracted using the Qiagen DNeasy Blood and Tissue kit (Cat# 69581, Manchester, UK), pooled and quality controlled in the same way as our DNA samples of interest. All samples, calibrators and NTC's are run in triplicate and all qPCR are performed using a Roche LC480 instrument using the following reaction protocol: 10 min at 95 °C (enzyme activation), followed by 50 cycles of 15 s at 95 °C (denaturation) and 30 s at 58 °C (primer annealing), then 30 s at 72 °C (signal acquisition). Melting curve protocol is 1 min at 95 °C, followed by 30 s at 58 °C, then 0.11 °C/s to 95 °C followed by 10 s at 40 °C.

We use the LinRegPCR software package (version 2016.0; Ruijter *et al*., 2009) to correct for baseline fluorescence, set a window of linearity for each amplicon group and to calculate well-specific reaction efficiencies and Cq values. A constant fluorescence threshold is set within the window of linearity for each amplicon group, calculated using the average Cq across all three plates. The threshold values used are 0.140 and 0.203, and the average efficiency across all plates are 1.91 and 1.93 for the B2M and telomere amplicon groups, respectively.

Samples are excluded from further analysis if the coefficient of variation (CV) across triplicate Cq values for either amplicon was > 5 %, or if at least one of their triplicate reactions had an efficiency that was 5 % higher or lower than the mean efficiency across all wells on that plate for the respective amplicon.

We calculate relative telomere length (RTL) for each sample following Pfaffl et al. (2001) as follows: $RTL = (E_{TEL}^{(CqTEL[Calibrator] - CqTEL[Sample])}) / (E_{B2M}^{(CqB2M[Calibrator] - CqB2M[Sample])})$. Where E_{TEL} and E_{B2M} are the mean reaction efficiencies for the respective amplicon group across all samples on a given plate; CqTEL[Calibrator] and CqB2M[Calibrator] are the average Cqs for the relevant amplicon across all calibrator samples on the plate; and CqTEL[Sample] and CqB2M[Sample] are the average of the triplicate Cqs for the sample for each amplicon.

During my PhD, sample preparation have been realized immediately in the field and DNA extraction have been performed at the LBBE/UMR 5558, Lyon (France) - by Corinne Régis, Benjamin Rey and myself. All downstream processing of samples (e.g. quality control (QC), telomere measurement) have been performed at the Institute of Evolutionary Biology in University of Edinburgh, Scotland (UK) - by Rachael V. Wilbourn, Hannah Froy and Marie-Christina McManus, under the supervision of Dan Nussey.

Determination of oxidative damages

A marker of oxidative state is assessed (on 2016-2017 plasma samples) using the Cayman's TBARS assay (Cayman Chemical Company, Ann Arbor USA). This assay give a general quantification of oxidative damage molecules found in the plasma, especially those generated by lipid peroxidation. The thiobarbituric acid in the assay reacts with the oxidative damage molecules under high temperature (90–100°C) and acidic conditions, which generates a colour reaction directly proportional to the concentration of oxidative damage molecules.

Box 2.4. Detailed protocol of TBARS measure.

100 µl of each plasma sample or standard were added to 10 µl of sodium dodecyl sulphate into 500 µl vials, and mixed. Then 400 µl of colour reagent (132.5 mg of thiobarbituric acid diluted into 12.5 ml of an acetic acid solution and 12.5 ml of a sodium hydroxide solution) were added to each plasma solution, and caped vials were kept in boiling water for one hour. After one hour the vials were removed from the boiling water and immediately put onto ice for 10 minutes in order to stop the reaction. Finally, 150µl of each solution was randomly pipetted in well plates and readings were taken at 530 nm. Standard curves were obtained from serial dilutions of an standard of MDA (from 0 to 50µM). All samples are tested in duplicate, and when the coefficient of variation among the two measures is >20%, samples are excluded of the analyses.

This measure of oxidative stress in roe deer have been performed at the LBBE/UMR 5558, Lyon (France) - by Benjamin Rey and myself.

CHAPTER 3

Development of immunity at young age, influence of growth and maternal effects?

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Does body growth impair immune function in a large herbivore?

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Author Contributions: JFL, JMG, EGF conceived the study and designed methodology; all authors collected the data; LC, EGF, BR, HV performed the immunological measures; LC and FD analysed the data; LC wrote the first draft of the paper and all authors contributed critically to the drafts and gave approval for the final version.

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ABSTRACT

According to the principle of allocation, trade-offs are inevitable when resources allocated to one biological function are no longer available for other functions. Growth, and to a lesser extent, immunity are energetically costly functions that may compete with allocation to reproductive success and survival. However, whether high allocation to growth impairs immune system development during the growing period or immune system performance during adulthood is currently unknown in wild mammals. Using three roe deer (*Capreolus capreolus*) populations experiencing contrasting environmental conditions, we tested for potential costs of growth on immune phenotype over both the short-term (during growth), and the long-term (during adulthood) over the course of an individuals' life. We investigated potential costs on a set of twelve immune traits that reflect both innate and adaptive responses, and compared them between sexes and populations. Although fast growth tended to be associated with low levels of some humoral traits (globulins) during the growing period and some cellular immune traits (i.e. eosinophil and neutrophil counts) during adulthood, evidence for a trade-off between growth and other immune components was limited. Unexpectedly, no detectable growth costs on immunity were found in females from the population experiencing the least favorable environment. We discuss our findings in the light of the complex interplay between resource allocation strategies among reproduction, maintenance and immunity, in relation to local environmental conditions experienced by roe deer.

Keywords: Body mass, Eco-immunology, Life history, Roe deer, Trade-off.

Introduction

Growth is a key life history trait that shapes reproductive success and both short-term and long-term survival in most organisms. Developing a large body rapidly can buffer the effects of fluctuating environmental conditions (Dmitriew 2011) and allows juveniles to spend less time at the critical early-life stage, when they are particularly vulnerable to predators (Metcalfe and Monaghan 2003, Ronget et al. 2018). Large mature males are generally more successful in intra- and inter-sexual competition, and have thereby higher reproductive success than small males (Andersson 1994), while large females often exhibit higher fecundity than small ones (Roff 1992). A large body size can be attained either by an extended developmental period, an accelerated growth rate, or both (Metcalfe and Monaghan 2003). However, growth entails substantial energy and nutrient requirements (Wieser 1994), which are expected to have deleterious consequences on individual physiological performance and survival during prime-age adulthood and old age (Arendt and Wilson 1997, Blanckenhorn 2000, Dmitriew 2011).

Physiological and survival costs associated with rapid growth have been documented for several decades, notably in some of the first contributions to the evolutionary biology of aging (Williams 1957). Such relationships are embedded in the principle of allocation (Cody 1966), which states that individuals must allocate the limited amount of energy they acquire from their environment among the competing functions of growth, reproduction and survival. Most studies on vertebrate populations performed in the wild to date have focused on the energy allocation trade-off between current reproduction and subsequent survival or reproduction (Stearns 1992, Lemaître et al. 2015, Gélin et al. 2015). So far, the long-term effect of fast growth has been investigated much less. Yet, high energy allocation to body growth could be at the expense of energy allocated to the maintenance of cellular and physiological functions (McDade 2005). In the wild, restriction of food resources likely exacerbates energy allocation trade-offs. Harsh environmental conditions and low levels of food resources slow down growth (Arendt and Wilson 1997) and lead to delayed sexual maturity or reduced adult size, or both (Bonenfant et al. 2009). However, compensatory growth (sensu Hector and Nakagawa 2012) can occur, enabling juveniles to recover partially or fully from food limitation during early life. This compensatory growth is also associated with various and pronounced physiological costs, both over the short- and long-term throughout the course of an individual's life (Metcalfe and Monaghan 2001). Thus, although it is possible to recover a normal growth trajectory at some stage, slow growth during early life can have profound, pervasive and long-standing effects on individual adult performance (Metcalfe and Monaghan 2001). The costs of compensating for a

bad start may include a faster rate of telomere attrition (Geiger et al. 2012), decreased physiological condition during adulthood (e.g. low tolerance to food restriction, Dupont-Prinet et al. 2010), an increased rate of senescence (e.g. more rapid loss of body mass in late adulthood, Douhard et al. 2017) or even a shortening of lifespan (Lee et al. 2013).

Immunity is a key physiological trait that potentially mediates life history trade-offs between growth and reproduction or between growth and survival (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Zuk and Stoer 2002, Lee 2006). Immune function determines an individual's sensitivity to infections and diseases, and thus affects both reproductive success and survival in the wild (Sheldon and Verhulst 1996, Schmid-Hempel 2003). The immune system is also a major physiological mechanism involved in cellular renewal and repair, and as such, is an essential component of somatic maintenance (McDade 2005). So far, the majority of studies that have focused on the trade-off between growth and immunity have been performed in birds (e.g. Soler et al. 2003, Brommer 2004, Mauck et al. 2005). Of these, experimental work based on food supplementation has shown that the immune response is resource dependent (Soler et al. 2003, Brommer 2004), a prerequisite for an energy allocation trade-off to occur. However, experiments based on parasite challenge or immune stimulation in young birds revealed that immune challenge affects the immune profile and body condition, but does not limit growth (Hõrak et al. 2000), and may even result in accelerated wing development (Saino et al. 1998, Szàp and Møller 1999). These previous studies, generally based on the manipulation of immunity in growing individuals, found limited impact on their growth, possibly because the amount of energy required for the immune response is much lower than that required for tissue growth (Klasing 1998). On the contrary, it is not known whether high allocation to growth could be costly over the short-term (i.e. for immune development) or over the long-term (i.e. for immune performance during adulthood). Specifically, rapid growth is more likely to compete with the most energy demanding traits such as cellular immunity than with less costly ones such as humoral functions (Klasing 2004). In addition, rapid growth may also carry a higher cost in terms of timing of immune development because individuals with low immune function in early life may accelerate their immune development later in life (Mauck et al. 2005, Rossi et al. 2013).

Here we tested for potential costs of *early* growth on immunity in young and adult roe deer, and of *late* growth on adult immunity. We collected data in both sexes on 12 immune traits encompassing both the innate and adaptive components of the immune response for 300 roe deer sampled during their growing period, and for 196 roe deer sampled during adulthood.

These animals originated from three populations which have been intensively monitored using Capture-Mark-Recapture for over 20-30 years (Gaillard et al. 2013, Hewison et al. 2009). The populations are subjected to very different environmental conditions, which leads to marked differences in life history (notably growth rate, see Gaillard et al. 1997) and immunological (Cheynel et al. 2017) traits. Based on the principle of allocation and knowledge gained from empirical studies, we predicted that (i) a negative association should occur between *early* growth and immune traits over the short-term (i.e. during the growing period) in young roe deer, in particular for adaptive immunity that develops more progressively compared to innate immunity; (ii) a negative association should occur between *early* and/or *late* growth and adult immune traits over the long-term (i.e. during adulthood); (iii) immunity costs of *late* growth should be higher than those of *early* growth; (iv) the trade-off between growth and long-term immunity should be stronger for the most costly immune functions (in particular, cellular innate immunity, e.g. neutrophils, monocytes) than for less costly ones (typically, antibody response); (v) the trade-off should be strongest in the population experiencing the poorest environmental conditions. Finally, we also controlled for sex-specific effects because studies performed in laboratory and natural systems have reported that levels of immune performance are generally higher in females than in males (Zuk and McKean, 1996). However, we expected the association between either *early* or *late* growth with immunity to be similar in males and females because roe deer only display a low intensity of sexual selection (Andersen et al. 1998), and because no clear sex differences were reported in physiological traits and intensity of parasitism in the studied populations (Cheynel et al. 2017).

Material and methods

Study populations and data collection

We used data from three populations of roe deer: two are located in enclosed forests in north-eastern France (48°43'N, 4°55'E, 1,360 ha) at Trois-Fontaines, and in western France (46°50'N, 0°25'W, 2,614 ha) at Chizé; and the third population is located at Aurignac, in a rural site in the southwest of France (43°17'N, 0°53'E, 7,500 ha).

The Trois-Fontaines population is exposed to a continental climate characterized by cold winters and warm rainy summers. This site has rich soils and provides high habitat quality for roe deer. In contrast, the Chizé population has a temperate oceanic climate with Mediterranean influences, and frequent summer droughts combined with poor soils, resulting in limited forest

productivity (Pettorelli et al. 2006). Hence, fawn survival, female fertility, adult body mass (Gaillard et al. 2013), age-specific telomere length (Wilbourn et al. 2017), and many markers of immune performance (Cheynel et al. 2017) are consistently lower at Chizé than at Trois-Fontaines. Aurignac has an oceanic climate with summer droughts and is a mixed landscape of woodland patches, meadows and crops offering high quality resources for roe deer (Abbas et al. 2011).

As part of the long-term Capture-Mark-Recapture program initiated in the late 70's for the two forest populations and in 2001 for the Aurignac population, 6-12 days of capture occur between December and March each year (see Gaillard et al. 1993 and Morellet et al. 2009 for details about the capture sessions). In May-June newborn fawns are also captured annually. Roe deer caught within their first year of birth (i.e. either as newborn or as 8 months-old individuals during winter captures) are identified using tooth eruption patterns (Hewison et al. 1999). At capture, we recorded body mass (to the nearest 50g) and performed a basic clinical examination. We collected blood samples from the jugular vein (up to 20 mL for a 20 kg roe deer). We performed cell counts within 52 hours of sampling on whole blood preserved on EDTA at 4°C. We also extracted and conserved serum at -20°C for measures of functional activities (see below).

The two forest populations of roe deer (Trois-Fontaines and Chizé) were used for the analyses of both short-term and long-term costs of rapid growth on immune performance. The population of Aurignac was included for the analysis investigating the short-term consequences of rapid growth on immune performance only since the exact age of most adults is unknown at this site (Hewison et al. 2009).

Dataset

We used data from known-aged individuals that were blood-sampled at least once during their lifetime between 2010 and 2016. Roe deer usually gain most of their adult body mass (more than 90%) within the first two years of life, but only reach their full adult mass at about 4 years of age (Hewison et al. 2011). When not faced with strong food limitation, females start to give birth at 2 years of age (Gaillard et al. 1992), while most males father their first offspring during their fourth year of life (Vanpé et al. 2009). Adult immune performance was measured as the median of each immune trait during adulthood (i.e. between 4 and 9 years of age). Based on these age-classes, data on immunity were collected for a total of 300 growing animals (i.e. sampled at 8 months of age) in the three populations (see details in Table 1). At Aurignac, we

only used data for growing roe deer (i.e. at 8 months of age, n = 41) since the sample size for known-aged adults was too small to perform a detailed analysis (n = 9). Data on immunity were also collected for a total of 196 prime-aged adults (i.e. sampled between 4 and 9 years of age) at Chizé and Trois-Fontaines. Data were approximately equally distributed between sexes (Table 1). These immune measures on roe deer at Trois-Fontaines and Chizé have already been the subject of a previous analysis on immunosenescence patterns (see Cheynel et al. 2017).

Owing to the relatively recent start of immunity assessments in the monitoring programs (2010) and to the imperfect detection rate during the capture-recapture season (i.e. recapture rate of about 0.5; Gaillard et al. 1993), only 32 out of the 196 adults with immunity measures also had immunity measures during their growing period (Table 1). Growth measures (i.e. *early* and *late* growth, see below for a definition of these metrics) were based on records of both body mass at first capture (at about 8 months of age) and on adult body mass (between 4 and 6 years of age, before body mass senescence starts; Douhard et al. 2017).

Table 1: Sex- and population-specific sample sizes.

Subset	Chizé		Trois-Fontaines		Aurignac		Total
	Females	Males	Females	Males	Females	Males	
Immunity measures at 8 months of age *(individuals with measure of early growth)*	59 *(59)*	71 *(71)*	68 *(68)*	61 *(61)*	20 *(20)*	21 *(21)*	300 *(300)*
Immunity measures during adulthood [4 – 9 years of age] *(individuals with early and late growth measures)*	61 *(43)*	39 *(28)*	51 *(33)*	45 (20)	-	-	196 *(124)*
Immunity measures during growth and adulthood *(individuals with growth measures)*	6 *(3)*	10 *(2)*	11 *(11)*	5 *(5)*	-	-	32 *(21)*

Immunity measures

We measured a set of 12 immune traits to depict both the innate and adaptive responses, represented by both humoral and cell-mediated components (Roitt et al. 1998). We assessed the cellular part of immunity by measuring the total white blood cell (WBC) count and assessing the composition of the white blood cell population (five different cell types, measured in 10^3 cells/mL), based on the identification of the first hundred WBC in Wright-Giemsa-stained blood smears (Houwen 2001, Gilot-Fromont et al. 2012). Neutrophils and monocytes are phagocytic cells of the innate response. Basophils and eosinophils participate in the inflammatory response and play a role in the innate response against parasites, but eosinophils

are also involved in the adaptive response. Finally, lymphocytes include T and B cells, B cells being specifically involved in the production of antibodies. Innate humoral immunity was assessed by measuring both the circulating levels of natural antibodies (NAbs) and the complement-mediated cell lysis activity. We used the hemagglutination-hemolysis (HAHL) assay (Matson et al 2005, see also Gilot-Fromont et al. 2012 for a previous study on roe deer). In this assay, the HA score (measured in titre) measures the ability of NAbs to agglutinate exogenous cells and provides a proxy of the NAbs concentration, and HL score measures the ability of these NAbs to cause hemolysis. Innate humoral immunity also includes numerous proteins involved in acute and chronic inflammatory processes. We thus measured the levels of alpha1-globulin, alpha2-globulin and beta-globulin, which include several acute phase proteins of the inflammatory response. Total protein content (in g/L) was first assessed by refractometry, followed by automatic agarose gel electrophoresis (HYDRASYS, Sebia, Evry, France) that separates albumin and the 4 fractions of globulins (alpha1, alpha2, beta, and gamma). We also measured the specific level of haptoglobin (HAP in mg/mL), a protein that belongs to the alpha2-globulin fraction which is synthesized in cases of chronic infection or inflammation. Haptoglobin analyses were performed on a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France) using phase Haptoglobin assay (Tridelta Development LTD, County Kildare, Ireland) chromogenic kit. Contrary to the other immune parameters, haptoglobin was only measured at Chizé and Trois-Fontaines. The humoral component of the adaptive immunity response was assessed by measuring the concentration of gamma-globulins (see above for details about the electrophoresis protocol), i.e., immunoglobulins, which represent the majority of circulating antibodies. Detailed protocols for measuring each of the immune parameter are provided in Appendix 3.1. For adult animals with repeated measures of immune traits between 4 and 9 years of age (82 individuals sampled twice or more), we estimated within-individual repeatability of our immune measures and their 95%-confidence interval (CI) using the R package rptR (Nagakawa and Schielzeth 2010). We found that the majority of adult immune traits were consistent over time (repeatability of between 0.22 and 0.41 for the various forms of white blood cells, between 0.03 and 0.46 for various forms of globulins and between 0.07 and 0.42 for HAHL, results provided in Appendix 3.1).

Growth measures

Early growth (i.e. adjusted body mass at 8 months of age, which was labelled as "early mass" in Douhard et al. 2017) and *late* growth (i.e. a compound of *early* growth and growth from 8 months of age to adulthood) were defined and calculated as in Douhard et al. (2017). Most births take place around mid-May (80% within less than 25 days, Gaillard et al. 1993), while annual winter captures occur from mid-December to early March. We could not measure postnatal growth rate *per se* (i.e. growth between birth and 8 months) because the exact birth date was unknown for those fawns that were first marked during the winter captures.

We indexed *early* growth as fawn mass at winter capture (i.e. about 8 months of age) adjusted to the median date of capture (i.e. January 27th). This adjustment is required because fawns in the studied populations continue to grow during their first winter (Hewison et al. 2002), although their mass at capture is mostly the accumulated product of growth from birth to early October, when weaning takes place (Andersen et al. 1998). From the body mass data, the average daily mass gain throughout the winter capture period was 12 ± 0.005 (SE) $g.day^{-1}$ at Chizé, 24 ± 0.008 $g.day^{-1}$ at Trois-Fontaines (Douhard et al. 2017), and 30 ± 0.005 $g.day^{-1}$ at Aurignac (linear regression with date of capture as the sole covariate; no sex-difference was detected). As individual variation in *early* growth was partly due to variation in environmental conditions during the year of birth (Gaillard et al. 1996), we further adjusted *early* growth relative to the average fawn mass of each cohort in each population.

Late growth corresponded to the post-weaning growth from about 8 months to 4 years of age. When calculating *late* growth we accounted for variation in mass at 8 months of age because fawns that grow slowly early in life subsequently tend to grow faster than fawns that grow fast early in life. This *late* growth was thus measured for each sex and each population as the residuals of the linear regression between early growth and subsequent mass gain, which corresponds to the difference between adult body mass (i.e. the median body mass between 4 and 6 years of age) and early growth. As such, *early* growth and *late* growth were statistically independent.

Assessing covariation among immunity traits

We performed a principal component analysis (PCA) on the 12 immune traits to identify the main axes of variation that determined their correlation structure. We thus examined whether the immune traits could be clustered into a few biologically meaningful and independent components (corresponding to PCs). We examined covariation among the 12 immune traits in both growing and adult animals to assess the consistency of the immune

phenotype over the lifetime at Chizé and Trois-Fontaines, and to identify potential differences in relation to sex and population (see Fig. 1 and Table 2). We did not include Aurignac in the PCA analysis because haptoglobin concentration was not assayed in this population. In the analyses, we removed individuals that contributed abnormally to the PCs, possibly because of a severe inflammatory state (i.e. 2 and 1 individuals that contributed to 28.7% and 17% of the first axis among growing and adult animals, respectively).

Finally, to assess whether 'slow' and 'fast' growing roe deer displayed an overall difference in immune traits, we calculated the average PC coordinates of the fastest growers (top 25%, n = 66 for growing and n = 37 for adult animals) and of the slowest growers (bottom 25%, n = 66 for growing and n = 37 for adult animals) after separating them within sex and population (see Fig. 1). To test whether the slowest growers differed in overall immune function from the fastest growers, we used a non-parametric PerMANOVA test ('adonis2' function in the R package vegan; Oksanen et al. 2013).

The PCA was performed using the R package 'ade4' (Dray and Dufour 2007). Differences in average trait measures between sexes and populations were assessed using a two-way ANOVA followed by Tukey's post hoc test using the multcomp R-package (Hothorn et al. 2008) (Table 2).

Assessing the short-term and long-term growth costs on immunity

We investigated, in both growing and adult animals, the effects of growth on the two first axes of covariation in immunity traits (i.e. first and second PCs, which roughly correspond to continuums of the level of globulins (PC1) and of the cellular part of immunity (PC2); see Fig. 1). Growth effects corresponded to *early* growth in growing animals, and to both *early* growth and *late* growth in adults. Firstly, we assessed the magnitude and the precision of these effects from their 'effect size' and CI (Nakagawa and Cuthill, 2007). Secondly, to assess whether variation in individual growth explained variation in immune function, we performed a model selection procedure using an information-theoretic approach (Burnham and Anderson, 2002).

Within each sex and population, we calculated 'effect size' as the partial correlation coefficients, which measure the standardized effect of one growth measure on an immune trait while controlling for the potential effects of other traits. To obtain the effect sizes, for each immune trait we first fitted the mixed-effect model including *early* growth, *late* growth (only in adults), and average cohort fawn mass as fixed factors and cohort as a random effect on the intercept (i.e. additive model). We then used equation (24) in Nakagawa and Cuthill (2007 p.

82) for mixed-effect models to calculate effect size. We calculated CI of effect sizes following Nakagawa and Cuthill (2007)'s recommendations (i.e. using the 'es calculator', see http://cebcp.org/practical-meta-analysis-effect-size for further information). We also calculated the effect size of growth on each of the 12 immune traits separately (see Appendix 3.1 for growing and adult roe deer). As data for growing roe deer at Aurignac could not be included in the PCA analysis (see above), we assessed effect size of *early* growth on individual immune traits separately in this population (results presented in Appendix 3.1).

Using the information-theoretic approach, we fitted several candidate linear mixed-effect models of immunity traits, which either included or did not include *early* and *late* growth as covariates (supplementary methods and best models selected in Appendix 3.1 for growing animals and adults). We investigated a possible population- or sex-specific effect of growth on the immune phenotype by fitting models including either three-way interaction (i.e. growth (*early* or *late*) × population × sex) or two-way interactions involving growth (see Appendix 3.1 for the full list of models). Models were ranked based on the Akaike Information Criterion corrected for small sample size (AICc, Burnham & Anderson, 2002) in the R package MuMIn (Bartoń, 2016). We selected the model with the lowest AICc. When the difference in AIC (denoted ΔAICc) of two competing models was less than 2, we retained the model with the lowest number of parameters in accordance with parsimony rules. In addition, we calculated AIC weights (ωi) to measure the relative likelihood that a given model was the best among the set of fitted models. All linear mixed-effect models were fitted using restricted maximum likelihood ('lmer' function in the R package lme4; Bates et al., 2015) to estimate model parameters. As data for growing roe deer at Aurignac could not be included in the PCA analysis (see above), we selected models separately for each immune trait in this population (Appendix 3.1). All analyses were carried out in R version 3.4.1 (R Core Team, 2017).

Results

Covariation among immune traits during growth and adulthood

The main axis of covariation among immune traits (PC1) accounted for 24.3% and 23.5% of the total inertia for growing and adult roe deer, respectively (Fig. 1). In both cases, PC1 was mostly influenced by the five globulin concentrations: gamma- (r = 0.81 and 0.75 in growing and adult roe deer, respectively), beta- (r = 0.90 and 0.90), alpha1- (r = 0.85 and 0.80), alpha2-globulin (r = 0.71 and 0.55), and haptoglobin (r = 0.38 and 0.57). PC1 could thus be directly interpreted as a continuum of the humoral part of innate immunity. In the following analyses, we used PC1 as a continuum for globulin levels.

PC2 captured around 13.2% and 13.3% of the total inertia in growing and adult roe deer, respectively (Fig. 1). It mostly reflected variation in cellular immunity. In both growing and adult roe deer, PC2 was positively correlated with markers of natural antibodies (HL: r = 0.72 and 0.75; HA: r = 0.69 and 0.46), neutrophil count (r = 0.38 and 0.29) and lymphocyte count (r = 0.14 and r = 0.45). PC2 was negatively correlated with monocyte (r = -0.34 and -0.55), eosinophil (r = -0.29 and r = -0.18) and basophil (r = -0.12 and -0.35) counts. Our analyses revealed clear consistency between the immune phenotype of growing and adult individuals.

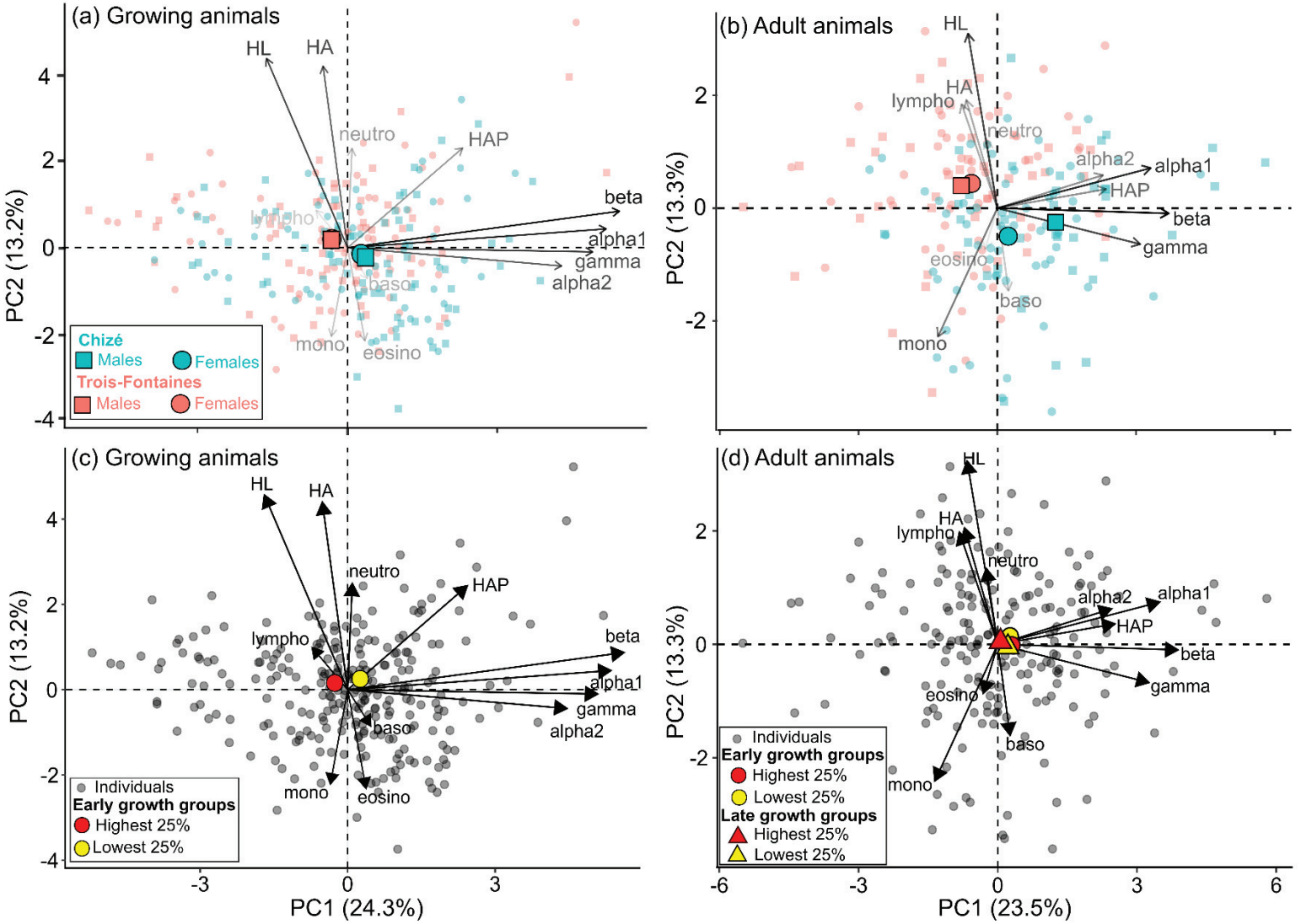
The similarity in the structure of correlations between immune traits (Fig. 1) most likely resulted from high and consistent between-population differences in the level of immune traits at both life stages (Table 2). As expected, both growing animals and adults had higher globulin levels at Chizé than at Trois-Fontaines. In contrast, both growing animals and adults had higher neutrophil and lymphocyte counts at Trois-Fontaines than at Chizé (Table 2). In growing animals, no sex-difference was detected for most immune traits except for lymphocytes, which were higher in females than in males. In adult animals, we detect sex-differences for eosinophils, which were higher in females than in males in adults of both populations; and for haptoglobin, which were higher in males than in females (Table 2). Finally, the growth type (*i.e.* slow or fast) did not influence the immune profile of either young or adult roe deer (Fig. 1). Thus, there were no differences between slowest (i.e. bottom 25%) and fastest (i.e. top 25%) growing roe deer in either *early* growth in growing ($F=1.37$, $df=12$, 132, $p=0.21$) and adult ($F=0.80$, $df=12$, 74, $p=0.56$) roe deer, or in *late* growth in adult roe deer ($F=0.40$, $df=12$, 132, $p=0.79$).

Table 2. Average sex-specific immune measures observed in the three roe deer populations, at Aurignac, Chizé and Trois-Fontaines.

Immune trait	Chizé		Trois-Fontaines		Aurignac		Effects		
	Females	Males	Females	Males	Females	Males	Population	Sex	Interaction
Growing animals									
Globulins levels (PC1)	0.27^{a}	0.36^{a}	-0.32^{a}	-0.33^{a}	-	-	**	-	-
Cellular immunity (PC2)	-0.15^{a}	-0.22^{a}	0.20^{a}	0.18^{a}	-	-	*	-	-
Alpha1-globulin	3.59^{a}	3.60^{a}	3.57^{a}	3.58^{a}	3.48^{a}	3.37^{a}	-	-	-
Alpha2-globulin	5.55^{ab}	6.09^{ac}	5.91^{a}	5.56^{ab}	4.47^{bc}	4.30^{b}	***	-	-
Haptoglobin	0.13^{a}	0.14^{a}	0.15^{a}	0.16^{a}	-	-	-	-	-
Beta-globulin	5.99^{a}	5.89^{a}	5.59^{a}	5.52^{a}	5.14^{a}	5.30^{a}	***	-	-
Gamma-globulin	18.24^{a}	17.88^{a}	13.31^{b}	14.11^{b}	10.08^{c}	9.69^{c}	***	-	-
Neutrophil count	4.34^{ac}	4.06^{a}	5.80^{d}	5.67^{d}	5.53^{bcd}	5.89^{bd}	***	-	-
Monocyte count	0.42^{ade}	0.45^{ad}	0.33^{cd}	0.26^{ce}	0.08^{bc}	0.17^{bce}	***	-	-
Basophil count	0.06^{a}	0.09^{a}	0.05^{a}	0.05^{a}	0.06^{a}	0.07^{a}	-	-	-
Lymphocyte count	2.38^{abd}	2.03^{a}	2.69^{bef}	2.37^{abd}	3.00^{cdf}	2.72^{ace}	***	*	-
Eosinophil count	0.06^{a}	0.07^{a}	0.05^{a}	0.07^{a}	0.05^{a}	0.04^{a}	-	-	-
HA	4.59^{a}	4.59^{a}	4.10^{a}	4.08^{a}	3.70^{a}	3.83^{a}	***	-	-
HL	1.78^{a}	1.98^{a}	2.02^{a}	2.30^{a}	3.05^{b}	3.33^{b}	***	-	-
Adult animals									
Globulins (PC1)	0.237^{b}	1.263^{a}	-0.56^{c}	-0.78^{c}	-	-	***	-	**
Cellular immunity (PC2)	0.50^{a}	0.25^{a}	-0.43^{b}	-0.40^{b}	-	-	***	-	-
Alpha1-globulin	3.03^{a}	3.35^{b}	3.16^{ab}	3.12^{ab}	-	-	-	-	*
Alpha2-globulin	5.83^{a}	5.76^{a}	5.70^{a}	5.35^{a}	-	-	-	-	-
Haptoglobin	0.21^{ab}	0.78^{a}	0.14^{b}	0.17^{c}	-	-	**	***	**
Beta-globulin	7.19^{ab}	8.36^{a}	6.61^{b}	6.44^{c}	-	-	***	*	**
Gamma-globulin	20.28^{a}	20.89^{a}	15.20^{b}	14.53^{b}	-	-	***	-	-
Neutrophils	5.18^{b}	5.05^{b}	6.42^{a}	6.01^{ab}	-	-	***	-	-
Monocytes	0.31^{a}	0.31^{a}	0.32^{a}	0.35^{a}	-	-	-	-	-
Basophils	0.08^{a}	0.08^{a}	0.05^{a}	0.07^{a}	-	-	-	-	-
Lymphocyte count	1.62^{b}	1.62^{b}	2.33^{a}	2.12^{a}	-	-	***	-	-
Eosinophil count	0.11^{a}	0.09^{a}	0.11^{a}	0.06^{a}	-	-	-	*	-
HA	3.96^{a}	3.90^{a}	4.04^{a}	4.20^{a}	-	-	-	-	-
HL	1.84^{b}	2.07^{ab}	2.25^{ab}	2.47^{a}	-	-	*	-	-

Level of statistical significance denoted by asterisk: *P <0.05, ** <0.01, *** <0.001. Means within rows sharing a common character in their superscript are not statistically different (P >0.05) based on pairwise comparisons (Tukey's test).

Figure 1. Biplots of PCA analyses of the 12 immunity traits measured during (a, c) the growing period, and (b, d) adulthood in (a, b) female and male roe deer of the two populations (Chizé and Trois-Fontaines), and in (c, d) individuals with extremely fast (top 25%) or slow (bottom 25%) growth. Arrows indicate the contribution of immune traits to each of the first two PCs (i.e. longer and darker arrows denote stronger correlations). Small symbols indicate individual coordinates on the first two PCs. Average PCs scores are reported by the larger symbols. See text for definition of variables.



Short-term effects of *early* growth on immunity traits in growing roe deer

Effect size

In growing roe deer with known values of *early* growth (n = 300, see Table 1), effect sizes for *early* growth in relation to immunity traits were mostly negligible (Fig. 2). At Trois-Fontaines and Chizé, no relationship was found between *early* growth and globulin level (PC1, Fig. 2), cellular immunity (PC2, Fig. 2) or immune traits separately (Appendix 3.1). At Aurignac, we found a negative effect of *early* growth on neutrophil count in males (effect size: -0.48 [-0.76;-0.06]). The magnitude of this effect was, however, not consistent with that observed at Chizé or Trois-Fontaines.

Selected models

Based on this approach, we only found support for an overall negative relationship between *early* growth and PC1 across both sexes and all populations (model selection and selected models detailed in Appendix 3.1). More specifically, the selected model for the two main immune traits contributing to PC1 included a negative effect of *early* growth, that is for alpha1-globulins at Chizé (Fig. 3(a), slope of -0.11 ± 0.04, $P < 0.01$) and gamma-globulins in all three populations (Fig. 3(b), slope of -0.36 ± 0.09, $P < 0.001$).

Figure 2. Effect size of *early* growth on the two main axes of covariation among immune traits (PC1, PC2) measured in growing roe deer (at 8 months of age) at Chizé and Trois-Fontaines. Effect sizes (symbols) are reported together with associated 95%-confidence interval.

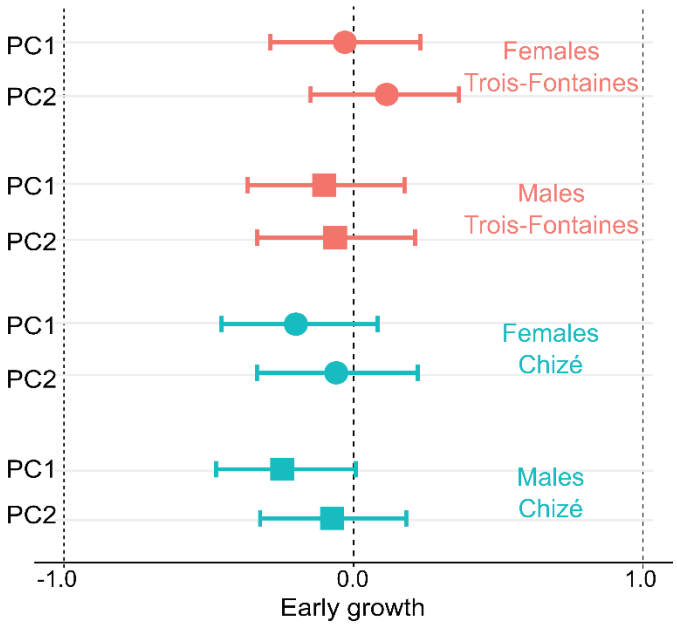
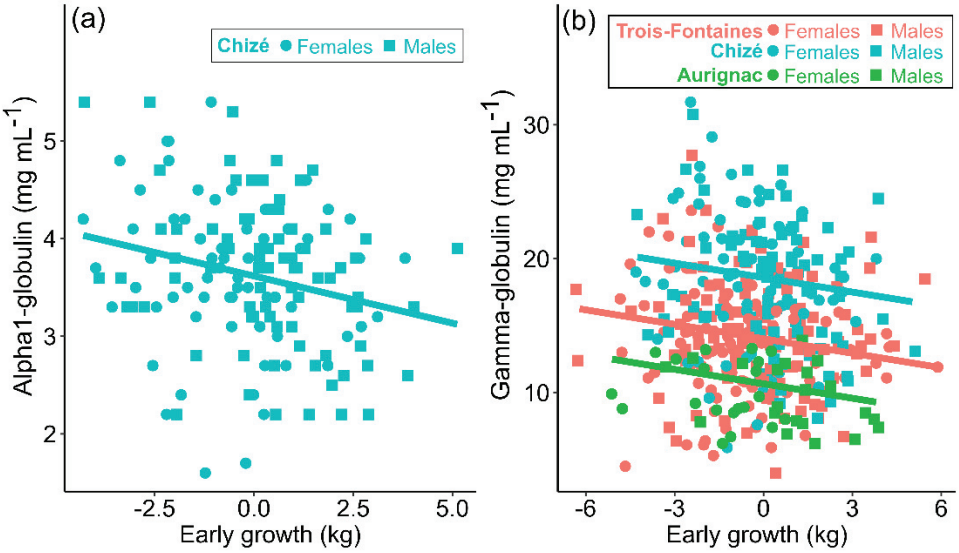


Figure 3. Negative effect of *early* growth on two traits of humoral immunity of fawns (8-months old) in a) Chizé, and b) in the three populations of Aurignac and Trois-Fontaines.



Long-term effects of *early* and *late* growth on immunity traits in adult roe deer

Effect size

Based on effect sizes for *early* and *late* growth in adult roe deer from both Trois-Fontaines and Chizé (124 out of 196 roe deer available to characterize immunity during adulthood, Table 1), evidence for substantial long-term growth costs on immunity was overall weak and heterogeneous (Fig. 4 and Appendix 3.1). In both Trois-Fontaines and Chizé, although several immune traits taken separately were negatively associated with early or late growth (Appendix 3.1), no significant association was observed between *early* or *late* growth and either globulin level (PC1) or cellular immunity (PC2).

Selected Models

The model selection procedure did not support any effect of our growth metrics on immune function, except for two immune traits that strongly contributed to PC2 (model selection and selected models detailed in Appendix 3.1): the eosinophil count in males (model selected: [*early* growth x sex], Appendix 3.1) and the neutrophil count (model selected: [*early* growth x pop + *late* growth], Appendix 3.1). More precisely, the eosinophil count was

negatively affected by *early* growth in males in the two populations (Fig. 5(a), slope of -0.03 ± 0.01, $P < 0.01$). Finally, the neutrophil count was negatively affected by *early* growth at Trois-Fontaines only (Fig. 5(b), slope of -0.24 ± 0.11, $P < 0.05$) and by *late* growth in both populations (Fig. 5(c), slope of -0.22 ± 0.09, $P < 0.05$).

Figure 4. Effect size of a) *early* growth and b) *late* growth on the two main axes of covariation among immune traits (PC1/PC2) measured in adult roe deer (from 4 to 9 years of age included) at Chizé and Trois- Fontaines. Effect sizes (symbols) are reported together with associated 95%-confidence interval.

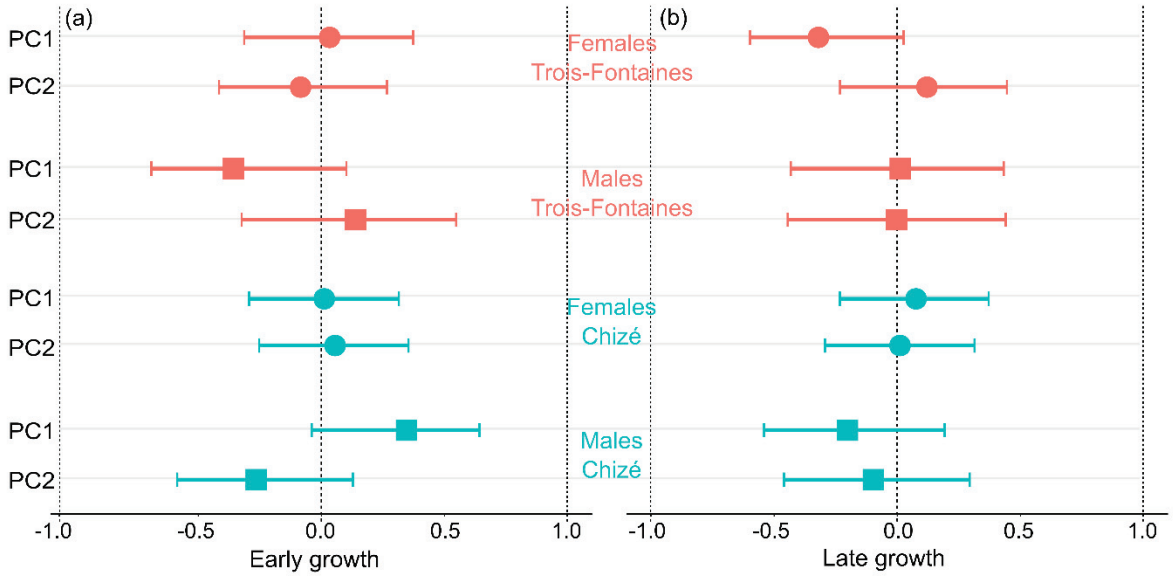
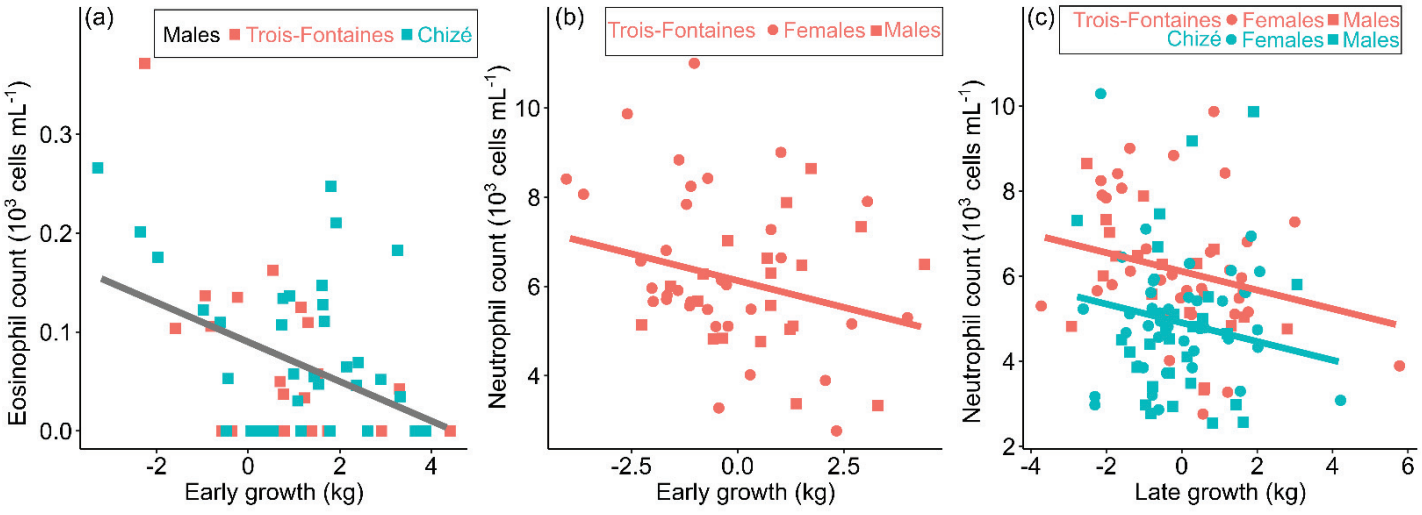


Figure 5. Negative effect of *early* or *late* growth on two traits of cellular immunity of adult roe deer (from 4 to 9 years of age included) in a) males of the two populations of Chizé and Trois-Fontaines, b) both sexes in Trois-Fontaines, and c) both sexes and both populations.



Discussion

We tested for potential short-term and long-term costs of growth on immunity in a large herbivore in three free-ranging populations facing markedly different ecological conditions. Overall, we found only limited evidence for the expected costs of growth on roe deer immunity, both over the short-term during immune development (at 8 months), and over the longer-term during adulthood (between 4 and 9 years of age). In growing animals, we found that a high rate of *early* growth led to a decrease in the levels of some globulins (gamma-globulins in all populations and alpha1-globulins at Chizé). Similarly, in adults, the level of some immune traits were slightly negatively impacted by a high rate of both *early* and *late* growth. In the adult males of two populations, both neutrophil and eosinophil counts were lower in individuals which had grown fast. In female adults of two populations, a high rate of *late* growth negatively impacted the neutrophil count, while a similar relationship with *early* growth was observed in females from Trois-Fontaines only. Long-term growth costs thus generally tended to affect the cellular part of the immune response, mostly the number of neutrophils and eosinophils. The fact that the various components of the immune system were differentially affected by growth is consistent with our hypothesis that immune functions are not equally costly to produce and maintain. As reported by previous studies (Klasing 2004), our results support the assertion that innate and cellular components of immunity are more costly to produce over the long-term than adaptive and humoral immune components. Consequently, although some cellular immune traits might be impaired by fast growth, the evidence for growth costs on other immune components is far from evident in roe deer. Our results also highlight that, contrary to our expectations, high *late* growth that potentially includes compensatory growth does not lead to higher costs for the immune response than *early* growth. Finally, although Trois-Fontaines provides clearly more suitable habitat for roe deer than Chizé (based on higher wood production, plant productivity and homogeneity of the forest, see details in Pettorelli et al. 2006), roe deer at Chizé did not seem to pay higher growth costs on immunity than those at Trois-Fontaines.

Obviously, our study had some limitations that might partly account for the overall findings of an absence of a marked trade-off between growth and immunity in roe deer. The two main axes of our PCA only accounted for 30% of the total variation in the immune measures, indicating that a substantial proportion of variation remains. Low resolution in our immune measures might account for this low explanatory power of the PCA, because measuring immunity in wild species is challenging. Indeed, access to wild animals is difficult, and

standardised measurement techniques have still to be developed for non-model species such as roe deer. The immune measures in our study provide a general pattern of energy allocation to the two components of the immune response, which is consistent over the long-term, as indicated by the substantial within-individual repeatability we found in adults. Although our measures were taken without considering the previous history of pathogen exposure, these baseline levels have been shown to be related to the response to previous challenges (Schneeberger et al. 2014), and to be strongly associated with survival in some mammals in the wild (Rossi et al. 2013 on wild boar *Sus scrofa*, Garnier et al. 2017 on Soay sheep *Ovis aries*). However, short-term variation in some immune parameters in response to specific immune challenges may obscure the observed pattern, in particular, for parameters displaying short-term responses such as haptoglobin or alpha-globulins. Some of these measures could also be improved to assess immune allocation more precisely, for example, by discriminating different populations of lymphocytes, and/or by conducting functional immunological tests. Lastly, our assessment of growth could potentially be improved and a more detailed assessment of the growth curve might have enabled a better detection of trade-offs. Since we did not have repeated measures of body mass in our populations, we used mass gain between 8 months of age and adulthood as a proxy of growth. Although this prevented us from deriving a very detailed growth trajectory, it still provided relevant information on individual allocation to growth for our model species. Indeed, roe deer display a monomolecular pattern of growth, involving a peak in growth rate at birth which then consistently decreases with increasing age (Portier et al. 2000).

Besides the methodological limitations reviewed above, a possible explanation for the absence of a clear trade-off between growth and immunity in the present study might be due to the lack of information on reproductive effort. While the patterns of covariation between growth and immune performance that have been reported so far are highly variable (see e.g. Saino et al. 1998, Hõrak et al. 2000, Mauck et al. 2005), most studies (including ours) ignored the fact that available resources have to be partitioned among growth, reproduction, and a broad range of physiological functions related to survival (Stearns 1992, Kirkwood and Rose 1991). High allocation to reproduction during early life is generally associated with long-term fitness (Lemaître et al. 2015, but see Panagakis et al. 2017) and physiological costs (e.g. Bauch et al. 2013, Hanssen et al. 2005) in wild vertebrates. Although most studies have been performed in females, recent evidence suggests that the same might be true in males (Lemaître et al. 2014, Beirne et al. 2015). Therefore, a decline in immune performance might, in some species, be

only apparent in individuals that allocate substantially to both growth and reproduction (e.g. gestation and lactation in females or energy allocation to traits associated with sexual competition in males). Studies on laboratory or farm animals that have been intensely selected for high productivity (growth or reproduction) have shown that, despite *ad libitum* access to nutrient-dense food, a down-regulation of immune function can occur when immunity is not purposely selected for (Van der Most et al. 2011). The trade-offs involving reproduction might be particularly pronounced in roe deer females that rely almost exclusively on available food resources during breeding and do not store body reserves to meet the energetic requirements of reproduction (i.e. income breeder reproductive tactic, Andersen et al. 2000). Cumulative costs of growth and reproduction might even be more pronounced in harsh environments such as Chizé, where roe deer females show higher between-year variation in reproductive success, than in Trois-Fontaines or Aurignac (Gaillard et al. 2013). Females of long-lived iteroparous species faced with harsh conditions are selected to favour their own survival over their offspring survival (Gaillard and Yoccoz 2003), which could explain the absence of costs paid on immunity by females at Chizé and their lower allocation to reproduction when faced with food shortage (Gaillard et al. 1992). The trade-off between growth and immune development may also be stronger in degraded habitats because habitat quality and biodiversity both contribute to shape pathogen burden and parasite selective pressure (Civitello et al. 2015, Young et al. 2013).

Optimal strategies relative to the maintenance or the regulation of the immune system also depend a lot on the pathogen pressure of the environment, and the optimal allocation of resources among different components of immunity is context-specific. Parasites and pathogens generate a threat to survival and infections are one of the causes potentially jeopardizing juvenile survival (Sams et al. 1996, Lynsdale et al. 2017). The 'antigen-exposure' hypothesis points to ecological variation in pathogen pressure as being a key determinant of observed variation in immunity (McDade 2016). This hypothesis predicts that, in environments with high pathogenic pressure, overall allocation to immune function will be greater, and/or that adaptive immunity will be prioritized over innate immunity if the costs of increasing allocation to all components of the immune function are exceedingly large. At Chizé, young roe deer faced high parasite pressure, for instance of *Trichuris sp.* (Cheynel et al. 2017). It is therefore crucial for them to allocate energy to immunity during body development. This strong allocation to immunity from the early stages until adulthood could explain the overall absence of a trade-off between growth and immunity, especially at Chizé. At Trois-Fontaines, roe deer are less exposed to parasites and have more resources available to mount an efficient immune response.

Additionally, the high parasite pressure on young roe deer at Chizé could result in the selective disappearance of lower quality individuals and especially young roe deer unable to allocate to both immunity and growth, a selection expected to be weaker at Trois-Fontaines. Finally, if nutritional resources are limited, as is expected to be the case at Chizé, immune components that require fewer resources, both in the short-term but also in the long-term, should be favoured. Even if allocating preferentially to adaptive immunity requires the payment of upfront costs, especially when food is limited, adaptive immunity should be favoured in the long run in anticipation of deferred future benefits and given the lower maintenance and per-response activation costs, compared to the innate responses (McDade 2016). In line with previous studies (Gilot-Fromont et al. 2012, Cheynel et al. 2017), these findings support the assertion that roe deer allocate more to adaptive immunity from the youngest age at Chizé than at Trois-Fontaines.

In this study, we showed that young roe deer pay only negligible costs of growing rapidly in terms of immune performance. However, it is likely that high allocation to growth, especially late in the growing period (after weaning), can affect physiological functions other than immunity, which might have deleterious consequences on fitness components later in life. For instance, roe deer that grow fast late in the growing period suffered from a steeper rate of mass senescence than slower growers (Douhard et al. 2017). These findings illustrate the complexity of detecting physiological costs and trade-offs in the wild, especially when immune responses are involved (Sandland and Minchella 2003).

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable institutional and/or national guidelines for the care and use of animals were followed. For Trois-Fontaines and Chizé populations, the protocol of capture and blood sampling of roe deer under the authority of the Office National de la Chasse et de la Faune Sauvage (ONCFS) was approved by the Director of Food, Agriculture and Forest (Prefectoral order 2009-14 from Paris). All procedures were approved by the Ethical Committee of Lyon 1 University (project DR2014-09, June 5, 2014). For the Aurignac population, the study was permitted by the land manager of both sites, the Office National des Forêts (ONF) and prefecture of Haute Garonne (Partnership Convention ONCFS-ONF dated 2005-12-23). All procedures were approved by the Ethical Committee 115 of Toulouse (project APAFIS#7880-2016120209523619v5).

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Maternal effects shape offspring condition and immunity in a wild mammal.

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ABSTRACT

In vertebrates, offspring survival often decreases with increasing maternal age. While many studies have reported a decline in fitness-related traits of offspring with increasing maternal age, the study of senescence in maternal effect through age-specific changes in offspring physiological condition is still at its infancy. We assessed the influence of maternal age and body mass on offspring physiological condition in two populations of roe deer (*Capreolus capreolus*) subjected to highly different ecological contexts. We measured seven markers to assess body condition and characterize the immune profile in 54 fawns recently emancipated from known age mothers. We found that maternal age did not impact offspring physiological condition measured at 8 months of age. This lack of evidence for senescence in maternal effects could be due to the strong viability selection observed in the very first months of life in this species. However, offspring physiological condition was strongly influenced by maternal body mass. Markers of body condition (fructosamine and hemoglobin) showed higher levels in fawns born to heavy mothers, as well as innate cellular immune traits (neutrophil count). These results shed a new light on the physiological pathway mediating the well-established relationship between mother's mass and offspring fitness. Finally, contrasted environmental conditions experienced by fawns of the two populations strongly influenced the levels of several markers of body condition and immune performance. Fawns facing food limitation displayed lower values in all markers of body condition (albumin, fructosamine and hemoglobin) than those living in good quality habitat. They also allocated preferentially in humoral immunity (i.e. gamma-globulins), which is much less expensive to develop; on the contrary to the population living in good conditions, where fawns allocated more in cellular response (i.e. neutrophil count).

Keywords: ageing, body condition, eco-immunology, roe deer.

Introduction

With increasing age, most vertebrates show a decline in reproductive traits, which is ultimately responsible for a decrease in reproductive success (Nussey *et al*., 2013; Lemaître and Gaillard, 2017). In females, such age-related decline in reproductive success, known as reproductive senescence, can involve a decline in birth rates, in clutch/litter size, or in offspring survival. A delayed date of birth/laying or a lower body mass of offspring with mothers age often accounts for the observed decrease in offspring survival (Lemaître and Gaillard, 2017). Empirical evidence from wild populations thus shows that senescent females are less efficient at raising offspring (*i.e.* less offspring weaned or offspring with low viability, *e.g.* Packer *et al*., 1998, Ericsson *et al.*, 2001; Descamps *et al.*, 2008; Sharp and Clutton-Brock, 2010; Karniski *et al.*, 2018).

The proximate causes of reproductive senescence are still poorly understood but a physiological deterioration of the female reproductive system over time leading to changes in age-specific maternal effects may be involved (Lemaître and Gaillard, 2017), independently of any age-specific changes in fertility (Moorad and Nussey, 2016; Lemaître and Gaillard, 2017; Karniski *et al.*, 2018). Maternal effects comprise a wide range of phenotypic influences of the mother on offspring phenotype that are unrelated to the offspring's own genotype (Bernardo, 1966). They can be direct through post-natal maternal care (Mousseau and Fox, 1998; Gouldsborough *et al.*, 1998; Cameron *et al.*, 2008a,b) or indirect through the transmission of antibodies that facilitates the ontogeny of the immune system in offspring (Halliday, 1955). Maternal traits strongly impact offspring size and growth (see *e.g.* Boonstra and Hochachka, 1997 on collared lemming, *Dicrostonyx groenlandicus*; Ellis *et al.*, 2000 on Harbour seal, *Phoca vitulina*), and thereby offspring survival. Such maternal effects on offspring traits are particularly strong in mammals compared to other taxa because of both the extended period of maternal care and the close association between mother and offspring during gestation and lactation (Reinhold, 2002). However, the efficiency of maternal effects can decrease with increasing mother age. For instance, diminished ability to forage (Catry *et al.*, 2006; but see Ricklefs, 2008) or to store body reserves in old females leads to decrease the amount of resources transferred to offspring (Lecomte *et al.*, 2010), especially during the lactation, when energy and nutrient demands peak (Sadleir, 1984; Clutton-Brock *et al.*, 1989). Such decline in resource acquisition is likely to affect both the quality and the quantity of milk produced by old females, and could explain why old females often produce lighter offspring (*e.g.* in northern fur seals, *Callorhinus ursinus*, Boltnev and York, 2001). Milk mostly contains water, lipids,

proteins, sugars and minerals such as calcium (Oftedal, 1984) and its composition varies with maternal body mass and condition in many species (reviewed in Skibiel and Hood, 2015). The variation of milk composition, especially of fat and protein content, influences mass, growth rate (Mellish *et al.*, 1999) and survival (Skibiel and Hood, 2015) of offspring in mammals.

The marked age-related changes of the immune profile in females (*e.g.* Cheynel *et al.*, 2017) may be another mechanism by which maternal effects vary during ageing. Mother-to-offspring transmission of immunity is a major determinant of immune capacities in young vertebrates (Grindstaff *et al.*, 2003). This transmission includes the transfer of IgG (or IgY in birds) immunoglobulins, but has also persistent effects on the offspring immune response that may far outreach the presence of maternally derived antibodies (Lemke *et al.*, 2003; Reid *et al.*, 2006). A decline in the transmission of immune defences with increasing maternal age could thus compromise the development of immunity in offspring and may affect their later survival. In children, the maternal transfer of antibodies declines with increasing mother age (Fu *et al.*, 2016). However the effect of maternal age may be confounded by the maternal immune status due to past vaccination schedule. More generally, the ability to transmit immune competence should depend on females' own immune status and history of antigen stimulation, which are both changing with age.

Many studies reported a decline in offspring quality with increasing maternal age - through fitness-related traits such as offspring body mass (Ericsson *et al.*, 2001; Nussey *et al.*, 2006; Descamps *et al.*, 2008; Sharp and Clutton-Brock, 2010). The effect of maternal age on offspring physiological condition has remained poorly investigated, although a study on blue-footed booby (*Sula nebouxii*) provided experimental evidence of a decreased egg quality with increasing maternal age, which was associated with a decline in offspring rearing capacities, growth and T-lymphocyte response (Beamonte-Barrientos *et al.*, 2010). To the best of our knowledge, similar studies in wild mammals are still lacking. To fill this gap, we assessed the effect of maternal age and body mass on offspring physiological condition in a wild mammal, the roe deer (*Capreolus capreolus*). In this weakly polygynous ungulate, females show senescence in many traits including body mass (Douhard *et al.*, 2017), haematological traits (Jégo *et al.*, 2014a), and immune competence (Cheynel *et al.*, 2017), all traits that influence offspring condition. We analysed seven markers to measure both physiological body condition and immune profile in 54 juvenile roe deer recently emancipated from known-age mothers. In particular, we measured circulating concentrations of albumin, fructosamine, haemoglobin and hematocrit as physiological markers of individual condition (Jégo *et al.*, 2014a). To assess

immune functions, we measured neutrophil and lymphocyte counts, and gamma-globulin and haptoglobin levels, as markers of cellular and humoral effectors of both innate and adaptive components (Gilot-Fromont *et al.*, 2012). Based on our current knowledge, we expected lower levels of physiological markers of roe deer born to old females compared to prime-aged ones. In addition, as adult body mass is a reliable proxy of individual quality in female roe deer (Plard *et al.*, 2014), we also expected that the deleterious effect of mother age on offspring condition should be more pronounced for offspring born to the lightest females. These latter likely have lower capacities to transfer resources to their offspring. Finally, our study took place in two different populations of roe deer subjected to highly different ecological context, which allow to test the importance of environmental conditions in the physiology of juvenile roe deer.

Materials and methods

Study population

Roe deer data were collected in two populations living in enclosed forests, at Trois-Fontaines located in north-eastern France (1,360 ha, 48°43'N, 4°55'E) and at Chizé located in western France (2,614 ha, 46°50'N, 0°25'W). The Trois-Fontaines forest offers habitats of high quality to roe deer, due to rich soils and a continental climate characterized by cold winters and warm rainy summers. In contrast, the Chizé forest offers a relatively poor habitat to roe deer because of the low productivity of the soils and a temperate oceanic climate with Mediterranean influences characterized by frequent summer droughts (Pettorelli *et al.*, 2006). The contrasted environmental conditions experienced by roe deer in the studied populations lead to marked differences in adult body mass (Gaillard *et al.*, 2013), offspring survival (Gaillard *et al.*, 1997), and immune profile (Cheynel *et al.*, 2017).

Roe deer populations were monitored using a long-term Capture-Mark-Recapture program. As roe deer females give birth in spring, systematic searches for newborn fawns were conducted between April and June. Upon capture, fawns were individually marked, and the filiation with the mother was assessed from field observations. In winter, between December and March, roe deer captures were organized each year (see Gaillard *et al.*, 1993 for details about the capture sessions). At the time of capture, offspring roe deer are approximatively 8-months of age. Lactation can last until September or October (Sempéré *et al*., 1988) but offspring orphaned early August are able to survive (D. Delorme, *pers. comm*.). Offspring roe deer captured in winter were thus fully emancipated from their mother. During captures, sex

and body mass (to the nearest 50g) were recorded and a basic clinical examination was performed. We also collected blood samples from the jugular vein (up to 20 mL for a 20 kg roe deer). Whole blood was EDTA-preserved for cell count and serum was extracted for other measures. Samples were received at the laboratory within 48 hours after sampling and analysed within 4 hours after reception.

Characterization of haematological and immune traits

We assessed body condition of offspring roe deer through four haematological traits that reflect energetic and protein reserves. Albumin is the most abundant plasma protein and its measure reflects the level of protein resources, independently of the immune status (Sams *et al.*, 1998; Stockham and Scott, 2008). It is thus considered as a relevant indicator of physiological status in ruminant species (Milner *et al.*, 2003; Perez *et al.*, 2006) and strongly correlated to other indices of body condition in roe deer (Gilot-Fromont *et al.*, 2012). Albumin was separated from other proteins and quantified by refractometry followed by electrophoresis, using an automatic agarose gel electrophoresis (HYDRASYS, Sebia, Evry, France) and expressed as mg/mL of serum. Fructosamine levels represent glycated proteins and indicate glycemia during the two-three weeks preceding sampling. This marker gives information on the level of carbohydrates reserves (Stockham and Scott, 2008). Fructosamine concentration was measured using Thermo scientific reagents and ABX Pentrafructosamine reagents on a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France) and expressed as µmol/ L of serum. Hemoglobin concentrations (in g/dL) reflects blood oxygen-carrying capacity and high concentrations of haemoglobin improve aerobic capacity (Minias, 2015). It is considered as a robust indicator of physiological condition and nutritional state of individuals (Minias, 2015). In roe deer, haemoglobin concentration was related to body mass and other body condition metrics (Gilot-Fromont *et al.*, 2012). Hemoglobin concentrations was issued from a complete blood count performed using an ABC Vet automaton (Horiba Medical, Montpellier, France) and was measured following cyan methemoglobin conversion at 550 nm, the most commonly used method in mammals (Stockham and Scott, 2008).

We assessed the immune phenotype of offspring roe deer by counting neutrophils and lymphocytes, and by measuring gamma-globulin and haptoglobin levels (see also Cheynel *et al.*, 2017). Neutrophils and lymphocytes represent between 70-80% and 20-30% of the total white blood cells, respectively. Neutrophil count is representative of the cellular innate immunity. Lymphocytes include both T and B cells, B cells being particularly involved in the production of antibodies, and therefore represent the cellular adaptive immunity. We

determined neutrophil and lymphocyte composition based on the identification of the first hundred white blood cells in Wright-Giemsa-stained blood smears (Houwen, 2001; Gilot-Fromont *et al.*, 2012). With this proportion of neutrophils and lymphocytes (in %) and the total white blood cell count measured by impedence technology (10^3 cells/mL), we obtained the count of neutrophils and lymphocytes (10^3 cells/mL). Gamma-globulins represent the majority of circulating antibodies and reflect the humoral adaptive immunity. Gamma-globulin levels (mg/mL) were separated from other proteins and quantified by refractometry, followed by electrophoresis, using an automatic agarose gel electrophoresis (HYDRASYS, Sebia, Evry, France). Finally, we measured the specific level of haptoglobin, a protein that belongs to alpha2-globulin fraction synthesized in case of chronic infection or inflammation, and that represents humoral innate immunity. Haptoglobin analyses were performed on a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France) using phase Haptoglobin assay (Tridelta Development LTD, County Kildare, Ireland) chromogenic kit.

Statistical analysis

We performed statistical analyses on 54 juvenile roe deer born between 2009 and 2015 and sampled at 8 months of age. This corresponds to 33 offspring sampled at Trois-Fontaines (18 females and 15 males) and 21 at Chizé (13 females and 8 males). All juveniles were born to identified and known-aged mothers. Mother age ranged between 3 and 12 years, which encompasses the entire reproductive life of roe deer females (Gaillard *et al.*, 1992). Detailed distribution of individuals according to sex, study site and maternal age is given in Appendix 3.2.

We tested the effect of maternal age and body mass on haematological and immunological traits of their offspring using linear mixed-effect models (LMMs). Each immune or haematological trait of offspring was analysed as a response variable. Maternal age and body mass were entered as explanatory variables, also with the two-way interaction between them. Two types of age functions were tested separately and compared: linear function and two classes (*i.e.* "prime-aged", i.e. females aged between 3 and 7 years and "old", *i.e.* females aged 8 years or more, Gaillard *et al.*, 1993). Considering the relatively small size of our dataset, we did not tested more complex age functions, as quadratic or threshold model. Maternal body mass was entered as two classes: "heavy", *i.e.* mother with a body mass higher than the median mass of her corresponding population and "light", *i.e.* mother with a body mass

lower than the median mass of her corresponding population. Sex, population, and body mass of offspring were also entered as explanatory variables. Offspring body mass was adjusted to the median date of capture (*i.e.* January 27th). This adjustment is required because juveniles grow throughout their first winter (Hewison *et al.*, 2002). The average daily mass gain throughout the winter was 12 ± 0.005 (SE) g.day^{-1} at Chizé and 24 ± 0.008 g.day^{-1} at Trois-Fontaines (linear regression with date of capture as the sole covariate; no sex-differences was detected; see Douhard *et al.*, 2017 for further details). Individual identity of the mother was included as a random effect to account for confounding effects of individual heterogeneity (van de Pol and Verhulst, 2006). The birth cohort was also included as a random effect to control for the marked differences in environmental conditions faced by roe deer during early life (Douhard *et al.*, 2014), which could ultimately influence physiological traits (Nussey *et al.*, 2007). The full list of fitted models is provided in Appendix 3.2 and detailed information on selected models is provided in Table 1. To select a model of age-specific variation in each immune or haematological metric, we used a model selection procedure based on the Akaike Information Criterion (AIC, Burnham and Anderson, 2002). For each trait, we retained the model with the lowest AIC, and when the difference of AICs between competing models was less than 2, we retained the simplest model to satisfy parsimony rules (Burnham and Anderson, 1982). In addition, we calculated the AIC weights (w_i) to measure the relative likelihood of each model to be the best among the set of fitted models. Finally, goodness-of-fit of the model was assessed through calculating conditional (total variance explained by the best supported model) and marginal (variance explained by fixed effects alone) R^2 formulations (Nakagawa and Schielzeth, 2013).

Finally, we calculated the effect size of maternal age on each offspring physiological trait. Effect sizes were calculated as partial correlation coefficients, which measure the standardized effect of maternal age on an offspring trait, while controlling for the potential effects of other traits. To obtain the different effect sizes, for each immune trait we first fitted the mixed-effect model including maternal age (linear) and body mass as fixed factors, and cohort and maternal identity as a random effect on the intercept (*i.e.* additive model). We then used the equation in Nakagawa and Cuthill (2007 p. 82) for mixed-effect models to calculate effect size. We calculated confidence intervals of effect sizes following Nakagawa and Cuthill (2007)'s recommendations (*i.e.* using the 'es calculator', see http://cebcp.org/practical-meta-analysis-effect-size for further information). We calculated effect sizes on the whole dataset

and in the two populations separately to compare the direction of the effect between populations.

All analyses were carried out in R version 3.2.3 (R Core Development Team, 2015) and using the function lmer from package lme4 (Bates *et al.*, 2015).

Results

We found no effect of maternal age on either body condition or immune offspring traits: whatever the physiological trait considered, mother age was never included in the selected model (Table 1). When looking at the effect size of maternal age on offspring traits (Fig. 1), no effect was detected either in the whole dataset or considering populations separately (Fig. 1). We only found a tendency for a negative effect of maternal age on gamma-globulin levels in offspring at Chizé (Fig. 1, average effect size [CI inf ; CI sup]: -0.45 [-0.74 ; -0.02]).

On the other hand, maternal body mass and population were generally included in the selected model (Table 1), which highlight their strong influence on offspring physiological performance at 8 months. Fawns born to heavier females had higher fructosamine level (+ 9%, Fig. 2a and parameter estimates in Table 1), haemoglobin concentration (+4%, Fig. 2b and Table 1) and neutrophil count (+22%, Fig 2c and Table 1) than those born to lighter ones; while the opposite pattern occurred for lymphocyte count (-23%, Fig. 2d and Table 1). All offspring markers of body condition and two immune traits strongly differed between populations, with offspring at Chizé showing lower levels of albumin (-14%, Table1), fructosamine (-14%, Table 1), haemoglobin (-13%, Table 1), and neutrophil count (-23%, Table1) but much higher levels of gamma-globulins than those at Trois-Fontaines (+52%, Table 1).

Table 1. Parameter estimates of the selected linear mixed-effect model of haematological and immune traits in the offspring (juvenile roe deer at 8 months of age). Potential fixed effects are offspring body mass, maternal age (linear function or classes, i.e. "prime-age" vs. "old"), maternal body mass ("heavy" vs. "light"), population (Chizé vs. Trois-Fontaines), offspring sex (males vs. females). The interaction maternal age x maternal body mass was also included. All models included the cohort of the offspring and maternal identity as random effects. Estimates are presented ± standard error (SE). Statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001. R^2m and R^2c are the marginal and conditional variance of the model, respectively.

	Selected model	Estimate ± SE	t-value	p	R^2m	R^2c
HAEMATOLOGICAL TRAITS						
Albumin	Intercept	37.54 ± 2.03	18.47	***	0.15	0.92
	Population (Chizé)	-5.08 ± 1.42	-3.58	***		
Fructosamine	Intercept	242.51 ± 13.36	18.36	***	0.22	0.62
	Maternal body mass (heavy)	21.08 ± 7.96	2.65	*		
	Population (Chizé)	-32.83 ± 9.68	-3.39	**		
Hemoglobin	Intercept	17.02 ± 0.32	53.60	***	0.43	0.48
	Maternal body mass (heavy)	0.75 ± 0.34	2.23	*		
	Population (Chizé)	-2.10 ± 0.38	-5.50	***		
IMMUNE TRAITS						
Neutrophil count	Intercept	5.05 ± 0.43	11.71	***	0.25	0.59
	Maternal body mass (heavy)	1.13 ± 0.45	2.51	*		
	Population (Chizé)	-1.47 ± 0.52	-2.86	**		
Lymphocyte count	Intercept	2.68 ± 0.21	12.90	***	0.10	0.81
	Maternal body mass (heavy)	-0.62 ± 0.30	-2.09	*		
Gamma-globulins	Intercept	13.65 ± 0.98	13.88	***	0.45	0.64
	Population (Chizé)	7.11 ± 1.23	5.77	***		
Haptoglobin	Intercept	0.12 ± 0.02	5.96	-	0.00	0.51

Figure 1. Effect size of maternal age on offspring haematological and immunological traits, in the two populations together (total) and in the two populations separately (Trois-Fontaines and Chizé). Effect of maternal body mass is taken into account as fixed factor. Effect sizes (symbols) are reported together with associated 95%-confidence interval. Blue colour represents a negative effect size and red colour a positive effect size.

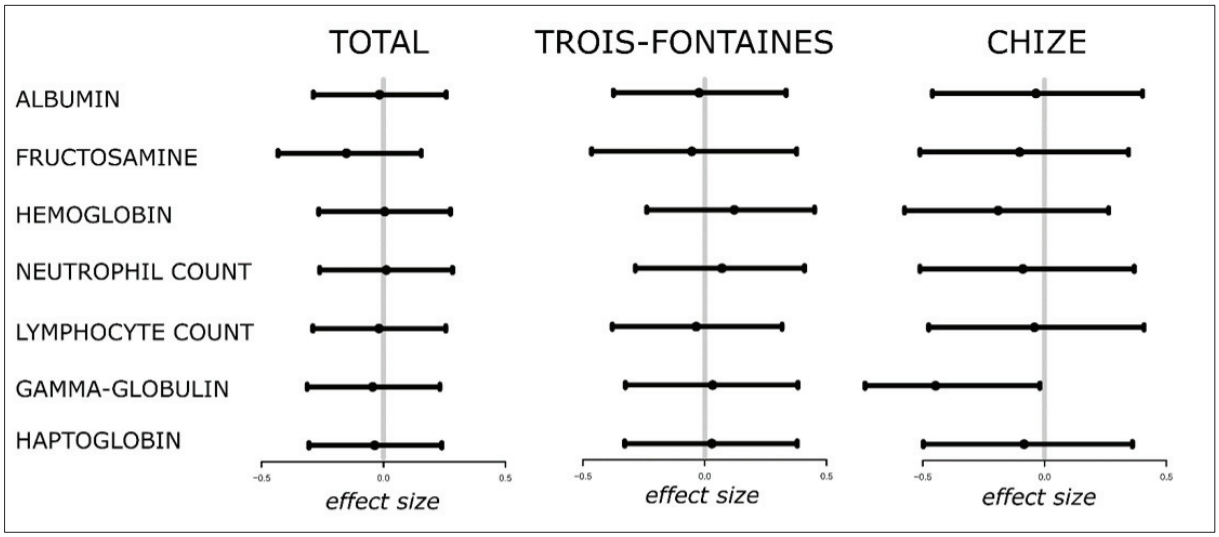
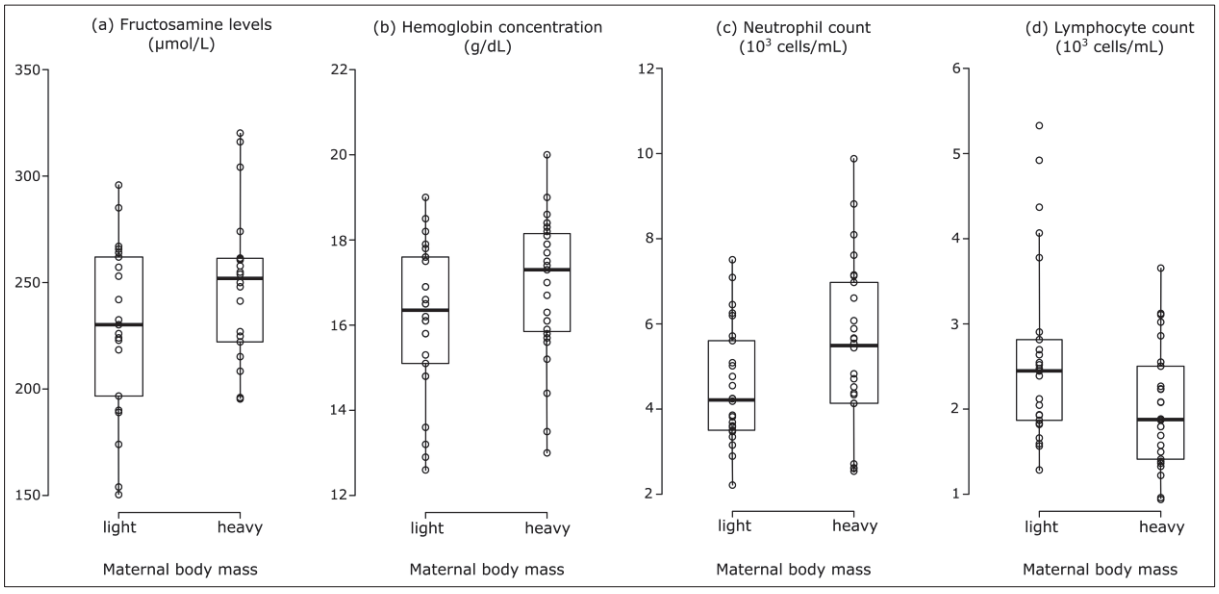


Figure 2. Effect of maternal body mass ("heavy" vs. "light") on offspring: a) fructosamine level, b) hemoglobin concentration, c) neutrophil count and d) lymphocyte count.



Discussion

Our results provide a thorough assessment of the effect of both maternal age and body mass on the physiology of recently emancipated offspring. We found that in roe deer, maternal body mass but not maternal age markedly influenced offspring physiological condition and immunity. We also highlight that environmental conditions experienced in the first months of their life play a key role in the physiology of roe deer fawns.

It is commonly reported in the literature that maternal age tends to be an important determinant of early survival across multiple animal taxa and notably in wild mammalian populations. Ivimey-Cook and Moorad (2018) recently compiled studies investigating the influence of maternal age on neonatal survival in 51 species, and found that 90% of these studies reported evidence for an deleterious effect of advanced maternal age, with an average effect of age estimates to -57.8% per standardized unit of increasing age in mammals (Ivimey-Cook and Moorad, 2018). These results suggest that born to old mothers might suffer from lower body condition and impaired physiological performance of offspring. However, in the present study, we did not find any detectable effect of maternal age, neither on markers of body condition, nor on immune profile of the offspring. Offspring immune traits were not down-regulated in fawns born to old mothers, contrary to our expectations and some case studies from the literature (*e.g.* reduced T-lymphocyte response in chicks born to old mothers in Beamonte-Barrientos *et al.*, 2010). However, we that the detrimental effect of maternal age offspring condition exists but was masked here by viability selection (sensu Fisher 1930). In roe deer, viability selection is particularly pronounced in early life, before weaning (Garratt *et al.*, 2015). If fawns from old females show poor physiological condition, they might die in the first weeks of their life (i.e. well before their first winter). Thus, the sample of 8-months old fawns captured might only include individuals with a quite high body condition. Quantifying physiological markers in newborn fawns, would be thus required to assess whether the effect of maternal age is at play before viability selection takes place. In addition, we cannot exclude the changes in offspring physiological trait with mother trait can take more complex forms, as observed for other traits (*e.g.* litter size, see Berger *et al.*, 2015). Because of our limited dataset, the effect of mother's age was tested with two age-classes or with a linear function, and may not fully describe age-related changes in physiological traits.

We found that the physiological condition of roe deer fawns is strongly influenced by both maternal body mass and environmental conditions. For many years, studies have reported

the positive influence of maternal body size on offspring size and development in vertebrates (Bernardo, 1996; Bowen *et al.*, 2001). More specifically in roe deer, maternal body mass is an important predictor of reproductive success (Gaillard *et al.*, 2000a,b) and is positively associated with both offspring birth mass and subsequent survival (Plard *et al.*, 2015). The positive influence of maternal body mass and offspring body condition is revealed here on a physiological basis as we found a positive association between maternal body mass and offspring physiological traits tightly linked to body condition. Fawns born from heavy mother present higher levels of fructosamine and hemoglobin than those born from light mothers. This reflects higher levels of carbohydrates reserves and aerobic capacity of these fawns, and thereby a higher physiological performance. In addition, maternal body mass was positively associated with offspring cellular immune trait (i.e. neutrophil count), but not associated with offspring humoral traits (*i.e.* gamma-globulin and haptoglobin). Neutrophils represent the majority of white blood cells (between 60 and 80%) and constitute an important part of the innate cellular immune response. The immune function is known to be energy-demanding and strongly dependant of the quality and quantity of nutritional resources, but the cellular part of the immune function entails particularly high costs of production compared to the humoral component (Klasing, 2004). This positive effect of maternal body mass on offspring condition, especially on the most energetic-demanding traits as cellular immune traits, may be the result of higher allocation of maternal resources such as a higher milk production or an increased protein content in milk of heavy mother (Landete-Castillejos *et al.*, 2003). Maternal milk production enhances offspring mass gain (Landete-Castillejos *et al.*, 2003) and immunity (Landete-Castillejos *et al*., 2002). Although 8 months roe deer are recently emancipated, the quality of food provisioning provided by their mother during the lactation period, and the energetic reserves induced, likely have lasting effects on their physiological condition.

Contrary to our prediction, we found a negative relation between maternal body mass and offspring lymphocyte count. However, the opposite effects of mother's mass on innate (neutrophil count) and adaptive immunity (lymphocyte count) are in line with previous findings suggesting that body condition positively correlates to innate immunity but negatively to adaptive immunity (Gilot-Fromont *et al*., 2012). These results suggest that maternal body mass can also influence offspring immune profile, in relation to their past infections. In roe deer, parasites are mostly found on light females (Jégo *et al.*, 2014b) that might thus develop high levels of specific immune response, which ultimately result in stronger immune priming of

offspring (Nystrand and Dowling, 2014). This could explain the higher level of markers od the adaptive immune system in fawn born from light mothers.

Finally, we found that environmental conditions influence mean physiological condition in roe deer fawns. Three markers of fawn body condition (albumin, fructosamine and hemoglobin) were lower at Chizé where roe deer experience poor living conditions. Fawns at Chizé also showed higher allocation to humoral immunity (*i.e.* higher levels of gamma-globulins), that are also less costly to develop (Klasing, 2004), than fawns at Trois-Fontaines that displayed higher level of cellular immunity (i.e. higher levels of neutrophils). These results are not surprising since at Chizé, roe deer experience an heterogenous environment of contrasted quality habitats, in a forest of overall low primary productivity, due to the poor soils and frequent summer droughts (Pettorelli *et al.*, 2006) and have thus less resources to devote to the immune function. At Chizé, roe deer also faced high parasite pressure (*e.g. Trichuris sp.* Cheynel *et al.*, 2017) and previous studies have shown that fawn survival, female fertility, adult body mass (Gaillard *et al*., 2013), age-specific telomere length (Wilbourn *et al.*, 2017) and many markers of adult immune performance (Cheynel *et al.*, 2017) are consistently lower at Chizé than at Trois-Fontaines. A large proportion of females at Chizé experience nutrient limitation and consequently low body mass (Gaillard *et al*., 2013) that should affect their milk production and quality, as milk is very dependent on diet quality (Sutton, 1989) and food availability (Oldham and Friggens, 1989; Landete-Castillejos *et al*., 2003). In addition, lactation period in roe deer takes place during summer, which is particularly dry in the Chizé forest, exposed to repeated drought episodes (Pettorelli *et al*., 2006). It could result in a lower ability to transfer resources to their offspring in their first months of life, and explain the low level of both cellular immunity and body condition markers compared to those at Trois-Fontaines.

Overall, our study revealed that increased maternal age at reproduction does not lead to impaired physiological condition of roe deer fawns after nutritional emancipation. It also provide strong evidence that maternal body mass influence offspring physiological performance and thus shed a new light on the physiological factors linking mother's mass and offspring fitness. Finally, we demonstrate that environmental conditions experienced in the first months of their life have a strong influence on fawns physiological performance. This first assessment of the effect of increasing maternal age and maternal body mass on a large variety of physiological traits in two populations of roe deer allowed us to assess better the role of maternal effects in wild mammalian populations.

Conflict of interest
The authors declare that they have no conflict of interest.

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Ethical approval
All applicable institutional and/or national guidelines for the care and use of animals were followed. The protocol of capture and blood sampling of roe deer under the authority of the Office National de la Chasse et de la Faune Sauvage (ONCFS) was approved by the Director of Food, Agriculture and Forest (Prefectoral order 2009-14 from Paris). All procedures were approved by the Ethical Committee of Lyon 1 University (project DR2014-09, June 5, 2014).

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CHAPTER 4

Age-related variation in immune profile

SCIENTIFIC REPORTS

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Immunosenescence patterns differ between populations but not between sexes in a long-lived mammal

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In animals, physiological mechanisms underlying reproductive and actuarial senescence remain poorly understood. Immunosenescence, the decline in the ability to display an efficient immune response with increasing age, is likely to influence both reproductive and actuarial senescence through increased risk of disease. Evidence for such a link has been reported from laboratory animal models but has been poorly investigated in the wild, where variation in resource acquisitions usually drives life-history trade-offs. We investigated immunosenescence patterns over 7 years in both sexes of two contrasting roe deer populations (*Capreolus capreolus*). We first measured twelve immune markers to obtain a thorough identification of innate and adaptive components of immunity and assessed, from the same individuals, the age-dependent variation observed in parasitic infections. Although the level of innate traits was maintained at old age, the functional innate immune traits declined with increasing age in one of two populations. In both populations, the production of inflammatory markers increased with advancing age. Finally, the adaptive response declined in late adulthood. The increasing parasite burden with age we reported suggests the effective existence of immunosenescence. Age-specific patterns differed between populations but not between sexes, which indicate that habitat quality could shape age-dependent immune phenotype in the wild.

With increasing age, most organisms experience senescence, a process characterised by progressive and irreversible decline in age-specific reproductive success (*i.e.* reproductive senescence) and survival (*i.e.* actuarial senescence)[1]. Reproductive and actuarial senescence have been repeatedly documented in laboratory animal models[2], captive[3] and wild[4] populations, and senescence appears to be the rule rather than the exception[4,5] in the living world. However, senescence patterns can be extremely diverse across species[5] and the reasons for such a high diversity remain poorly understood. It seems that variation in senescence patterns is influenced by environmental conditions[3,6,7] and often differs between sexes[8,9]. It is thus of particular importance to identify the underlying physiological mechanisms that shape the diversity of senescence patterns between sexes and in relation to environmental conditions.

Many physiological mechanisms potentially underlying senescence have been proposed. They notably include telomere attrition[10], oxidative stress[11] and dysregulation of the immune response with increasing age, called immunosenescence[12]. Among these processes, immunosenescence is likely to play an important role on the variation observed in life history traits[13,14]. The deterioration of immune function with age makes individuals more sensitive to infections and diseases, and is thus expected to affect reproductive success and survival under natural conditions[15,16]. Although immunosenescence has been well studied in laboratory conditions, much less is known in the wild because of the difficulty of measuring within-individual changes in the field[17] (but see[18,19]). For the time being, observations suggest that patterns of age-specific changes in the immune response are similar in wild and laboratory conditions[20].

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In vertebrates, the immune system has two different but complementary components: the innate and the adaptive system. The innate response mostly corresponds to the unspecific cellular response mediated principally by monocytes, natural killer and dendritic cells, while the adaptive response provides an antigen-specific response mediated by T and B lymphocytes. A decline in the adaptive response with increasing age has been reported in humans, laboratory animal models[21], as well as in the wild (*e.g.* collared flycatchers *Ficedula albicollis*[22]; Soay sheep *Ovis aries*[20]). On the other hand, the innate component of the immune response such as the inflammatory processes seems to be maintained over ages[21,23] or even enhanced[20,24], throughout the lifetime, causing a persistent low-grade inflammation referred to as 'inflammaging'. Inflammaging has multiple origins and may have many detrimental effects on organisms[25]. These age-related dysregulations and the subsequent reshaping of both components of the immune system can deeply affect the resistance against parasitism and infectious diseases of old individuals as suggested by the consistent increase in parasite burden with age reported so far (*e.g.* in Soay sheep[26,27]; in house martins *Delichon urbica*[28]).

Studies conducted on humans also revealed sex differences in immunosenescence patterns[29] and generally pointed out that adaptive immune traits decline at a lower rate in women than in men. In the wild, available evidence of sex-differences in immunosenescence patterns are rather indirect (*e.g.*, age-specific changes in parasitism[26,30]). A better assessment of sex-specific immunosenescence in the wild is thus required. In addition, it could provide important insights to better understand differences in longevity between sexes of many animals such as mammals, where males generally show shorter lifespan[31] and earlier onsets of senescence[32] than females. Finally, whether environmental conditions influence immunosenescence profiles remains unknown. In natural conditions, animals are subjected to varying amounts of resources, which likely influence their allocation to immunity[33]. As developing an efficient immune response is a highly energetically demanding process, long-lasting caloric restriction can lead to the suppression of the immune system[34]. Moreover, in mammals, increasing tooth wear with age leads food to be increasingly difficult to acquire (*e.g.*[35] for large herbivores), and even more when resources are limited. This decrease of nutrient input with increasing age could have direct consequences on immune responsiveness and may contribute to accelerate immunosenescence.

We aimed at investigating age-related changes of immune parameters and parasitic load, in males and females of two populations of roe deer *Capreolus capreolus*, subjected to highly different ecological contexts in the wild. In this weakly polygynous ungulate, both actuarial[36] and reproductive[37] senescence have been reported, as well as senescence in body mass[38], home range size[39] and haematological parameters[40]. Both parasite prevalence and load are higher in old age classes than in young age classes in roe deer[41], suggesting the existence of some immunosenescence. However, the exact age-specific changes in parasite burden remain to be identified. We thus measured a large set of immune markers, encompassing both innate and adaptive components of the immune response, to obtain a comprehensive picture of the age-related changes in the immune system of known-aged roe deer. We also assessed age-specific patterns in the parasite burden of the same individuals. Based on our current knowledge we predicted: (i) a progressive decline of the adaptive immune component but a stable innate response and an increased inflammatory markers in old individuals, (ii) an earlier and more pronounced immunosenescence in males than in females, (iii) an earlier and sharper immunosenescence in the population with poor and limited food resources (Chizé) than in the population with rich and abundant food resources (Trois-Fontaines), and (iv) an increase in parasitic load with age for individuals of a given sex in a given population.

Results

Senescence patterns of immune response were analysed from 615 measures on 325 known-aged (from 2 to 16 years) roe deer captured between 2010 and 2016 (Trois-Fontaines n = 166, sex ratio (*i.e.* male/female ratio) 0.80:1; Chizé n = 159, sex ratio 0.83:1). Among the 325 studied individuals, 163 were captured once, 80 twice, 47 three times, 25 four times and 10 five or six times. Most individuals captured (76%, *i.e.* 467 capture events) were prime-aged adults between 2 and 7 years old, with a sex ratio of 0.86:1 at Trois-Fontaines and of 0.83:1 at Chizé. Old adults (*i.e.*, older than 7 years) were less numerous (24%, *i.e.* 148 capture events), with a more female-biased sex ratio, especially at Chizé (0.57:1 at Trois-Fontaines and 0.26:1 at Chizé). Full information on age- and sex-specific sample size is given in supporting information (Table S1).

As in many studies in wild animal populations, we collected relatively few data from very old individuals (*i.e.* older than 10 years, see in supporting information Table S1). This is particularly true for males in Chizé with only two males older than 10 years (captured at 11 and 14 years old). These two individuals had values very different from those of younger individuals, likely due to a degraded physical condition at the end of their life, and these extreme values drove the model selection. We therefore decided to report results without these oldest males. However, we provide results from analyses including these individuals in the supporting information (Table S2). In females at Chizé and in both sexes in Trois-Fontaines, we consistently had at least 3 individuals in each age class.

Innate immune response. At two years of age, roe deer of both sexes showed higher levels of neutrophils at Trois-Fontaines than at Chizé (see Table 1 for the predicted values at 2 years old). The neutrophil count increased with age in females in both populations (Table 1; Fig. 1a), whereas it slightly decreased in males in both populations (Table 1; Fig. 1a). Hemagglutination (HA) ability changed with age from 8 years onwards, with marked between-population differences (Table 1; Fig. 1b): it increased at old ages at Trois-Fontaines but decreased at Chizé. Hemolysis (HL) ability showed both similar age-related changes from 10 years and similar between-population differences (Table 1; Fig. 1c). Monocyte, basophil and eosinophil counts all remained constant from 2 years of age onwards in both sexes and populations (Table 1).

Inflammatory markers. At two years of age, males had higher levels of haptoglobin than females in both populations, with higher values at Chizé than at Trois-Fontaines in both sexes (see Predicted values at 2 years in Table 1). Age-related changes in haptoglobin concentration differed between the populations (Table 1; Fig. 1d). In

Immune trait	Best model selected	Age function	Variable	Parameter estimate ± SE	t-value	p	R^2m	R^2c	Predicted value at two years			
									♂ TF	♀ TF	♂ CH	♀ CH
INNATE TRAITS												
Neutrophil count	I(age^2)*sex + pop	quadratic	Intercept	6.00 ± 0.25	23.97	***	0.09	0.51	6.25 ± 0.29	6.52 ± 0.15	5.34 ± 0.34	5.14 ± 0.18
			I(age^2)	0.01 ± 0.003	3.97	***						
			Sex (M)	0.57 ± 0.29	1.96	—						
			Pop (CH)	−1.16 ± 0.22	−5.15	***						
			I(age^2): sex (M)	−0.02 ± 0.01	−3.69	***						
Monocyte count	Constant	—	Intercept	0.29 ± 0.09	3.12	—	0.00	0.46	0.30 ± 0.10	0.31 ± 0.10	0.29 ± 0.07	0.30 ± 0.10
Basophil count	Constant	—	Intercept	0.07 ± 0.02	4.33	—	0.00	0.28	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.08 ± 0.01
Eosinophil count	Sex	—	Intercept	0.12 ± 0.01	10.03	***	0.02	0.15	0.07 ± 0.01	0.12 ± 0.02	0.10 ± 0.02	0.11 ± 0.01
			Sex (M)	−0.03 ± 0.01	−2.90	**						
Hemagglutination	Age * pop	threshold (8 years)	Intercept	2.43 ± 0.65	3.76	***	0.01	0.44	4.03 ± 0.33	4.03 ± 0.25	4.26 ± 0.35	3.98 ± 0.36
			Age	0.19 ± 0.07	2.85	**						
			Pop (CH)	2.67 ± 0.88	3.04	**						
			Age: Pop	−0.32 ± 0.10	−3.01	**						
Hemolysis	Age * pop	threshold (10 years)	Intercept	−1.52 ± 1.31	−1.17	—	0.01	0.62	2.24 ± 0.41	2.35 ± 0.37	2.10 ± 0.40	1.97 ± 0.38
			Age	0.38 ± 0.12	3.07	**						
			Pop (CH)	5.11 ± 2.06	2.48	*						
			Age: Pop	−0.53 ± 0.20	−2.57	*						
INFLAMMATORY TRAITS												
Alpha1-globulin	Age + I(age^2) + pop + sex	quadratic	Intercept	3.35 ± 0.19	17.45	***	0.04	0.5	3.35 ± 0.20	3.11 ± 0.19	3.24 ± 0.16	2.94 ± 0.15
			Age	−0.09 ± 0.03	−2.54	*						
			I(age^2)	0.01 ± 0.003	3.29	**						
			Pop (CH)	−0.13 ± 0.05	−2.76	**						
			Sex (M)	0.18 ± 0.05	3.79	***						
Alpha2-globulin	Constant	—	Intercept	5.79 ± 0.30	19.62	–	0.00	0.26	5.63 ± 0.30	5.84 ± 0.34	5.66 ± 0.33	5.88 ± 0.29
Beta-globulin	I(age^2) + sex + pop	quadratic	Intercept	5.84 ± 0.38	15.22	***	0.12	0.43	6.20 ± 0.54	5.73 ± 0.36	7.12 ± 0.51	6.41 ± 0.41
			I(age^2)	0.02 ± 0.002	8.71	***						
			Sex (M)	0.63 ± 0.16	3.81	***						
			Pop (CH)	0.64 ± 0.16	3.87	***						
Haptoglobin	Age * pop + sex	threshold (9 years)	Intercept	−4.24 ± 0.74	−5.71	***	0.07	0.15	0.34 ± 0.10	0.15 ± 0.06	0.69 ± 0.14	0.30 ± 0.11
			Age	0.48 ± 0.08	6.04	***						
			Pop (CH)	3.18 ± 1.20	2.66	**						
			Sex (M)	0.25 ± 0.08	3.05	**						
			Age: Pop	−0.33 ± 0.13	−2.50	*						
ADAPTIVE TRAITS												
Lymphocyte count	Age + I(age^2) + pop	quadratic	Intercept	2.81 ± 0.17	16.49	***	0.14	0.37	2.25 ± 0.14	2.42 ± 0.13	1.90 ± 0.17	1.89 ± 0.15
			Age	−0.15 ± 0.05	−2.76	**						
			I(age^2)	0.01 ± 0.004	2.12	*						
			Pop (CH)	−0.65 ± 0.08	−8.27	***						
Gamma-globulin	Age + pop	threshold (4 years)	Intercept	12.32 ± 1.14	10.5	***	0.21	0.65	13.86 ± 0.90	13.54 ± 0.93	18.55 ± 1.66	18.43 ± 1.57
			Age	0.52 ± 0.08	6.79	***						
			Pop (CH)	4.40 ± 0.39	11.43	***						

Table 1. Linear mixed effect models selected for 12 immune parameters. The effect of different age functions (factor, linear, threshold, quadratic), of sex (F: Female, M: Male), of population (TF: Trois-Fontaines, CH: Chizé), with all two and three-way interactions between them, were tested. All models included individual identity, the year of capture and the cohort of individuals as random effects. When the age threshold model was selected, we have indicated the age at which the parameter begins to vary, and the "Parameter estimate" of the age function is the slope of the variation with age after the threshold age. Statistical significance is represented by *for $p < 0.05$, **for $p < 0.01$ and ***for $p < 0.001$. R^2m and R^2c are the marginal and conditional variance of the model, respectively. Values are presented ± Standard Error.

both sexes at Trois-Fontaines, haptoglobin levels increased markedly from 9 years of age (Fig. 1d). Likewise, haptoglobin levels also increased from 9 years of age in both sexes at Chizé, but displayed quite lower values (Fig. 1d). Beta-globulin levels varied in relation to both population and sex, but consistently increased from 2 years of age (see population effect estimates in Table 1, Fig. 1e). Levels of alpha1-globulin increased with advancing age in both sexes of the two populations (Table 1; Fig. 1f). Finally, alpha2-globulin levels remained constant whatever the sex and the population considered (Table 1).

Adaptive response. At two years of age, roe deer had lower levels of lymphocytes at Chizé than at Trois-Fontaines (see Predicted values at 2 years in Table 1). Lymphocyte counts declined with increasing

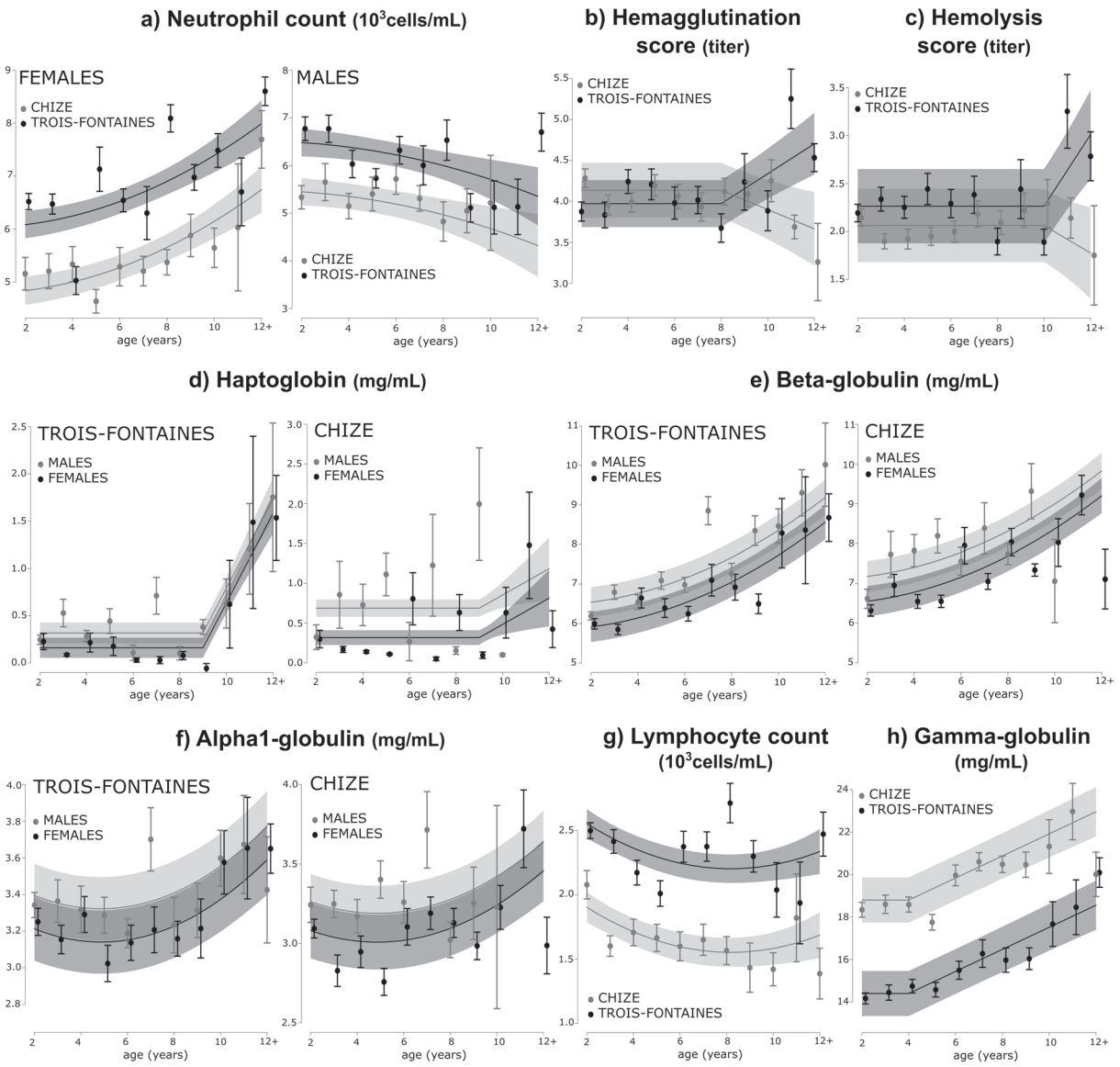

Figure 1. Predicted age-related changes in immune parameters in two populations of roe deer (Trois-Fontaines and Chizé). Plots are based on predicted effects from the selected model for each parameter (see Table 1). The lines represent model predictions and the shaded areas the 95% CIs. The points correspond to the average value per age and the bars correspond to ± Standard Error. All individuals older than 12 years of age were pooled within a "12+" age class.

age in both sexes of the two populations (Table 1; Fig. 1g). On the contrary, at two years of age, roe deer had higher gamma-globulins levels at Chizé than at Trois-Fontaines (see Predicted values at 2 years in Table 1). Gamma-globulins concentration increased from 4 years of age in both populations (Table 1; Fig. 1h).

Parasitism. The abundance of gastro-intestinal strongyles increased with age in both sexes of the two populations (Table 2; Fig. 2a). The abundance of *Trichuris* sp. markedly increased from 5 years of age in males of the two populations (Table 2; Fig. 2b). The abundance of *Trichuris* sp. also increased from 5 years of age in females of the two populations, but more slightly (Fig. 2b). The abundance of protostrongylids increased from 9 years of age in both sexes at Trois-Fontaines and in females at Chizé (Table 2; Fig. 2c). In Chizé males, the abundance of protostrongylids remained constant with age, but we did not have data in males older than 9 years (Fig. 2c). Finally, we did not find any age-related changes in coccidia abundance in either sex of the two populations (Table 2).

Discussion

Our results provide the first assessment of age-related and sex-specific changes of a large array of immune parameters (12 markers) and parasitic load (4 parasitic traits) in two contrasted populations of a wild vertebrate. Our findings demonstrate that both components of the immune response do show senescence, as characterized by

Parasitic trait	Best model selected	Age function	Variable	Parameter estimate ± SE	t-value	p	R^2m	R^2c	Predicted value at two years			
									♂TF	♀TF	♂CH	♀CH
Gastro-intestinal strongyles	(age + I(age^2)) * pop + sex	quadratic	Intercept	45.63 ± 16.02	2.85	**	0.06	0.77	34.99 ± 10.81	15.05 ± 4.93	34.48 ± 10.61	22.01 ± 16.35
			Age	−15.30 ± 5.16	−2.97	**						
			I(age^2)	1.55 ± 0.42	3.72	***						
			Pop (CH)	−48.27 ± 21.40	−2.26	*						
			Sex (M)	19.86 ± 8.27	2.4	*						
			Age: Pop (CH)	20.35 ± 7.76	2.62	**						
			I(age^2): Pop (CH)	−1.42 ± 0.64	−2.23	*						
Trichuris sp.	age * pop * sex	threshold (5 years)	Intercept	−20.85 ± 40.58	−0.51	—	0.15	0.18	7.97 ± 4.75	7.35 ± 3.40	81.42 ± 32.81	86.40 ± 29.43
			Age	4.19 ± 5.66	0.74	—						
			Pop (CH)	−4.21 ± 55.94	−0.08	—						
			Sex (M)	−35.95 ± 60.37	−0.60	—						
			Age: Pop (CH)	10.28 ± 8.02	1.28	—						
			Age: Sex (M)	8.13 ± 9.20	0.88	—						
			Pop (CH): Sex (M)	−222.44 ± 99.69	−2.23	*						
			Age: Pop (CH): Sex (M)	49.66 ± 16.08	3.09	**						
Protostrongylids	age * pop * sex	threshold (9 years)	Intercept	−24.67 ± 4.09	−6.04	***	0.13	0.15	0.17 ± 0.02	0.41 ± 0.37	1.40 ± 0.74	0.38 ± 0.18
			Age	2.76 ± 0.44	6.3	***						
			Pop (CH)	22.83 ± 6.14	3.72	***						
			Sex (M)	17.84 ± 8.22	2.17	*						
			Age: Pop (CH)	−2.52 ± 0.66	−3.82	***						
			Age: Sex (M)	−1.94 ± 0.90	−2.17	*						
			Pop (CH): Sex (M)	−213.75 ± 38.07	−5.61	***						
			Age: Pop (CH): Sex (M)	23.83 ± 4.22	5.65	***						
Coccidia	constant	—	Intercept	2560.00 ± 2296	1.12	—	0.00	0.98	233.38 ± 194.70	244.10 ± 202.30	26.07 ± 13.75	27.48 ± 8.86

Table 2. Linear mixed effect models selected for 4 parasite abundances. The effect of different age functions (factor, linear, threshold, quadratic), of sex (F: Female, M: Male), of population (TF: Trois-Fontaines, CH: Chizé), with all two and three-way interactions between them, were tested. All models included individual identity, the year of capture and the cohort of individuals as random effects. When the age threshold model was selected, we have indicated the age at which the parameter begins to vary, and the "Parameter estimate" of the age function is the slope of the variation with age after the threshold age. Statistical significance is represented by *for $p < 0.05$, **for $p < 0.01$ and ***for $p < 0.001$. R^2m and R^2c are the marginal and conditional variance of the model, respectively. Values are presented ± Standard Error.

marked changes with increasing age in many measured immune parameters. We also observed an age-specific increase in the parasitic burden of both sexes in the two populations studied.

Different patterns of senescence occurred between sexes and populations (see also[27]). As some individuals may experience specific conditions associated with particular immune responses, variation in the health status of roe deer provides a first plausible explanation for these different patterns. Although the clinical examination of roe deer did not reveal any sign of pathology at the time of capture, some individuals might have experienced inflammatory state, dehydration or malnutrition that could have influenced the measures[42].

The cellular innate immune traits (*i.e.* monocyte, eosinophil and basophil counts) did not decline with increasing age in both populations. The neutrophil counts even increased in females in both populations. We only found a decline in the neutrophil count in males of the two populations. While the stability in the number of innate immune cells over age has also been reported in humans[21] and in wild Tree swallows *Tachycineta bicolor*[23], this does not imply that the performance of the innate immune system remains stable over the individual life course. The increase in neutrophil counts observed in females of the two populations could be a way to compensate[25] not only for the decline of the adaptive response often occurring with increasing age, but also for the reduction of intrinsic functional activity of leukocytes. Indeed, the phagocytic ability of neutrophils or monocytes often decreases in old individuals[43]. The decline in adaptive response (*i.e.* lymphocyte count) is actually observed in the two populations. We also brought evidence of an increase of inflammatory markers with age (*i.e.* haptoglobin, alpha1-globulin and beta-globulin) in both sexes of the two populations. The increase of inflammatory markers has been observed in old humans[24,44] and in Soay sheep older than 7 years[20]. It suggests that a progressive dysregulation of the inflammatory response occurs at old ages, leading to an increase in the production of related inflammatory products. While an acute and transient inflammation allows neutralizing invading pathogens and facilitates repair and turn-over of injury tissues, chronic inflammation causes tissue degeneration affecting body condition and weakening individuals[25]. Circulating pro-inflammatory molecules are considered as strong predictors of age-related morbidity and mortality[44]. This increased inflammatory state could also be linked to the decline in survival in the older age classes previously reported in roe deer[36].

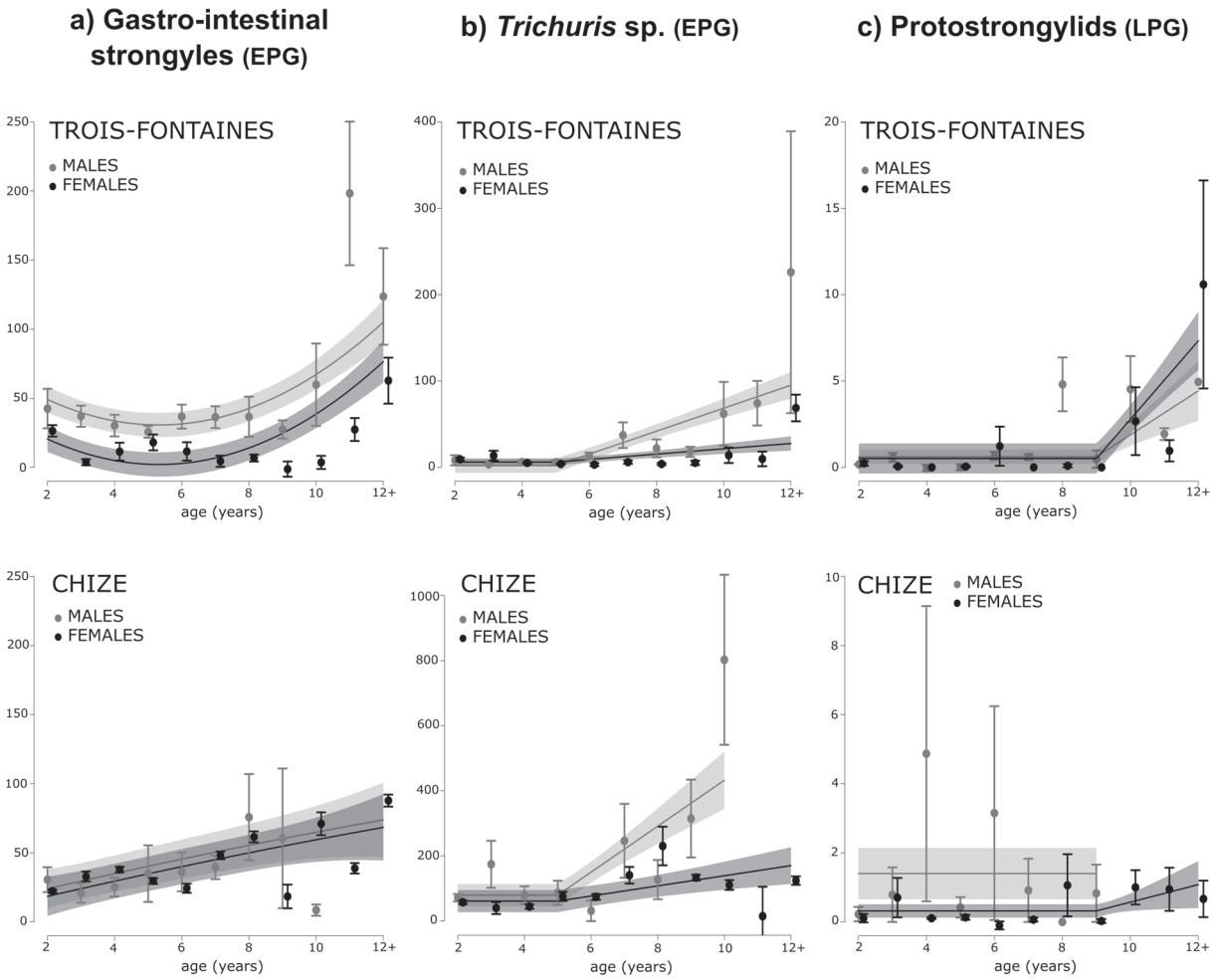

Figure 2. Predicted age-related changes in parasite abundance in two roe deer populations (Trois-Fontaines and Chizé). Plots are based on predicted effects from the selected model for each parameter (see Table 2). The lines represent model predictions and the shaded areas the 95% CIs. The points correspond to the average value per age and the bars correspond to ± Standard Error. All individuals older than 12 years of age were pooled in a "12+" age class.

The decline in adaptive response at old ages we report on roe deer has previously been documented in humans[21], mice[45] and in two vertebrates in the wild (Tree swallows[23]; and Soay sheep[20]). Although our method does not discriminate T and B lymphocytes, a decline in naïve T lymphocytes is expected to occur with ageing, and could thus possibly occur in old roe deer. Such decline may be due to the involution of the thymus, occurring quite early in life in all vertebrates and leading to a decline of the naïve T cell production[46]. On the other hand, we observed an increase of the gamma-globulin concentration with age in both sexes of the two populations. Thus, the capacity of B lymphocytes to produce gamma-globulins seems to be maintained, or maybe even increased, with age.

Our findings attest the existence of immunosenescence in roe deer in the wild. In this species, actuarial senescence begins at around 7 and 8 years of age in the two studied populations[36,47], reproductive senescence at around 11 and 12 years of age for females[37] and body mass senescence between 7 to 9 years of age[38]. However, some haematological parameters like creatinine or albumin start to decline earlier in life[39], and some immune traits (*e.g.* beta-globulin, gamma-globulin, lymphocyte count) begin to show age-related changes prior to 8 years of age. Haematological and biochemical traits, which show quite early senescence, could play a crucial role in the senescence of body mass or female reproduction, which both start at older ages. The assessment of a decline of physiological traits with increasing age could also provide a reliable measure of individual body condition and allow more accurate detection of the onset of the decline in physiological mechanisms, ultimately leading to the death of individuals. The assessment of age-related changes in parasitic burden, which occurred in both roe deer populations, also suggests that immunosenescence may impact individual's physiological condition. Indeed, in both sexes of the two populations, at least two out of four parasitic traits involving digestive or pulmonary nematodes increased with age. Like the immune traits, the increase of gastrointestinal strongyles and *Trichuris* sp. started quite early in life, prior to 8 years of age in both sexes of the two populations, which suggests that immune functions are effectively declining with increasing age. A covariation between immune and parasite traits might involve a direct functional relationship. Thus, an immune trait may indicate the strength of the parasite exposure

(positive association) or resistance (negative association) to infection. However, a detailed analysis of the relationships between parasitism and immune profile is beyond the scope of this work because it is required to account for likely confounding effects of yearly variation in environmental conditions and the past parasitic status of individual roe deer to obtain firm results. The descriptive analysis of the relationships between immune and parasite trait we reported in Supplementary Information should thus be interpreted with great caution.

Importantly, our study also allows detecting differences in age-related changes in immunity and parasitism between the two populations. Although we cannot identify the exact driver of this between-population difference, environmental conditions offer a potential candidate. The marked differences in food resources between these populations have been previously shown to influence strongly body size[48] and demographic traits[49] and could likely affect the immune phenotype as well. Two years-old roe deer had higher levels of adaptive humoral response (antibodies) at Chizé than at Trois-Fontaines, which indicates a higher allocation to the adaptive response (see also[50]). As the adaptive response is likely to be less energetically costly than innate immune response[51], the higher adaptive response observed at Chizé matches the predicted response to low resource availability in an environment with predictable pathogens. Males at Chizé also had high values of inflammatory proteins at two years of age (haptoglobin). Then, statistically significant interactive effects between age and population occurred in humoral innate (*i.e.* hemagglutination and hemolysis) and inflammatory (*i.e.* haptoglobin) markers: these three traits markedly increased with age in Trois-Fontaines, but increased much less (or even decreased) at Chizé. One cannot exclude that the two studied populations may differ in several aspects, such as genetic background and pathogenic environment. However, the latter was found to be relatively similar[40]. In contrast, environmental conditions (especially nutritional resources) clearly differ between both sites, and this difference is expected to affect both life history and immunity[52]. In the wild, organisms may have access to a limited amount of resources, leading them to share the energy gained from these resources among growth, reproduction, and soma-maintenance[33,53]. As the development, maintenance and use of efficient immune responses are costly and require nutrients[34], poorer nutritional resources and starvation experienced more often by roe deer at Chizé could account for the between-population difference observed in their immunosenescence patterns.

Contrary to studies in humans[29], we did not find clear evidence of sex differences in immunosenescence patterns. While immune differences between sexes occur from early life onwards[54], patterns of age-related changes appear to be quite similar between sexes. Thus, the decline in immune response with increasing age is probably not an underlying cause of observed sex differences in longevity, at least in the two roe deer populations we studied. However, in these populations, roe deer males exhibited higher levels of parasitism than females and a steeper increase in parasite burden with age, in accordance with previous studies[55]. As roe deer males and females do not segregate spatially[56], the exposure to parasites is expected to be similar in both sexes. The increase of parasite burden with age might involve a weaker immune ability of males compared to females, which may have not been detected.

Our first assessment of age-specific variation in immune traits and parasitism in two populations of roe deer offers a comprehensive view of age variation in physiological performance in the wild, and appeals for future studies to uncover the demographic consequences of this pattern. A key challenge would thus be to investigate how the age-related changes we highlighted in immunological and parasitological traits relate to both reproductive and actuarial senescence patterns.

Methods

Ethics. The protocol of capture and blood sampling of roe deer under the authority of the Office National de la Chasse et de la Faune Sauvage (ONCFS) was approved by the Director of Food, Agriculture and Forest (Prefectoral order 2009–14 from Paris). The land manager of both sites, the Office National des Forêts (ONF), permitted the study of the populations (Partnership Convention ONCFS-ONF dated 2005-12-23). All experiments were performed in accordance with guidelines and regulations of the Ethical Committee of Lyon 1 University (project DR2014-09, June 5, 2014).

Study population. We focused on two populations of roe deer in the wild, 'Trois-Fontaines' and 'Chizé'. Both sites are enclosed forests. Trois-Fontaines (1,360 ha), located in north-eastern France (48°43′N, 4°55′E), has a continental climate characterized by cold winters and warm rainy summers. This site has rich soils and offers habitat of high quality to roe deer. In contrast, in Chizé (2,614 ha) located in western France (46°50′N, 0°25′W), the climate is temperate oceanic with Mediterranean influences. This site presents low productivity due to poor quality soils and frequent summer droughts[57] and thus offers a relatively poor habitat to roe deer.

Roe deer from these two populations have been monitored using a long-term Capture-Mark-Recapture program since 1975 and 1977 for Trois-Fontaines and Chizé, respectively. Every year and for each site, 10-12 days of capture are organized between December and March (see[36] for details about the capture sessions). Once an individual is captured, its sex and body mass (to the nearest 50 g) are recorded and a basic clinical examination is performed. In this study, we only used data from known-age individuals (*i.e.*, caught during their year of birth, identified using tooth eruption patterns[58]). Since 2010, we collected blood samples from the jugular vein (up to 20 mL for a 20 kg roe deer). Whole blood was EDTA-preserved for cell count and serum was extracted for other measures. We also collected fecal samples. After sampling, roe deer were released at the location of capture within a couple of hours. Samples were received at the laboratory within 48 hours after sampling and analysed within 4 hours after reception.

Characterization of immune phenotype. We measured a set of 12 immune parameters in order to depict both the innate and the adaptive responses. These two responses are represented by both humoral and cell-mediated components[59].

First we assessed innate cellular immunity by counting total white blood cells (WBC, in 10^3 cells/mL), which is considered as a proxy of the allocation to immunity, using a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France). We also determined the composition of the WBC population (five different cell types), based on the identification of the first hundred WBC in Wright-Giemsa-stained blood smears[60]. Among these, neutrophils and monocytes are phagocytes involved in the innate response. Basophils, which are quite rare, play a key role against macroparasites such as ticks[61] while eosinophils are associated with defence against internal parasites and inflammatory response. Total white blood cells (WBC) and neutrophil count were highly correlated ($r = 0.91$, see pairwise correlations displayed in Table S3), because neutrophils represent the majority of white blood cells (between 60 and 80% of the total WBC). However, we reported estimates of the relationship between WBC and age in supporting information (Table S4) to allow future potential comparisons or meta-analyses across species because WBC is a commonly used marked in immunosenescence studies. Finally, lymphocytes represented the adaptive cellular part of immunity (see below).

Innate humoral immunity was assessed by measuring the circulating levels of natural antibodies (NAbs) and the complement-mediated cell lysis activity following the hemagglutination-hemolysis (HAHL) assay[62] previously performed on roe deer[50]. In this assay, the HA score (titer) measures the ability of NAbs to agglutinate exogenous cells and provides a proxy of the NAbs concentration, and HL score measures the ability of the complement system to cause hemolysis.

Innate humoral immunity also includes numerous proteins involved in acute and chronic inflammatory processes. We thus measured alpha1-globulins, alpha2-globulins and betaglobulins; globulins fractions including several acute phase proteins of the inflammatory response[63]. Total protein content (in g/L) was first assessed by refractometry followed by automatic agarose gel electrophoresis (HYDRASYS, Sebia, Evry, France) that separates albumin and the 4 fractions of globulins ($\alpha 1$, $\alpha 2$, β, and γ). We also measured the specific level of haptoglobin (in mg/mL), a protein that belongs to alpha2-globulin fraction synthesized in case of chronic infection or inflammation. Haptoglobin analyses were performed on a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France) using phase Haptoglobin assay (Tridelta Development LTD, County Kildare, Ireland) chromogenic kit.

The humoral component of the adaptive immunity response was assessed by measuring the concentration of gamma-globulins (see above for details about the electrophoresis protocol), or immunoglobulins, which represent the majority of circulating antibodies. The cellular component of adaptive immunity was assessed by lymphocyte counts including both T and B cells, B cells being particularly involved in the production of antibodies.

Measures of parasitic load. We investigated fecal propagule counts of parasites frequently occurring in roe deer[41]: nematodes parasite from the lung (protostrongylids), from the digestive tract (gastro-intestinal strongyles, *Trichuris* sp.), and coccidia (*Eimeria* sp., Protozoa). The McMaster protocol[64] was used for the count of gastro-intestinal nematode eggs/g (EPG) and coccidian oocysts/g (OPG); and the Baermann fecal technique[65] for the count of first stage larvae of pulmonary nematode (protostrongylids, in larvae/g, LPG). We previously provided evidence that egg counts in faeces allow a reliable estimate of the number of parasites in roe deer from the studied populations during the capture period[41].

Statistical analysis. In all the analyses, we only included known-aged male and female roe deer from 2 years of age onwards, which corresponds to the minimum age of reproduction in roe deer[66,67]. We pooled individuals aged 12 years and older in a single age class '12+', as supported by survival analyses[68]. This class included 3 males (aged 12, 12 and 13) and 10 females (8 aged 12–13; 2 aged 15 and 16) at Trois-Fontaines. At Chizé, 1 male (14 years old) and 6 females (aged 12–13) were the oldest.

To assess age-specific changes in immunological parameters and in parasitic load, analyses were performed using linear mixed-effect models (LMMs). Individual identity was included as a random effect, to avoid pseudo-replication issues[69] and to account for confounding effects of individual heterogeneity when assessing age-specific changes[70]. The cohort was also included as a random effect to take into account the marked differences roe deer faced during early life in response to high variation in environmental conditions among years[71], which could influence senescence rates[72]. Although, there is no clear evidence of any density response in a life history trait during the study period[49], we controlled for between-year variation in population density that could potentially influence immune or parasitic traits markers. We thus included the year of capture as a random effect. Each immune or parasitic trait was analysed as a response variable. Age, sex and population were entered as explanatory variables, with all two and three-way interactions between them. Four types of age functions were tested separately and compared: full age dependence (11 separated age classes from 2 to 12+), linear, quadratic, or threshold. For the "threshold model" model, the threshold was determined by maximum likelihood estimation over a grid of values between 3 and 11 years of age (see[75] and Fig. S1 for the deviance profile). The full list of fitted models is provided in supporting information (Table S5 for immune traits, Table S6 for parasitic traits). To select the best model of age-specific variation in each immune or parasitic variable, we used a model selection procedure based on the Akaike Information Criterion (AIC[73]). For each trait, we retained the model with the lowest AIC, and when the difference of AICs between competing models was less than 2, we retained the simplest model to satisfy parsimony rules[74]. In addition, we calculated the AIC weights (w_i) to measure the relative likelihood of each model to be the best among the set of fitted models. The normality of the residuals for the selected model was tested (Shapiro–Wilk normality test) and visually assessed with histograms. Goodness-of-fit was assessed through calculating conditional (total variance explained by the best supported model) and marginal (variance explained by fixed effects alone) R^2 formulations (Table 1, Table 2) and standard residual plot techniques[76]. From the selected model, we then estimated the value of the explained variable ($\pm$SE) at two years of age (Table 1, Table 2). Model selection and results of similar analyses performed separately per sex and population (along with additional fixed effect of body mass) are provided in supporting information (Table S7).

When analysing the parasite load, we considered for each parasite its presence (individual presence/absence, analysed with a GLMM with a binomial error), intensity (mean faecal egg or oocyst counts in infested hosts) and abundance (mean faecal egg or oocyst counts per host). Some models could not be fitted to intensity or presence data because of too little data that lead to optimization problems over which classical numerical methods failed to converge. As abundance encompasses both presence and intensity, we only present results on abundance.

To assess the relationships between the 12 immune and the 4 parasite traits, analyses were performed using linear mixed-effect models (LMMs). Each immune trait was analysed as a function of parasite load, population and the interaction parasite load*population, considering 4 different parasite groups. Models included individual identity as a random effect. This descriptive analysis is provided in Supplementary Information (Table S8).

Blood sampling started in 2010 in both study sites and the age at death was known for 37% of the individuals (120 out of 325) included in our analyses. It was thus not possible to fully account for the possible selective disappearance of individuals with poor immune performance or high pathogens prevalence, by including longevity as a covariate[70]. However, to control for such effect, we replicated our analyses on a subset of individuals ($n = 120$) by including as a fixed factor whether or not individuals reached 8 years of age. Models including this longevity metric were never retained (see Table S9 for a description of these models), suggesting that selective disappearance did not influence the outcome of our analyses.

All analyses were carried out in R version 3.2.3[77] and using the function lmer from package lme4[78].

Data accessibility. All data will be deposited in Dryad.

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Author Contributions

E.G.F., J.F.L., J.M.G. conceived the study and designed methodology; all authors collected the data on both study sites; E.G.F., L.C., M.J., B.R., performed the immunological measures; G.B. and H.F. performed the parasitic measures; L.C., J.F.L., E.G.F., J.M.G., B.R., L.J. analysed the data; L.C. wrote the first draft of the paper and all authors contributed critically to the drafts and gave approval for the final version.

Additional Information

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CHAPTER 5

Associations between immune profile and telomere length

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Physiology

Age-dependent associations between telomere length and environmental conditions in roe deer

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Telomere length (TL) represents a promising biomarker of overall physiological state and of past environmental experiences, which could help us understand the drivers of life-history variation in natural populations. A growing number of studies in birds suggest that environmental stress or poor environmental conditions are associated with shortened TL, but studies of such relationships in wild mammals are lacking. Here, we compare leucocyte TL from cross-sectional samples collected from two French populations of roe deer which experience different environmental conditions. We found that, as predicted, TL was shorter in the population experiencing poor environmental conditions but that this difference was only significant in older individuals and was independent of sex and body mass. Unexpectedly, the difference was underpinned by a significant increase in TL with age in the population experiencing good environmental conditions, while there was no detectable relationship with age in poor conditions. These results demonstrate both the environmental sensitivity and complexity of telomere dynamics in natural mammal populations, and highlight the importance of longitudinal data to disentangle the within- and among-individual processes that generate them.

1. Introduction

Understanding how environmental variation shapes organismal physiology and life history in wild systems is fundamental to evolutionary ecology, but identifying physiological biomarkers relevant to life history and fitness is challenging. Recently, telomere length (TL) has emerged as a potential biomarker of an individual's physiological state and past environmental experiences [1]. Telomeres are repetitive DNA segments that maintain genomic integrity by capping the ends of eukaryotic chromosomes and forming complexes with proteins [2]. Telomeres shorten with each cell division and are sensitive to oxidative damage, and critically short telomeres trigger cellular senescence *in vitro* [3]. In humans, average TL decreases with age and short TL in adulthood predicts late-onset disease and mortality [3], while past experience of stressful events is associated with shortened adult TL [4]. In birds, short TL predicts increased mortality risk [5], and experimentally induced competition for food or physiological stress accelerates telomere attrition during early life [6,7]. Accordingly, there is growing interest in how natural variation in environmental conditions

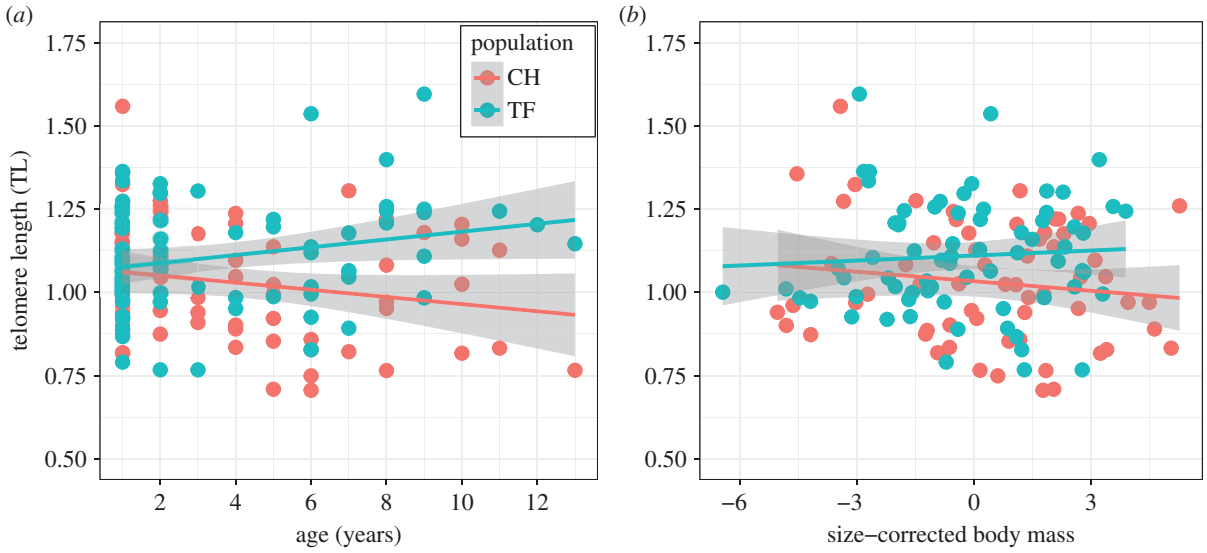

Figure 1. Changes in relative leucocyte telomere length (TL) with age (*a*) and size-corrected body mass (*b*) in two different populations of roe deer. Raw data for CH (red) and TF (blue) are presented with a linear regression (red and blue lines, respectively) and associated standard errors (grey shading). (Online version in colour.)

influences telomere dynamics, and recent studies in wild birds and fishes suggest that populations experiencing physiologically challenging environments, particularly in early life, have shorter TL [8–12]. Although associations between TL, age, sex and survival have recently been reported in wild mammals [13–15], the relationship between environmental conditions and mammalian telomere dynamics is currently unknown.

Here, we test how broad differences in environmental conditions influence telomere dynamics in a wild mammal by comparing patterns of TL variation between two populations of European roe deer (*Capreolus capreolus*). These populations experience markedly different environmental conditions [16], with consequences for various life-history and demographic parameters [17], as well as body condition and immune phenotype [18]. Based on trends emerging from the recent literature on humans and birds, we hypothesized that persistent experience of a poor environment would result in shorter TL at any given age, owing to lower initial TL in early life and a faster rate of TL shortening over an animal's lifetime. Since body mass at a given age is thought to reflect overall physiological condition in this species [18], we also predicted a positive association between body mass and TL within populations.

2. Methods

Blood samples were collected from roe deer at two long-term study sites that differ markedly in environmental conditions (January–March 2016). Both deciduous woodland habitats, the Trois-Fontaines site (TF; 1360 ha) in northeastern France (48°43′ N, 4°55′ E) has more fertile soils, a continental climate and higher forest productivity than Chizé (CH; 2614 ha) in western France (46°50′ N, 0°25′ W). Poor-quality soils and summer droughts at Chizé result in low forest productivity [16], and deer living there consequently have reduced growth rates, adult size and fecundity, and markers of physiological condition [17,18]. Body mass was taken at capture, and all individuals were of known age and sex (TF: 34 females, 39 males; CH: 36 females, 30 males). Buffy coat fractions, comprising mainly leucocytes, were prepared in the field and immediately frozen at −80°C until DNA extraction. Relative TL was measured by quantitative PCR as described previously [19] and in the electronic supplementary material.

We ran linear models in R v.3.3.3. We tested our first hypothesis by running a model of TL including sex and population as two-level factors, age as a linear covariate and all possible two-way interactions among these terms. A backward elimination approach was used to remove non-significant terms from the maximal model. We used a similar approach to confirm previously established differences in body mass between the two populations, while accounting for the effects of sex and age (linear and quadratic terms). Finally, we tested whether a measure of body condition explained variation in TL, independent of its associations with age, sex and population, by adding size-corrected body mass (residuals from a regression of body mass on hind foot length, see electronic supplementary materials) as a covariate to the minimal model for TL, and applying the same backward simplification.

3. Results

There was an interaction between the effects of age and population on TL ($F_{1,135} = 6.294$, $p = 0.013$; electronic supplementary material, table S1): shorter telomere lengths were observed in the poor environment of CH but only among older individuals (figure 1*a*). This was underpinned by a marginally non-significant increase in TL with age in TF ($F_{1,71} = 3.849$, $b = 0.012 \pm 0.006$ s.e., $p = 0.054$) and non-significant decline with age in CH ($F_{1,64} = 2.562$, $b = -0.011 \pm 0.007$ s.e., $p = 0.114$). There was no evidence for sex differences in TL, or interactions between sex and age or population (electronic supplementary material, table S1). Individuals from TF were heavier regardless of age or sex (electronic supplementary material, figure S1 and table S2), as has been previously documented [17]. There was no evidence for a relationship between size-corrected body mass and TL (figure 1*b*; electronic supplementary material, table S3), and including size-corrected mass in the TL model did not alter the magnitude of the age-by-population interaction (electronic supplementary material, table S3).

4. Discussion

In this study, we provide, to our knowledge, the first evidence for contrasting telomere dynamics in wild mammal populations experiencing different environmental conditions. As predicted, we found shorter TL in the population experiencing

a poorer environment, but this difference was only apparent at older ages and was underpinned by a cross-sectional increase in TL with age in the population experiencing good environmental conditions. Our study adds to an emerging literature on wild birds and fishes documenting associations between TL and environmental conditions, although most studies have focused on early life. For instance, experimental brood enlargements, expected to increase competition for parental provisioning and physiological stress in developing birds, are associated with shortened TL [7], and being raised in urban or higher altitude populations reduced TL in nestlings [8,10]. Likewise, in salmon, young-of-the-year from higher average temperature rivers (i.e. higher thermal stress) had shorter TL [9]. By contrast, we found no evidence of a difference in TL among roe deer populations in the youngest age groups, despite fawns in different locations experiencing marked differences in climatic conditions and food availability *in utero* and during early life [17]. Previous studies in birds have detected shorter TL in adults experiencing more challenging environments [11,12]. However, our data encompass the full natural age range in both populations and imply that the environmental effect on TL is a cumulative one that is apparent only later in adulthood at the population level.

We predicted, assuming that increased environmental stress drives more rapid telomere attrition, that declines in TL with age should be greater in CH than TF. However, TL actually increased with age in TF and tended to decline in CH. There is growing appreciation that within-individual lengthening of TL can and does occur [20], although the process remains poorly understood. Cross-sectional changes in telomere length, however, are not necessarily driven by within-individual changes, and the selective disappearance of individuals with short telomeres has been observed to increase average TL with age in wild mammals [13]. It is possible that both our study populations are experiencing selective disappearance, but that poor environment at CH may drive more rapid TL shortening compared with TF, making the increase in TL in older individuals not detectable in this population. Overall, our results highlight the potential complexity of telomere dynamics in natural systems, and the importance of long-term longitudinal studies to disentangle the contributions of within- and among-individual processes to these dynamics.

We found no evidence that size-corrected body mass was associated with TL in either study population (figure 1*b*), despite marked differences in average body mass across all ages between populations (electronic supplementary material, figure S1). We predicted a positive relationship between TL and body mass within populations, but note that previous studies in birds and mammals have reported conflicting associations between TL and either early-life growth rates or body mass [14,15]. A previous study comparing the same two roe deer populations found that while CH had lower levels of metabolic markers (e.g. haemoglobin and albumin levels) associated with body condition than TF, immunological markers were not consistently lower at CH [18]. In contrast to other vertebrates, mammals have enucleated red blood cells and TL measurements from blood only include leucocytes, which means immune status could have a much greater influence on telomere dynamics. Although a recent study of Soay sheep found little evidence that leucocyte TL and leucocyte cell composition were associated [14], the role of infection history and immune phenotype in the population differences in TL reported here remains to be determined.

We have presented important evidence for sex and body mass independent differences in TL among populations experiencing contrasting environments. The crucial next step for the application and understanding of TL as a biomarker in wildlife ecology will be to understand the particular aspects of environmental conditions and physiological status that TL responds to and how these in turn relate to life history and fitness.

Ethics. The land manager of both sites, the Office National des Forêts (ONF), permitted the study of the populations (Partnership Convention ONCFS-ONF dated 2005-12-23). All procedures were approved by the Ethical Committee of Lyon 1 University (project DR2014-09, 5 June 2014).

Data accessibility. Data available as electronic supplementary material.

Authors' contributions. D.H.N. and J.F.L. conceived and designed the study. M.P., J.M.G., E.G.F., J.F.L., B.R. and L.C. performed fieldwork. C.R., B.R. and L.C. extracted DNA, R.V.W. and M.C.M. ran telomere assays, and H.F., M.C.M., R.V.W. and D.H.N. analysed the data. R.V.W., D.H.N. and H.F. wrote the manuscript with input from all other co-authors. All authors gave final approval for the manuscript, and agree to be held accountable for its publication.

Competing interests. The authors declare no competing interests.

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Telomere length vary with level of inflammatory markers, but not with proportions of different leukocyte cell types, in two populations of a wild mammal.

- PRELIMINARY ANALYSES AND RESULTS –

Part 1: Relationship between telomere length and leukocyte cell types proportion.

Context

Telomeres are repeated sequences of non-coding DNA, which have a crucial role in the protection of the ends of chromosomes, subjected to progressive loss during cell replication process. In the absence of restoration and repair processes, telomere length declines with each cell division. However, telomere length may be maintained or restored by telomerase, a reverse transcriptase present in all vertebrates (Chan and Blackburn, 2004). In a large range of species including humans, telomere length have been associated with age of individual, at least in some tissues (Monaghan and Haussmann, 2006). But strong variations exists in the relationship between telomere length and age among species, populations or individuals and these variations remains poorly understood.

In mammalian studies, telomere length is usually measured in DNA of leukocytes, extracted from blood samples. As red blood cells do not contain DNA in mammals, the immune cells (*i.e.* the leukocytes) are the only nucleated blood cells that allow to measure telomere length. In other vertebrate groups (*e.g.* birds), telomere length can be measured in their nucleated erythrocytes. It represents a certain limitation in the study of mammalian telomere dynamics, because it also have been described that leukocyte telomere length differ between the different leukocyte cell types. For instance in adults, granulocytes (*i.e.* neutrophils, basophils and eosinophils) have longer telomere length than lymphocytes (Aubert *et al.*, 2012). Different cell types display strong differences in their proliferative capacity and in their telomerase expression, which results in these differences in telomere length (Weng, 2001). In mammalian species, the measure of leukocyte telomere length is performed on a pool of

leukocytes that differ in their telomere length. It implies that variations in average leukocyte telomere length, observed wih age and/or with sex, could also reflect changes in underlying cell population structure (Weng, 2001). Many raisons can lead to changes in proportion of leukocytes in the blood. During an inflammatory response or in situation of stress, the composition of circulating leukocytes vary in organisms and influence global telomere length measure. An immune response can lead to an increase in the ratio of granulocytes to lymphocyte, leading to an increase of average leukocyte telomere length. Stressful conditions often up-regulate telomerase expression in leukocytes, and thus influences average telomere length. Finally, the age-related changes in immune cell proportions described in many mammals including humans could exert a strong influence on the measure of leukocyte telomere length. This aspect is often neglected in the analyses of telomere dynamics. The study of the association of leukocyte telomere length with age and sex - taking into account to the variation in leukocyte population structure - are very rare in non-primate mammals. One study on the Soay sheep however found that variation in leukocyte telomere length was independent of variation in the proportions of different leukocyte cell types (Watson *et al*., 2017).

In previous studies presented in this manuscript, we have found an effect of age and environmental conditions, but not of the sex, on roe deer leukocyte telomere length (Chapter 5; Wilbourn *et al.,* 2017). We also have described that immune cells proportion vary with age in roe deer (Chapter 4; Cheynel *et al*., 2017). In this complementary study, we thus aimed at assessing whether leukocyte telomere length could vary in association with proportions of the different leukocyte cell types, which are known to differ in telomere length. We tested the effect of neutrophil, lymphocyte, eosinophil, basophil and monocyte proportions on relative leukocyte telomere length, in adult roe deer of two populations living in contrasted conditions.

Methods

Study population

Roe deer data were collected in two populations living in enclosed forests, at ‘Trois-Fontaines’ located in north-eastern France (1,360 ha, 48°43’N, 4°55’E) and at ‘Chizé’ located in western France (2,614 ha, 46°50’N, 0°25’W). Trois-Fontaines forest offers habitat of high quality to roe deer, due to rich soils and a continental climate characterized by cold winters and warm rainy summers. In contrast, the Chizé forest offers a relatively poor habitat to roe deer, because of the

low productivity of the soils and a temperate oceanic climate with Mediterranean influences characterized by frequent summer droughts (Pettorelli et al., 2006). The contrasted environmental conditions experienced by roe deer in the studied populations lead to marked differences in adult body mass (Gaillard *et al*., 2013), offspring survival (Gaillard *et al*., 1997), and immune profile (Cheynel *et al.,* 2017). Roe deer were monitored using a long-term Capture-Mark-Recapture program. In winter, between December and March, roe deer captures were organized each year (see Gaillard *et al.,* 1993 for details about the capture sessions). During captures, sex and body mass (to the nearest 50g) are recorded and a basic clinical examination is performed. We also collected blood samples from the jugular vein (up to 20 mL for a 20 kg roe deer). Whole blood sampled at captures was EDTA-preserved for cell count and serum was extracted for other immunological measures. Samples were received at the laboratory within 48 hours after sampling and analysed within 4 hours after reception.

Determination of relative leukocyte telomere length

Within 30 min of blood sampling, whole blood was spun at approximately 3000 rpm for 10 min and the plasma layer drawn off and replaced by the same quantity of 0.9% w/v NaCl solution and spun again. The intermediate buffy coat layer, comprising mainly leukocytes (white blood cells) was collected into a 1.5-mL Eppendorf tube and stored at −80 °C until further use. Relative telomere length was measured by quantitative PCR as described in Wilbourn *et al*. (2017).

Determination of different leukocyte cells types

We manually estimated the proportion of the various leukocytes forms (i.e. neutrophil, lymphocyte, monocyte, eosinophil and basophil count) by counting the 100 first cells on blood smears with a microscope under 200x magnification (in %). Blood smears are stained with May-Grünwald's (#T863.2, Carl Roth GmbH) and Giemsa (#T862.1, Carl Roth GmbH) solution, a method that has been used before in studies on mammalian immunology (Durbin *et al.*, 2009). A complete blood count was also performed using an ABC Vet automaton (Horiba Medical, Montpellier, France). It measured the total leukocyte count (10^3 cells/mL) by impedance technology, considering parameters for bovine samples, since the size of blood cells is comparable between the two species (Ursache *et al*., 1980). With both the proportion of each leukocyte forms (in %) and the total leukocyte count (10^3 cells/mL), we obtained the count of

each leukocyte (10^3 cells/mL). Neutrophil to lymphocyte ratio (NL ratio) was also found with a mathematical calculation of the ratio of neutrophils with lymphocytes.

Statistical analyses

In these analyses, we included roe deer sampled in 2016 and 2017, with both a measure of leukocyte telomere length and a measure of the different leukocyte cell type proportion and from 2 years of age onwards (*i.e.* in sexually mature individuals). It represents 162 samples, equally distributed between populations and sexes (74 individuals at Trois-Fontaines *vs.* 88 at Chizé; 91 females *vs.* 71 males). We calculated correlations between RTL and the proportion of different leukocyte cell types with Spearman rank correlation. Spearman rank correlation is a non-parametric test used to measure the degree of association between two variables, which does not carry any assumptions about the distribution of the data. To test whether RTL varied with proportion of the different leukocyte cell types, analyses were performed using linear mixed-effect models (LMMs). RTL was included as the response variable. The five different leukocytes cells types (i.e. neutrophil, lymphocyte, monocyte, basophil and eosinophil) were entered as explanatory variables. Age (years) and population, with their interaction (age x population), sex and body mass (kg) of the individuals were also entered as explanatory variables. Finally, we included individual identity as random effects (23 roe deer sampled both in 2016 and 2017), cohort of birth (between 2003 and 2015), qPCR plate and date of run (seven plate, three days of measures) as random effects. We fitted the full model (*i.e.* RTL ~ neutrophil + lymphocyte + monocyte + basophil + eosinophil + age x population + body mass + sex) and all submodels (see Table S1 for the list of the most competitive models). To select the best model explaining RTL variation, we used a model selection procedure based on the Akaike Information Criterion (AIC, Burnham and Anderson, 2002). For each trait, we retained the model with the lowest AIC, and when the difference of AICs between competing models was less than 2, we retained the simplest model to satisfy parsimony rules (Ulm, 1989). Finally, the goodness-of-fit of the selected models was assessed through calculating conditional (total variance explained by the best supported model) and marginal (variance explained by fixed effects alone) R2 formulations (Nakagawa and Schielzeth, 2013). All analyses were carried out in R version 3.2.3 (R Core Development Team, 2015) and using the function lmer from package lme4 (Bates *et al.,* 2015).

Results

RTL showed differences between the two populations of our study: roe deer at Chizé displayed shorter RTL than those at Trois-Fontaines (see best model selected in Table 1). RTL was independent of age, sex, body mass of individuals and was not associated with variation in the levels of different leukocyte cell types measured in the samples (Table 1; Fig. 1).

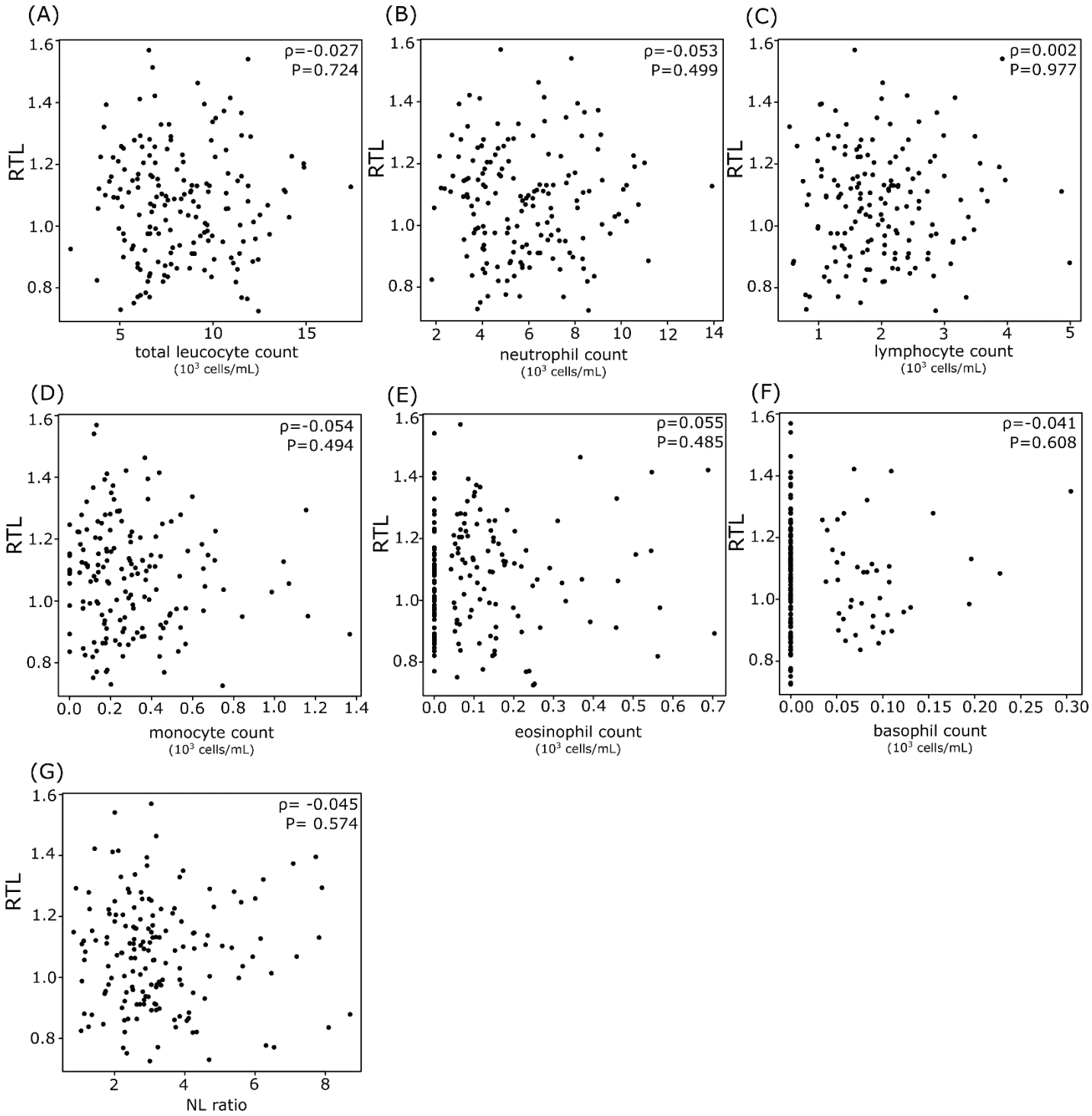
Spearman's correlation coefficients between RTL and the count of leukocyte cell types ranged between +0.1 and -0.1 (see correlation with total white blood cells in Fig. 1(A), neutrophil in Fig. 1(B), lymphocyte in Fig. 1(C), monocyte in Fig. 1(D), eosinophil in Fig. 1(E), basophil in Fig. 1(F) or NL ratio in Fig. 1(G)).

Table 1. Parameter estimates of the selected linear mixed-effect models assessing the association between leukocyte relative telomere length (RTL) and the different leukocyte cell types. The potential fixed effects tested were population (Chizé *vs*. Trois-Fontaines), age (years) and their interaction (age x population), body mass (kg), sex (males *vs*. females) and the count of each different leukocyte cell types (*i.e*. neutrophil, lymphocyte, monocyte, eosinophil and basophil). All models included individual identity, years of capture, years of birth, and assay plate as random effects. Estimates are presented ± standard error (SE). Statistical significance is represented by *for $p < 0.05$, **for $p < 0.01$ and ***for $p < 0.001$. R^2m and R^2c are the marginal and conditional variance of the model, respectively.

Relationship with different leukocyte cell types

	Selected model	Estimate ± SE	t-value	p	R^2m	R^2c
RTL	Intercept	1.122 ± 0.027	41.474	***	0.040	0.077
	Population (Chizé)	-0.069 ± 0.027	-2.618	**		

Figure 1. Scatter plots illustrating correlations among relative leukocyte telomere length (RTL) and measures of different leukocyte cell types. Scatter plots show relationships between RTL and: (A) total leukocyte count, (B) neutrophil count, (C) lymphocyte count, (D) monocyte count, (E) eosinophil count, (F) basophil count and (G) neutrophil to lymphocyte (NL) ratio. Spearman's correlation coefficients and associated P values presented for each plot.



Discussion

In this analysis, we investigated whether relative telomere length in adult roe deer is associated with variation in the different leukocyte cell types. We found that variation in leukocyte telomere length was independent of variation in the proportions of different leukocyte cell types. This result is in accordance with a study in another mammal, the Soay sheep (Watson *et al*., 2017).

The absence of association between lymphocyte, which levels vary with age (Cheynel *et al*., 2017) and telomere length can be due to the fact that we did not discriminate levels of different populations of lymphocytes in lymphocyte populations. Different types of lymphocytes differ in their telomere length, with naïve T cells having longer telomere lengths in comparison with memory T cells (Weng, 2001; Aubert *et al*., 2012). A global measure of lymphocyte level could masked variations of the different lymphocytes populations and therefore masked associations with telomere length.

Surprisingly and contrary to previous results, relative telomere length do no either vary with the age of individuals. In Wilbourn *et al*. (2017), we found a significant increase in telomere length with age in Trois-Fontaines populations, which lead to a significant difference in telomere length between roe deer of the two populations at older ages (*i.e*. shorter telomere length at Chizé). This results was not confirmed here, and the effect of the interaction "age x population" was never retained in the list of best fitting models (Table S1). However, it is to note some differences in the analyses. In this analysis, we do not included juvenile roe deer (*i.e*., 1 year old) because of their immune system is likely not fully developped, and we have access to two years of data on telomere length. Studying two years of data on telomere length is still a cross-sectional study, Here again, to fully understand the telomere dynamics in the wild, longitudinal data would be needed, to

In accordance with previous results in roe deer, we did not found differences in telomere length between males and females. In wild mammals, very few studies have tested sex-differences in telomere length and they found contradictory results. A study on European badgers did not found any differences between male and female mean telomere length (Beirne *et al*., 2016); whereas a study on Soay sheep provide evidence for sex-differences (*i.e.* longer leukocyte telomere length in females at adulthood, Watson *et al*., 2017). Here again, it highlights the strong need of more mammalian studies on telomere dynamics.

Finally, this analysis also confirmed the influence exert by environmental conditions on telomere length in roe deer (Wilbourn *et al*., 2017). At adulthood, roe deer experiencing harsh environmental conditions at Chizé display shorter telomere compared to individuals at Trois-Fontaines that are living in good conditions.

Part 2: Relationship between telomere length, levels of inflammatory markers and oxidative damages

Context

With increasing age, individuals are subject to senescence, *i.e.* a progressive and irreversible decline in physiological functions. Several mechanisms have been suggested to contribute to this process, and two mains candidates are telomere shortening and the installation of a chronic low-grade inflammatory state with increasing age. Telomere shortening with age is a part of the "normal' process of ageing, but while age-dependent telomere loss have been often described, a large proportion of the variation in the relationship between telomere length and age remains unexplained (Monaghan and Haussmann, 2006). Telomere shortening show indeed a higher rate of attrition than expected as a simple consequence of the end replication problem (Takai *et al*., 2003; Landsdorp, 2005). Inflammation, oxidative stress and others stressors such as pathogenic infections seems to increase the rate of attrition of telomeres and could be the cause of accelerated ageing processes (Aubert and Lansdorp, 2008; Zhang *et al*., 2016). The G-rich telomeric sequences are indeed very vulnerable to oxidative damages (Von Zglinicki, 2002). Oxidative stress can also accelerated telomere shortening by interfering with telomere repair by the telomerase enzyme. Short telomeres have been then associated with premature aging syndromes and with common age-related diseases, such as cancer and cardiovascular disease (Zhang *et al*., 2016).

While negative associations between inflammation and telomere length have already been documented in humans (Zhang *et al*., 2016), the relationship between telomere length and inflammation has rarely been studied in wild species. Some studies have rather focused on the relationship between telomere length and infectious status. A study in a wild-derived house mice (*Mus musculus musculus*) have for instance proved that telomere attrition can be

accelerated during an infectious state, an attrition supposed to be caused by inflammatory responses by the authors (Ilmonen *et al*., 2008). Here, we assessed whether the level of inflammatory markers is associated with telomere length, in two populations of a wild mammal, the roe deer. We also performed a first measure of a marker of oxidative damages (malondialdehyde, a by-product of lipid peroxidation) in the same individuals, and we tested whether the levels of oxidative damages could also be associated with telomere length.

Methods

Study population

Roe deer data were collected in two populations living in enclosed forests, at 'Trois-Fontaines' located in north-eastern France (1,360 ha, 48°43'N, 4°55'E) and at 'Chizé' located in western France (2,614 ha, 46°50'N, 0°25'W). Trois-Fontaines forest offers habitat of high quality to roe deer, due to rich soils and a continental climate characterized by cold winters and warm rainy summers. In contrast, the Chizé forest offers a relatively poor habitat to roe deer, because of the low productivity of the soils and a temperate oceanic climate with Mediterranean influences characterized by frequent summer droughts (Pettorelli *et al*., 2006). The contrasted environmental conditions experienced by roe deer in the studied populations lead to marked differences in adult body mass (Gaillard *et al.,* 2013), offspring survival (Gaillard *et al.,* 1997), and immune profile (Cheynel *et al.,* 2017). Roe deer were monitored using a long-term Capture-Mark-Recapture program. In winter, between December and March, roe deer captures were organized each year (see Gaillard *et al*., 1993 for details about the capture sessions). During captures, sex and body mass (to the nearest 50g) are recorded and a basic clinical examination is performed. We also collected blood samples from the jugular vein (up to 20 mL for a 20 kg roe deer). Whole blood sampled at captures was EDTA-preserved for cell count and serum was extracted for other immunological measures. Samples were received at the laboratory within 48 hours after sampling and analysed within 4 hours after reception.

Determination of relative leukocyte telomere length

Within 30 min of blood sampling, whole blood was spun at approximately 3000 rpm for 10 min and the plasma layer drawn off and replaced by the same quantity of 0.9% w/v NaCl solution and spun again. The intermediate buffy coat layer, comprising mainly leukocytes (white blood cells) was collected into a 1.5-mL Eppendorf tube and stored at −80 °C until further use.

Relative telomere length was measured by quantitative PCR as described in Wilbourn *et al*. (2017).

Determination of inflammatory markers

We measured alpha1-, alpha2- and beta-globulins (mg/mL) that are globulin fractions including several acute phase proteins (APPs), a group of proteins which concentration changes following external or internal challenges such as trauma, inflammation or infection (Cray *et al*., 2009). We also measured the specific level of haptoglobin (in mg/mL) which belongs to alpha2-globulin fraction. Total protein content (in mg/mL) was first assessed by refractometry followed by automatic agarose gel electrophoresis (HYDRASYS, Sebia, Evry, France) that separates albumin and the 4 fractions of globulins (α1, α2, β, and γ). Haptoglobin analyses were performed on a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France) using phase Haptoglobin assay (Tridelta Development LTD, County Kildare, Ireland) chromogenic kit.

Determination of oxidative damages

In order to evaluate the oxidative status of individuals in this study, we performed a measure of oxidative damage. We measured plasma malondialdehyde levels (µmol/mL), a by-product of lipid peroxidation (Halliwell and Gutteridge, 2007), as a proxy of oxidative damage (Mateos *et al*., 2005; Halliwell and Gutteridge, 2007; Sepp *et al*., 2012; Lopez-Arrabé *et al*., 2018). We used the Cayman TBARS assay kit (Cayman, MI, USA): malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) under high temperature (90-100°C) and acidic conditions to form the TBARS. The TBARS was then measured colourimetrically at 530-540 nm. All samples are measured in duplicate and we used in analyses mean MDA levels. All samples were assayed in duplicated, MDA mean value of each sample is used below in the analyses.

Statistical analyses

In these analyses, we included roe deer sampled in 2016 and 2017, with both a measure of leukocyte telomere length and a measure of the level of inflammatory markers (*i.e.* alpha1-, alpha2-, beta-gloublins and haptoglobin) and of oxidative damages (*i.e*. malondialdehyde, MDA), from 2 years of age onwards (*i.e.* in sexually mature individuals). It represents 176 samples, equally distributed between populations and sexes (77 individuals at Trois-Fontaines vs. 99 at Chizé; 101 females vs. 75 males). We calculated correlations between RTL and the

measure of inflammatory markers or oxidative damages levels with Spearman rank correlation. Spearman rank correlation is a non-parametric test used to measure the degree of association between two variables, which does not carry any assumptions about the distribution of the data. To test whether RTL varied with the level of inflammatory markers and oxidative damages, analyses were performed using linear mixed-effect models (LMMs). RTL was included as the response variable. The levels of inflammatory markers (*i.e*. alpha1-, alpha2-, beta-gloublins and haptoglobin) and of oxidative damages (*i.e*. MDA) were entered as explanatory variables. Age (years) and population, with their interaction (age x population), sex and body mass (kg) of the individuals were also entered as explanatory variables. Finally, we included individual identity as random effects (29 roe deer sampled both in 2016 and 2017), cohort of birth (between 2003 and 2015), qPCR plate and date of run (seven plates, three days of measures), MDA assay plate (ten plates) as random effects. We fitted the full model (*i.e*. RTL ~ alpha1-globulin + alpha2-globulin + beta-globulin + haptoglobin + MDA + age x population + body mass + sex) and all submodels (see Table S2 for the list of the most competitive models). To select the best model explaining RTL variation, we used a model selection procedure based on the Akaike Information Criterion (AIC, Burnham and Anderson, 2002). For each trait, we retained the model with the lowest AIC, and when the difference of AICs between competing models was less than 2, we retained the simplest model to satisfy parsimony rules (Ulm, 1989). Finally, the goodness-of-fit of the selected models was assessed through calculating conditional (total variance explained by the best supported model) and marginal (variance explained by fixed effects alone) R2 formulations (Nakagawa and Schielzeth, 2013). All analyses were carried out in R version 3.2.3 (R Core Development Team, 2015) and using the function lmer from package lme4 (Bates *et al*., 2015).

Results

When testing the effect of inflammatory markers (alpha1-, alpha2-, beta-globulin and haptoglobin levels) and of oxidative damages (malondialdehyde) on RTL using LMMs, we found a negative effect of alpha1-globulin levels on RTL (effect of -0.056 ± 0.025, $p < 0.05$, Table 2).

Spearman's correlation also show negative correlations between RTL and alpha1-globulins levels (Fig. 2(A); $\rho = -0.152$, $p < 0.05$) and with beta-globulin levels (Fig. 2(B); $\rho = -0.216$, p

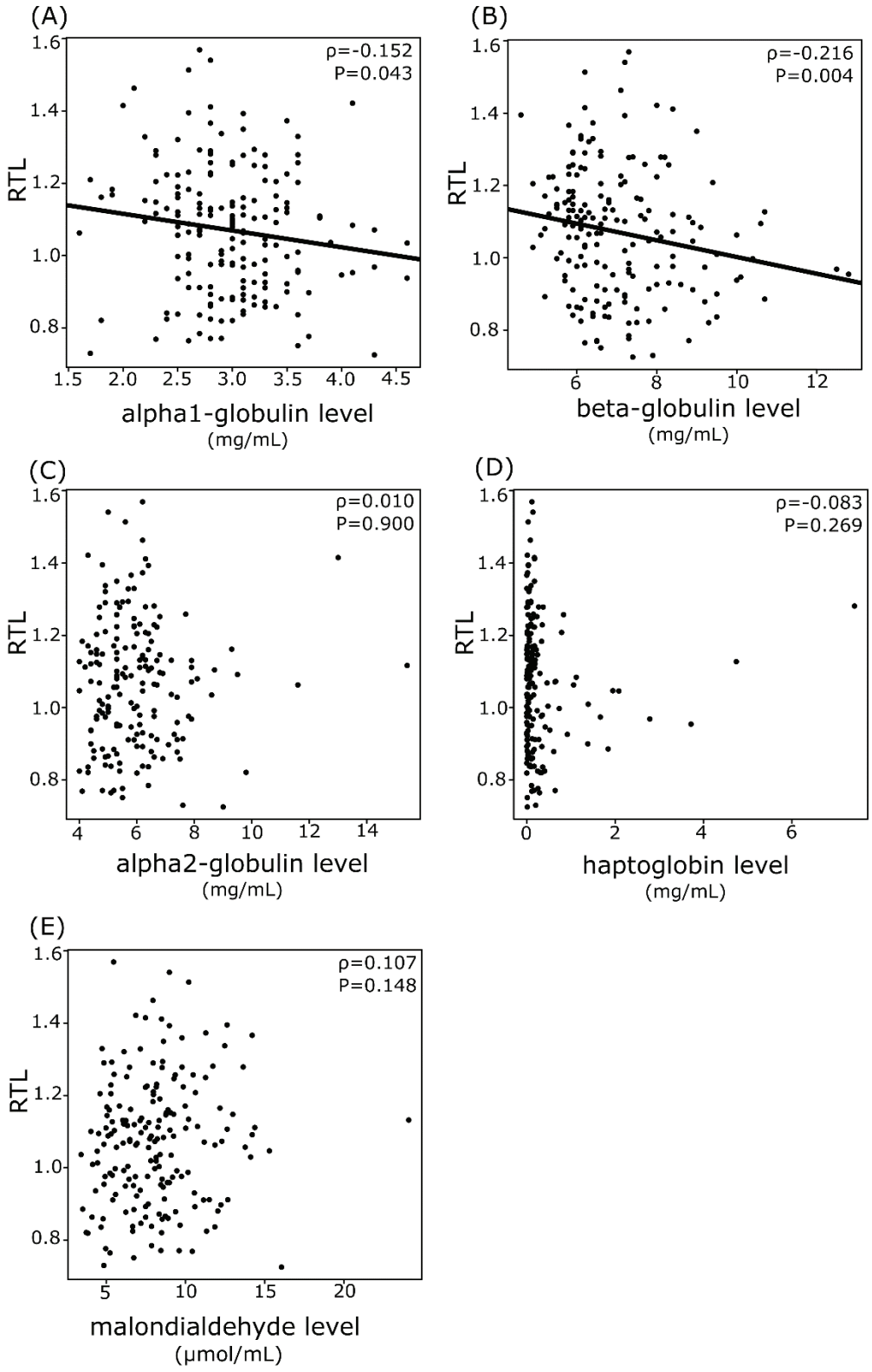
< 0.05). We found no correlation of RTL with alpha2-globulins levels (Fig. 2(C)), haptoglobin levels (Fig. 2(D)) and malondialdehyde levels (Fig. 2(E)).

Table 2. Parameter estimates of the selected linear mixed-effect models assessing the association between leukocyte relative telomere length (RTL) and the level of inflammatory makers (i.e. alpha1-, alpha2-, beta-globulin and haptoglobin). The potential fixed effects tested were population (Chizé *vs.* Trois-Fontaines), age (years) and their interaction (age x population), body mass (kg), sex (males *vs*. females), the levels of inflammatory markers (*i.e*. alpha1-, alpha2-, beta- and haptoglobin) and the levels oxidative damages (*i.e*. malondialdehyde). All models included individual identity, years of capture, years of birth, and assay plate as random effects. Estimates are presented ± standard error (SE). Statistical significance is represented by *for $p < 0.05$, **for $p < 0.01$ and ***for $p < 0.001$. R^2m and R^2c are the marginal and conditional variance of the model, respectively.

Relationship with inflammatory markers and oxidative damages

	Selected model	**Estimate ± SE**	**t-value**	**p**	**R^2m**	**R^2c**
RTL	Intercept	1.285 ± 0.078	16.416	***	0.078	0.237
	Population (Chizé)	-0.087 ± 0.026	-3.294	**		
	Alpha1-globulins	-0.056 ± 0.025	-2.248	*		

Figure 2. Scatter plots illustrating correlations among relative leukocyte telomere length (RTL) and measures of inflammatory markers and oxidative damages. Scatter plots show relationships between RTL and levels of: (A) alpha1-globulin, (B) beta-globulin, (C) alpha2-globulin, (D) haptoglobin, (E) malondialdehyde (MDA). Spearman's correlation coefficients and associated P values presented for each plot.



Discussion

Because of a potential link between telomere length and longevity and age-related diseases, recent studies aims to investigate which factors are influencing telomere length shortening. These preliminary analyses performed in a wild mammal, the roe deer, provide first insights that inflammatory status and telomere length could be associated.

This result is in accordance with studies in humans that describe negative associations between telomere length and inflammatory status (Zhang *et al.,* 2016). However, even in humans, the precise causal relationship between inflammation and telomere length is not very well understood. Indeed, oxidative stress and inflammatory substances, both closely related, are known to impair directly telomeres, because of the guanine nucleobases that constitute a major part of vertebrate telomeres are particularly sensitive to oxidative attack (Von Zglinicki, 2002). From another side, cells with short telomere could lead to an increased inflammatory status (Campisi, 2005), could underlie immunosenescence processes and lead to an overall decline in individuals health and survival. Both relationships are not mutually exclusive, and could lead to a vicious circle of inflammation and telomere shortening (Zhang *et al*., 2016).

To our knowledge, this association have not been yet described in a wild mammal. A very recent study performed measures of corticosterone, inflammation (haptoglobin levels), immune status and telomere length on nestlings of the magnificent frigatebird (*Fregata magnificens*) facing herpesvirus infection. They found associations between the plasma concentration of haptoglobin (*i.e.* a marker of inflammation) with the infectious status of birds and their probabilities of survival, but not with telomere length (Sebastiano *et al*., 2017). More studies on the link between inflammation and telomere length, in relation with infectious status of animals, would therefore be needed to draw general trends.

In these preliminary analyses, we did not found associations between telomere length and the measure of oxidative damages we performed. Oxidative stress in organisms is a balance between the production of reactive oxygen species (ROS), antioxidant defences, oxidative damages and oxidative repair mechanisms (Beckman and Ames, 1998). However, some measures are very challenging to be performed *in vivo* (*e.g.* the production ROS that have a very short half-life), so biologists often focused on indirect biomarkers such as damages to proteins, lipids or DNA. Here, we focused on MDA, a by-product of lipid peroxidation (Halliwell and Gutteridge, 2007) as a proxy of oxidative damages. MDA is a very commonly used marker of oxidative damages but it is obviously totally insufficient to focus on an unique

measure to describe the oxidative status of individuals (Christensen *et al*., 2015). This absence of correlation between MDA levels and telomere length should not be generalized without testing other markers of oxidative damages and markers of antioxidant defences.

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CHAPTER 6

General discussion and perspectives

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Immunity is a key physiological function for survival that however entails diverse developmental, maintenance or activation costs. In natural conditions, resources allocation trade-offs occur among different functions (*e.g.* immunity, growth and reproduction). On the long-term, resource allocation strategies between different functions are likely to have consequences on the age-specific reproductive and survival tactics displayed by individuals.

The aim of this thesis was to describe age-related variations of the immune phenotype of a wild and long-lived mammal, the roe deer, and to provide a better understanding of the resource-based allocation trade-off between immunity and other processes or traits (*e.g.* growth, senescence or telomere dynamics). As discussed below, our results also pave the road for future studies that would allow deciphering the role of immunity - as a function at the core of life-history trade-offs.

In this Chapter, I provide a summary of the results obtained during my PhD, detailed in Table 6.1 and in relation with other life-history traits in Fig. 6.1. Following this summary, I discuss two main perspectives of this work:

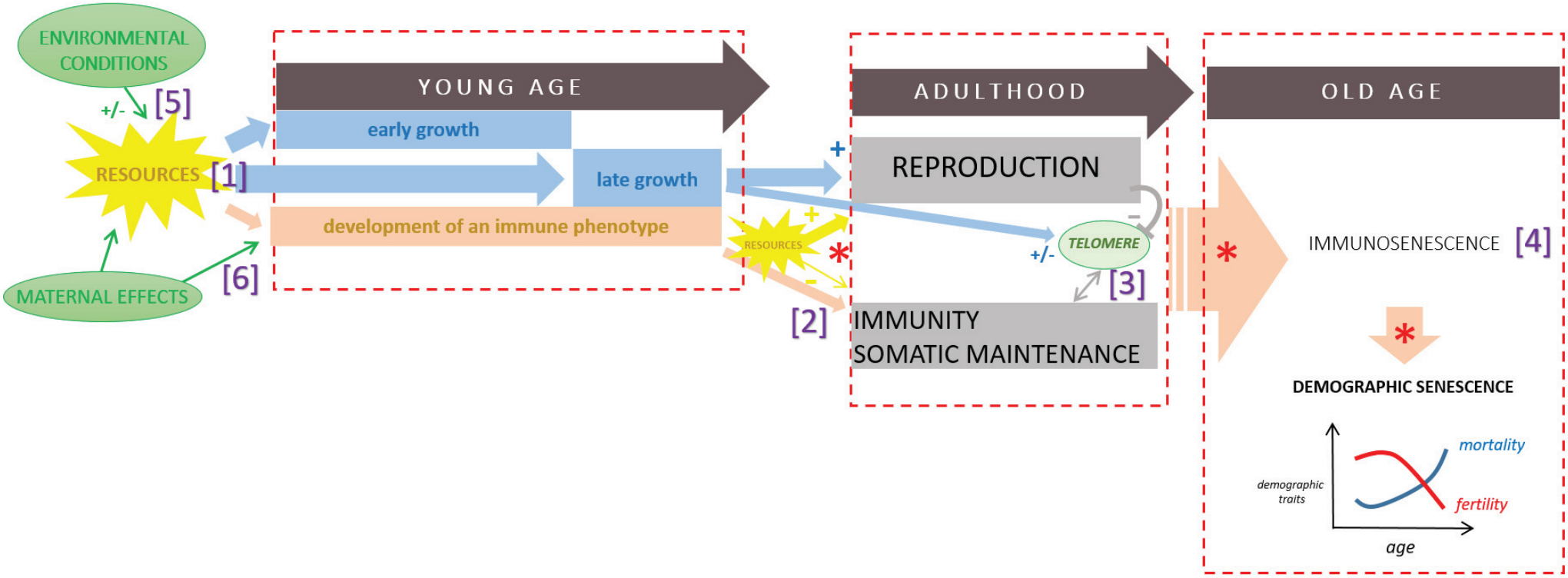
(i) how my results provide promising avenues of research for a better understanding of the underlying mechanisms of demographic senescence in the wild

(ii) studying immune function in relation to age-specific reproductive performance to assess whether reproductive allocation could decrease immune performance at both short- (*i.e.* during adulthood) and long-term (*i.e.* immunosenescence, reproductive and actuarial senescence).

Table 6.1. Summary of the results obtained during my PhD. Numbers in square brackets are referring to the following Fig. 6.1.

DESCRIPTION OF THE MAIN RESULTS		Variation with age?	Sex-specific pattern?	Population effect?	Publication
CHAPTER 3: Development of immunity at young ages					
Does a trade-off exist between growth and immunity?	A high allocation to growth (early or late) does not impair allocation to immunity of young roe deer, neither on the short-term (8 months of age [1]), nor on long-term (at adulthood [2])	*not tested*	***no***	***yes***	Cheynel *et al.* *In press* Oecologia
	The cost of growth in terms of immune performance does not differ between sexes				
	The allocation to immunity in young roe deer differs according to the environment: higher allocation to adaptive immunity at CH (poor access to resources and high parasite pressure), higher allocation to innate immunity at TF (higher access to nutritional resources and low parasite pressure)				
Do maternal effects senesce and impair juvenile condition?	Maternal age does not impact offspring physiological condition measured at 8 months of age [6]	***no***	***no***	***yes***	Cheynel *et al.* *In prep.*
	Offspring physiological condition is strongly influenced by maternal body mass: fawns born to heavy mothers have higher levels of markers of body condition and innate cellular immune traits [6]				
	Environmental conditions strongly influence the levels of markers of body condition and immune performance: juveniles at CH display lower levels of physiological markers of body conditions and allocate more to humoral immunity than those at TF [5]				
CHAPTER 4: Senescence in immune traits					
Are roe deer subject to immunosenescence?	Innate traits are overall maintained at old age, but some functional traits decline with increasing age at CH [4] [5]	***yes***	***yes*** ***no***	***yes***	Cheynel *et al.* (2017) Scientific Reports
	Inflammatory markers increase with age in both populations [4]				
	Adaptive response declines in late adulthood [4]				
	Parasite burden sharply increases at old age [4]				
CHAPTER 5: Immunity and relative telomere length (TL)					
Is there a decline in telomere length with age?	At older ages, TL are shorter at CH (poor environmental conditions) than at TF [5]	***yes***	***no***	***yes***	Wilbourn *et al.*(2017) Biology Letters
	With increasing age, TL increase at TF but no detectable relationship with occurs age at CH [5]				
	Sex and body mass re not related to TL				
Association between TL and immunity?	Variation in relative TL is independent of variation in leukocyte counts [3]	***no***	***no***	***yes***	Preliminary analyses *In prep.*
	Relative TL does no vary with age and sex of individuals				
	Telomeres are shorter at CH compared to TF [5]				
	Higher levels of some globulins (alpha1- and beta-globulins) are associated with shorter TL [3]				
	High level of oxidative damage (malondialdehyde) is not associated with shorter telomeres				

Figure 6.1. Age-related relationships between immune function and life-history traits. Results obtained during my PhD are figured by numbers in square brackets, they are detailed above in Table 6.1. The perspectives for future work are referred by red stars and are explained all along the main text of the discussion.



Promising results for a better understanding of the mechanisms underlying demographic senescence in the wild

For years, it has been considered that wild animal populations were not subject to senescence. Because of the high mortality risk experienced by animals in nature, it was assumed that very few individuals would survive long enough to experience senescence (Medawar, 1952; Comfort, 1956). However, in recent years, the increasing availability of longitudinal studies on wild species has provided strong evidences that senescence is the rule rather than the exception in free-ranging populations of vertebrates (Nussey *et al.,* 2013; Gaillard *et al*., 2017). Indeed, these studies proved that the probabilities of survival and reproduction are decreasing with increasing age in wild vertebrates, which constitutes *actuarial* and *reproductive* senescence, respectively (Jones *et al*., 2008b; Nussey *et al.*, 2013; Lemaître and Gaillard, 2017). The study of senescence then focused on these two main components of individual fitness (*i.e.* survival and fecundity).

More recently, studies in free-ranging animals have also reported age-related changes in others traits, such as phenotypic traits (*e.g*. body mass in Nussey *et al*., 2011 or Douhard *et al.,* 2017; secondary sexual characters in Nussey *et al*., 2009a; tooth wear in Gaillard *et al*., 2015) or physiological traits (*e.g.* hormones levels in Angelier *et al*., 2007; oxidative stress in Nussey *et al*., 2009b; telomere length in Monaghan and Haussmann, 2006; haematological traits in Jégo *et al*., 2014). It appears that a large number of phenotypic and physiological traits also decline with increasing age in vertebrates. This phenotypic and physiological deterioration is likely to impair the performance of individuals with increasing age and should ultimately influence individual fitness. Whether the decline in fitness-related traits with increasing age is a direct consequence of senescence in physiological traits or functions remains a central question in the study of ageing (Promislow *et al*., 2006).

A growing knowledge on senescence in roe deer

Unsurprisingly, like other wild vertebrates, roe deer shows a decline in fitness-related traits with increasing age. First, senescence in survival have been reported, occurring from 7-8 years of age onward in both sexes at Chizé and Trois-Fontaines (Loison *et al*., 1999; Gaillard *et al*., 1993, 2004). There is also evidence of reproductive senescence, occurring from 8 years of age for female fecundity (measured *via* implantation failure, Hewison and Gaillard, 2001; or *via* pregnancy rates, Gaillard *et al.*, 2003) and for male breeding success (Vanpé *et al*., 2009).

Furthermore, studies have described age-related decline in body mass (from 8 years of age, Douhard *et al*., 2017) and in physiological traits (*i.e*. haematocrit, albumin and creatinine that senesce from 7-9 years of age according to the trait, Jégo *et al.,* 2014), suggesting that a decline in individual performance does occur with increasing age in roe deer. The results obtained during my PhD, on immunosenescence and age-related changes in telomere length provide additional insights for a better understanding of the mechanisms underlying demographic senescence in this wild mammal. One major perspective would be indeed to understand why senescence in all these traits is not always synchronous (Promislow *et al.*, 2006; Hayward *et al.*, 2015; Gaillard and Lemaître, 2017).

Our work provides an accurate picture of age-related variations in a large number of immune traits, reflecting both innate and adaptive immune responses of roe deer. As reviewed in the introduction, previous studies that assessed age-related changes in large number of immune traits are rare, especially in mammals (only 5 studies, *i.e*. Nussey *et al*., 2012; Schneeberger *et al*., 2014; Watson *et al*., 2016; Singleton *et al*., 2018; Abolins *et al.*, 2018; see Table 1.1), and they have often reported contradictory results. In roe deer, we found clear evidence that both males and females are subject to profound changes in their immune profile with increasing age, *i.e*. principally a decline in the adaptive response (lymphocytes) and a strong increase in the production of inflammatory markers (haptoglobin, beta-globulin levels), which suggests the installation of a chronic state of inflammation. It proved that male and female roe deer are subjected to immunosenescence. In the same individuals, a parallel marked increase in their parasite burden with increasing age suggested the existence of a cost of immunosenescence in terms of infections. We also know that when aging, roe deer are subject to tooth wear that causes a decreased food intake (Gaillard *et al.,* 2015). This decrease of nutrient input with increasing age could have direct negative consequences on immune responsiveness and body condition, and therefore in the ability of individuals to display efficient parasitic resistance. At old ages, the interplay among immunosenescence, increased parasitism and decreased body condition could give the rise to a vicious circle (*i.e.* increased parasitism leads to lower condition, which predisposes to host infections and so on), which is likely to have impact on the probability to survive and reproduce (Beldomenico and Begon, 2008).

We also found evidences of age-related changes in roe deer leukocyte telomere length. Some preliminary analyses suggested in addition that a link exists between inflammatory status and telomere length of individuals, *i.e*. that individuals with high levels of some inflammatory markers (beta- or alpha1-globulins) have shorter telomeres, independently of age. Negative

associations between inflammation and telomere length have been documented in humans (see Zhang *et al*., 2016 for a review). Telomere shortening with increasing age is naturally occurring during the ageing process, but factors such as inflammation and oxidative stress (themselves closely related) increase the rate of telomere attrition, possibly leading to an accelerated ageing process (Zhang *et al.,* 2016). It is however very difficult to identify causal relationships between inflammation and telomere length. Indeed, oxidative stress and inflammatory substances are known to impair directly telomeres, because guanine nucleobases that constitute a major part of vertebrate telomeres are particularly sensitive to oxidative attack (Von Zglinicki, 2002). On the other hand, cells with short telomeres could also overexpress inflammatory cytokines (Zhang *et al.,* 2016). Both relationships are not mutually exclusive, and could lead to a vicious circle of inflammation and telomere shortening (Zhang *et al*., 2016). In humans, these mechanisms are assumed to accelerate ageing and thereby increase the risk of age-related diseases (Zhang *et al*., 2016) but so far, the relationships linking senescence, telomere and inflammation have rarely been studied in the wild. Until now, studies have rather focused on associations between telomere length and infectious status (Ilmonen *et al.,* 2008; Beirne *et al*., 2014; Ashgar *et al*., 2015). A study in a wild-derived house mice have for instance proved that telomere attrition can be accelerated during an infectious state, and this attrition is supposed to be caused by inflammatory responses (Ilmonen *et al*., 2008).

In wild mammals, associations between telomere length, age and survival have also recently been reported (in badgers, Beirne *et al.,* 2014; in Soay sheep, Fairlie *et al*., 2016). However, while age-dependent telomere loss is often found, a large proportion of the variation in the relationship between telomere length and age remains unexplained (Monaghan and Haussmann, 2006). Inflammatory state, oxidative stress, but also challenging environmental condition (particularly during early life, Stier *et al*., 2016; Salmón *et al*., 2016) would thus be strong candidates in the acceleration of age-related telomere loss. Our results in roe deer, on the associations between telomere length and inflammation, but also in relation to environmental conditions (Wilbourn *et al.,* 2017), offer interesting avenues of research for a better understanding of telomere dynamics in the wild.

Perspectives on the relationship between physiological and demographic senescence

Our results on age-related variation in immunity, parasite burden and telomere length brings new elements into our knowledge of the variation in roe deer physiological performance with age. Added to previous results on age-related changes in body mass or in physiological traits

reflecting body conditions (*e.g*. haematocrit, albumin or creatinine), we bring a quite precise picture in the phenotypic and physiological decline experienced by roe deer with increasing age, which is likely to participate to its demographic senescence (*i.e.* decline in reproduction and/or survival). However, for now, all these traits have been studied separately. The next step would thus be to combine these descriptions of demographic, phenotypic and physiological metrics of senescence. For future work, I propose two main lines of investigations that would provide great advances in the understanding of senescence process in the wild.

In the wild, associations between some immune traits and survival over the subsequent winter have been reported (Watson *et al.,* 2016). In farm animals, correlations have been found between the levels of some immune traits, health and the productivity of animals (Banos *et al*., 2013). However, to my knowledge, the longitudinal associations between immune, parasitic resistance and demographic senescence patterns have been not been yet studied in any wild population. While correlations can never prove causation, path analysis and structural equation modelling might allow different models of causal relationships between the age-related patterns of traits measured to be considered (and in some cases statistically compared) (Graham *et al*., 2011). Based on my findings, it could be tested whether the age-related changes in immunological performance and parasitic burden match demographic senescence in roe deer. The hypothesis would be that the levels of specific measures of immune or parasitic traits could be correlated with parasitic resistance of individuals, and be associated with health and fitness. The idea would be to identify thresholds of immune traits that once attained lead to the strong and late increase in parasitic burden (as reported in Cheynel *et al*., 2017), and to the decline in reproductive success and survival. Additionally, these results would help to determine which traits are the most important in predicting fitness under natural conditions. We could also hypothesize that high rates of immunosenescence are associated with an early age at the onset of senescence and/or high rates of demographic senescence. Because female roe deer does not display reproductive cessation at old ages, in these analyses we could assess reproductive senescence in female roe deer through a decline in birth rate, fecundity or fawn body mass (*i.e*. good predictor of body condition and survival).

Finally, a more general study analysing age-related associations between all different traits measured in males and females roe deer during later adulthood would be very interesting (like done by Hayward *et al*., 2015 on Soay sheep). The aim of these analyses would be to compare ageing trajectories among functionally-linked groups of traits to determine the extent to which ageing rates are synchronous among traits. This previous study found an asynchrony of

senescence among phenotypic traits (Hayward *et al*., 2015). A better understanding of these patterns would help understand the cause of variation in ageing in the wild (Nussey *et al*., 2013).

Allocation to reproduction and disposable soma theory

Early- vs. late-life trade-offs play a central role in the life-history theory of ageing (Kirkwood, 2017). The disposable soma theory is based on the principle of energy allocation: in a situation of limited resources (as it is the case in the wild), if an individual allocates heavily in reproduction, the amount of energy still available to insure its own maintenance or its immune function will be lower (Kirkwood and Rose, 1991). A lower allocation in maintenance (*e.g.* in repair mechanisms) leads to very high levels of damages accumulation and a lower allocation in immunity leads to higher sensitivity in response to parasite and pathogen attacks. In both cases, at a longer term, it could lead to an increased rate/or an earlier onset of senescence in reproduction or survival. For a better understanding of senescence, it is of high importance to investigate how the trade-off between reproductive allocation and other traits at young age (somatic maintenance, immune function) influences reproductive and survival senescence on the long term.

Until recently, evidence for trade-offs between early-life reproduction and either survival or late life performance remained scarce in the wild, and particularly for mammals. A review demonstrated that high allocation to reproduction (or growth) early in life is associated with earlier or faster senescence late in life, in accordance with the general principle of allocation and thereby provides support for the disposable soma theory (evidence in 21 of 26 studies in vertebrate populations reviewed by Lemaître *et al*., 2015). More recently, some studies have provided further support to the theory (Tarwater and Arcese, 2017; Froy *et al*., 2017; Hämäläinen *et al*., 2017; Fay *et al*., 2018).

Now that a large number of studies proved that elevated reproductive effort have often negative effects on the long-term, a better understanding of the physiological mechanisms that underlie this trade-offs is needed, and various mechanisms have been proposed as candidates. Up to now, a vast majority of studies investigating physiological costs of reproductive expenditure have focused on the accumulation of oxidative stress (Metcalfe and Monaghan, 2013). However, as explained in the introduction of this manuscript, reproductive effort and immunity are also strongly related. Based on the resource allocation principle (Cody 1966; Williams 1966), to maximize reproductive success in a context of limited resources, individuals may shift resources from parasite defence when making a large reproductive effort (Sheldon

and Verhulst, 1996). As a result, these individuals may experience a reduced resistance and ability to fight parasites, leading to greater levels of parasitic infections. On the long-term, it may lead to reduce probability of individuals to survive and reproduce (Sheldon and Verhulst, 1996). Additionally, higher levels of parasitic infection may increase inflammatory status of these individuals, that are known to produce oxidative damages (*immunopathology*) that may also have long-term negative effects. Immune function therefore is a strong candidate to involve in early-late trade-offs. Recently, a large number of studies conducted on birds have focused on the relationship between reproductive effort, parasitism and/or immune function, but results were contradictory and thus appeal for more studies (Knowles *et al*., 2009). Finally, a potential mechanism, that may underlie trade-off between early reproduction and longevity is telomere shortening (Monaghan and Haussmann, 2006). High reproductive allocation would elevate turnover rate of cells and consequently increase oxidative stress, leading to faster telomere attrition. A study conducted on zebra finches (*Taeniopygia guttata*) proved that an experimentally increased level of reproductive effort causes telomere reduction (Reichert *et al*., 2014). In the wild, studies were also mostly conducted on birds, and confirmed these results (Bauch *et al*., 2012; Sudyka *et al*., 2014). The rate of telomere shortening in blood cells seems to be inversely correlated with longevity in many species of birds and mammals (Haussmann *et al.,* 2003; Tricola *et al*., 2018).

Following the results we obtained during this work, an interesting path of research linking immunity and life-history traits would be to assess whether reproductive allocation decreases immune performance and telomere length in the short term (*i.e.* during adulthood) and senescence patterns on the long term (*i.e*. immunosenescence, reproduction, survival).

In roe deer, both male and female allocate a large amount of energy to the reproductive effort that is likely to have long-term costs. The majority of female roe deer display a high reproductive output by producing heavy and fast-growing twins every year between 2 and 11 years of age (Gaillard *et al*., 1998). Roe deer are income breeders and females thus do not rely on body reserves for gestation and lactation. Their allocation to reproduction is considered as one of the highest reported among large herbivores (Andersen *et al*., 2000). Male roe deer also incur strong costs of reproduction. Roe deer are territorial during the rut, *i.e.* for 6 month between March and September (Andersen *et al*., 1998). In seasonally breeding mammals, the mating season is associated with strong energy expenses and consequently loss of body mass in males (McElligott *et al*., 2003; Mysterud *et al*., 2005). Due to strong energy expenditures, males have lower body condition right after the breeding period. It is likely that high allocation

to reproduction during repeated breeding seasons leads to accumulated damages for males, limits energy allocation to somatic maintenance (*e.g*. in immunity or antioxidant defences), and would be associated on the long-term with more rapid senescence (Lemaître *et al*., 2014a). In addition, male roe deer systematically grow antlers each year from their first year of life onwards. Antlers show a particularly fast growth for an animal tissue (Price and Allen, 2004) and are therefore supposed to be energetically costly to develop and maintain (Lemaître *et al.*, 2014b). However, some studies failed to detect long-term costs associated with male expenditure on antlers (in red deer in Lemaître *et al*., 2014b; in roe deer in Lemaître *et al.*, 2018). In roe deer, the antler cycle is principally controlled by testosterone secretions (Sempéré, 1982) and in all vertebrates, the level of testosterone is generally positively correlated with male reproductive effort (Altalo *et al*., 1996; Negro *et al*., 2010). Androgens are however known to modulate the immune function (Folstad and Karter, 1992; Malo *et al*., 2009; Ezenwa *et al*., 2012), which can partly explain high levels of parasites found in males (as explained in the *Introduction*). Hormones linked to sexual competition such as testosterone but also IGF-1, could play a strong role between traits such as reproduction and immunity during early life and, at long-term, on ageing (Brooks and Garratt, 2017).

We could investigate in roe deer how reproductive allocation influence senescence trajectories, with the following hypotheses: (i) on the short-term, individuals that allocate a lot to reproduction should less allocate to maintenance (*i.e*. immune function and telomere maintenance) (ii) on the long-term, these individuals should suffer from an early and/or intense level of immunosenescence and telomere attrition (iii) finally leading to earlier or faster reproductive and/or survival senescence. Ideally, it would be also very interesting to take into account the allocation in growth of roe deer. We could hypothesized that a decline in physiological performance (*i.e.* in immune function or telomere maintenance) might be exacerbate in individuals that allocate substantially to both growth and reproduction. Cumulative costs of growth and reproduction might even be more pronounced in harsh environments such as Chizé, where roe deer females show higher between-year variation in reproductive success, than in Trois-Fontaines (Gaillard *et al*., 2013). To evaluate reproductive investment and senescence in females, as in roe deer almost all females produce at least one fawn at birth every year, we could use litter mass as a proxy of female allocation. In males, we could assess reproductive allocation through antler growth or through paternity analyses that are planned to be developed in the following years.

The influence of environmental conditions on life-history strategies

Finally, my PhD focused on two contrasted populations of roe deer has shown the importance of habitat quality on the development of an immune phenotype, the overall physiology and the life-history traits of individuals. My results are thus in line with previous studies suggesting that eco-immunological results obtained on a particular species or on a single population, cannot be easily generalized (Pigeon *et al*., 2013).

Between two environmentally contrasted populations, such as Trois-Fontaines and Chizé, it is indeed obviously impossible to determine which parameters drive differences observed in immune profiles of roe deer between these populations, and more generally the differences observed in a large variety of life-history traits. Immunity is a function developed in response to a particular context, *i.e.* in response to the richness and density of pathogens, the level of resources availability or climatic conditions; and more likely an interplay among all these effects. Further research is strongly needed to determine the relative importance of each environmental factor such as resources and/or pathogens in immune profile of wild animals. It would necessitates much more longitudinal follow-ups conducted on several populations (more than two) experiencing different habitats, to be able to assess general trends.

A better understanding of the effect of environmental conditions on immune function of wild populations would also be needed to understand the biology and dynamics of pathogen populations. Immune function of hosts exerts indeed a selective force on pathogens of its environment and therefore, the immunological state of wild populations modulates the transmission of diseases. Differences in immune selection drive evolutionary trajectories of pathogens. Zoonotic diseases, *i.e.* pathogens transmit from vertebrate animals to humans, represents actually a major concern in public health as their frequency and prevalence increase worldwide (*e.g*. outbreaks of pathogens such as Ebola virus, influenza A virus, transmission of endemic pathogens such as Salmonella *sp*…. see Plowright *et al*., 2017). Several factors determine the probability of zoonotic cross-species spillover of infections, such as disease dynamics in the reservoir host, pathogen exposure and susceptibility within human populations. To understand dynamics and spreading of these pathogens, more studies on the heterogeneity that could exist in immune function of different populations of wild animals would be very useful.

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Appendices

Appendix 1. *The influence of early-life allocation to antlers on male performance during adulthood: evidence from contrasted population of a large herbivore.*

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LINKING ORGANISMAL FUNCTIONS, LIFE HISTORY STRATEGIES AND POPULATION PERFORMANCE

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The influence of early-life allocation to antlers on male performance during adulthood: Evidence from contrasted populations of a large herbivore

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Abstract

1. To secure mating opportunities, males often develop and maintain conspicuous traits that are involved in intrasexual and/or intersexual competition. While current models of sexual selection rely on the assumption that producing such traits is costly, quantifying the cost of allocating to secondary sexual traits remains challenging.
2. According to the principle of allocation, high energy allocation to growth or sexual traits in males should lead to reduced energy allocation to the maintenance of cellular and physiological functions, potentially causing them to age faster, with impaired survival.
3. We evaluated the short-term and delayed consequences of energy allocation to antlers early in life in two contrasted populations of roe deer, *Capreolus capreolus*. Although most males mate successfully for the first time in their fourth year, antlers are grown annually from the first year of life onwards. We tested the prediction that a high level of allocation to antler growth during the first two years of life should lead to lower body mass, antler size and survival during the early and late prime stages, as well as to reduced longevity overall.
4. Growing and carrying long antlers during the first years of life was not associated with any detectable cost in the late prime stage. The positive association between antler growth in early life and adult body mass instead supports that fawn antler acts as an honest signal of phenotypic quality in roe deer.
5. For a given body mass, yearling males growing longer antlers displayed impaired performance during their late prime. We also found a trend for a short-term survival cost of allocation to relative antler length during the second year of life.

6. Yearling males that grow long antlers relative to their mass might display a fast life-history tactic. We argue that differential allocation to secondary sexual traits generates a diversity of individual trajectories that should impact population dynamics.

KEYWORDS
ageing, *Capreolus capreolus*, life history, roe deer, secondary sexual traits, senescence

1 | INTRODUCTION

The evolution of complex secondary sexual traits in males is generally explained by the positive relationship between the intensity of their expression and breeding success (Andersson, 1994), as is the case for plumage coloration in many birds (Brommer, Ahola, & Karstinen, 2005). All models of sexual selection that have been developed thus far (Kuijper, Pen, & Weissing, 2012) share the common and mandatory assumption that growing and maintaining secondary sexual traits is costly (Kotiaho, 2001), although these costs may vary in relation to individual condition (Rowe & Houle, 1996). However, measuring the fitness costs of bearing extravagant sexual traits empirically is challenging and most studies of life-history (e.g. higher mortality due to higher predation risk) or physiological (e.g. lower performance of the immune system) costs to date have reported contrasting patterns (Kotiaho, 2001). A meta-analysis across birds, spiders, insects and fish revealed that the expression of secondary sexual traits is strongly condition-dependent as the expression of a given sexual trait (known to predict mating success) is generally positively associated with longevity (Jennions, Møller, & Petrie, 2001). However, while males in good condition during the early stages of life are able to develop the most conspicuous sexual traits, they may suffer from a higher degree of somatic deterioration compared to males that are in low condition during early life, which can lead to an accelerated rate of ageing (Adler, Telford, & Bonduriansky, 2016). In line with these results, it has recently been argued that the costly nature of secondary sexual traits should be rooted in the theories currently proposed to explain the evolution of ageing (Hooper, Lethonen, Schwanz, & Bonduriansky, 2018; Tidière et al., 2015).

Our understanding of the ageing process takes its root in the principle of allocation (Cody, 1966), a cornerstone of life-history theory (Stearns, 1992). This principle states that individuals need to share a finite pool of resources among competing functions such as growth, reproduction and survival. When it concerns a long-lasting or delayed cost of a given allocation, this principle is closely related to the disposable soma theory of ageing (Kirkwood & Holliday, 1979). This theory postulates that individuals that allocate a substantial amount of resources to reproduction will have less resources to devote to the set of molecular mechanisms (e.g. DNA repair mechanisms) involved in somatic maintenance (Kirkwood & Rose, 1991). Therefore, individuals that allocate much to reproduction early in life will accumulate a variety of damages that will ultimately cause a shortened life span or an increased rate of reproductive senescence (Lemaître & Gaillard, 2017). The central role played by early- vs. late-life trade-offs in the evolution of ageing constitutes the core of the life-history theory of ageing (Kirkwood, 2017) and has been supported by many empirical studies (Hammers, Richardson, Burke, & Komdeur, 2013; Jankowiak, Zyskowski, & Wysocki, 2018). However, most of these studies have focused on females (Lemaître et al., 2015) and there is no consensus yet on whether early- vs. late-life trade-offs are prevalent in males. During the reproductive season, males devote a substantial part of their resources to sexual competition (Andersson, 1994). While sexual competition can take different forms, male breeding success is determined by allocation to secondary sexual traits in most species (Bonduriansky, 2007; Emlen, 2008).

Several studies have revealed that the allocation to secondary sexual traits can be associated with diverse physiological costs (Garratt & Brooks, 2012). In the Australian painted dragon (*Ctenophorus pictus*), males that maintained a high level of head coloration suffered from a rapid rate of telomere loss (Giraudeau et al., 2016). This finding suggests that males with particularly conspicuous traits might have impaired fitness later in life. While current theories predict that males should suffer from long-term or delayed costs of growing costly sexual traits, such allocation can levy immediate fitness costs. Thus, successful reproduction often has short-term reproduction and survival costs in male birds (Bleu, Gamelon, & Saether, 2016), which might potentially be driven by strong allocation to sexual traits.

Overall, measuring costs of allocation to secondary sexual traits during early life is important because the expression of secondary sexual traits varies over the lifetime (Lemaître & Gaillard, 2017), and also because allocation to secondary sexual traits early in life increases the absolute level of allocation to the entire growth process, which potentially leads to detrimental fitness consequences later in life (Lee, Monaghan, & Metcalfe, 2013; Metcalfe & Monaghan, 2003). This avenue of research should boost our understanding of the relationships linking individual development with population dynamics.

So far, the few studies that assessed the long-term fitness consequences of early allocation to secondary sexual traits in mammalian males have mostly been performed on bovids (see Table 1 for a review), and showed that the intensity of early horn growth is rarely associated with a delayed survival cost (Table 1). While the horns of bovids grow continuously over most of their lifetime, the amount of energy allocated to the annual horn growth is quite limited. On the contrary, male cervids grow antlers that are cast

TABLE 1 Summary of published studies that have evaluated the long-term life-history consequences of horn growth in male bovids

Species	Population	Horn growth	Late-life trait	Relationship	References
Alpine ibex (*Capra ibex*)	Belledonne-7 Laux Reserve, France	Early annual horn increment	Longevity	Null	Bergeron, Festa-Bianchet, von Hardenberg, and Bassano (2008)
	Gran Paradiso National Park, Italy	Second horn increment	Age-specific survival	Null (tend to be negative at very old age)	Toïgo, Gaillard, and Loison (2013)
Bighorn sheep (*Ovis canadensis*)	Banff National Park, Canada	Horn increment between 3 and 8 years of age	Survival at 7–11 vs. 12–17 (years of age)	Negative (greater horn growth in the short-lived rams)	Geist (1966)
	Ram Mountain, Canada[a]	Horn increment between 1 and 4 years of age	Age-specific survival	Null (once the negative bias due to hunting mortality is removed)	Bonenfant, Pelletier, Garel, and Bergeron (2008)
Chamois (*Rupicapra rupicapra*)	Swiss National Park, Switzerland	Horn increment in the first 2 years of horn growth (L2 segment)	Longevity	Null	Corlatti, Storch, Filli, and Anderwald (2017)
	Comprensorio Alpino di Sondrio, Switzerland[a]			Negative	
	State Forest district of Oberammergau, Germany[a]			Null	
Dall sheep (*Ovis dalli*)	Kluane National Park, Canada[a]	Horn volume from the first 5 summers of horn growth	Longevity	Negative	Loehr, Carey, Hoefs, Suhonen, and Ylonen (2007)
Soay sheep (*Ovis aries*)	St Kilda, Scotland	Horn type: normal (N) or scurred (S)	Longevity	Lower for N than S	Robinson, Pilkington, Clutton-Brock, Pemberton, and Kruuk (2006)
			Lifetime breeding success	No difference between N and S	
		Horn size	Longevity	Negative (N) Null (S)	
			Lifetime breeding success	Null (N) Null (S)	

[a]Hunted populations.

and fully regrown every year (Chapman, 1975). This repeatedly high annual allocation of resources to antler growth is thus expected to be particularly costly. So far, the only study that quantified delayed costs of growing antlers reported that male red deer (*Cervus elaphus*) that allocated substantially to antler growth between 4 and 9 years of age did not suffer any detrimental consequences on harem size or antler length later on (Lemaître, Gaillard, Pemberton, Clutton-Brock, & Nussey, 2014). However, young males grow antlers from their first year of life, well before they can successfully mate and at a time when they must allocate intensively to body growth (Douhard, Gaillard, Pellerin, Jacob, & Lemaître, 2017). Furthermore, delayed fitness costs of early allocation to antler growth might impact survival at the end of the prime-aged adulthood (i.e. when the onset of actuarial senescence occurs; Gaillard, Garratt, & Lemaître, 2017) rather than reproductive success (Lemaître & Gaillard, 2017). Surprisingly, the long-term fitness consequences of early development of secondary sexual traits have not yet been investigated. Moreover, while there is increasing evidence that early-life environmental conditions mediate trade-offs between early- and late-life performance (Balbontín & Møller, 2015), whether the interplay between environmental conditions and the expression of secondary sexual traits early in life shapes the magnitude of delayed fitness costs remains unknown.

We propose to fill this knowledge gap by investigating the consequences of allocation to antlers in early life (defined here as the first two years of life) on survival, body mass and antler size during adulthood in two populations of European roe deer (*Capreolus capreolus*) living in contrasted habitats. In this slightly polygynous mammal, males are territorial from early spring until mid-July–late August and generally begin mating during their fourth year, although some males can sire offspring in their third year of life (Vanpé et al., 2009). Male roe deer systematically grow antlers each year from their first year of life onwards. Growing large antlers in early life well before mating opportunities occur should thus come at a cost in terms of survival, body mass or antler growth later in life. Finally, as resource availability mediates the trade-off between growth and somatic maintenance, such costs should be more pronounced in populations experiencing poor environmental conditions.

2 | MATERIALS AND METHODS

2.1 | Study population

We studied the populations of roe deer at Trois-Fontaines (TF) and Chizé (CH) living in enclosed forests. Trois-Fontaines (1,360 ha), located in north-eastern France (48°43′N, 4°55′E), has a continental climate characterized by moderately severe winters and warm rainy summers. This site has rich soils and provides high-quality habitat for roe deer (Pettorelli et al., 2006). In contrast, in Chizé (2,614 ha), located in western France (46°50′N, 0°25′W), the climate is temperate oceanic with Mediterranean influences. This forest has low productivity due to poor-quality soils and frequent summer droughts (Pettorelli et al., 2006) and thereby provides a quite poor habitat for roe deer in most years.

Roe deer have been intensively monitored using a long-term capture–mark–recapture programme since 1975 and 1977 for Trois-Fontaines and Chizé, respectively. Every year and for each site, 10–12 days of capture is organized between December and March (Gaillard et al., 1993a). Once an individual is captured, its sex and body mass (to the nearest 50 g) are recorded and a basic clinical examination is performed.

2.2 | Allocation to antler growth during the first two years of life

Antlers grow each year from the end of November until early March (i.e. before the velvet is shed). Roe deer are in hard antler until the end of October/early November when they cast their antlers (Sempéré & Boissin, 1981). During their first year of life, males develop a small "button" (i.e. a very short antler with a round top instead of a spike) on top of their pedicle before growing their first full antlers at 1 year of age (Figure 1). Then, males generally develop a four-point (in yearlings) or five-/six-point (in adults) head. Antler length was measured to the nearest 0.5 cm along the external side of the main beam, from the base of the antler to the top of the main beam (Vanpé et al., 2007). Antler length was used as a proxy for allocation to antler growth because there is a strong covariation among the different antler traits in deer (Lemaître et al., 2014). When an individual was caught more than once in a given year, only the last measure was considered. When both antlers were intact, the average length was computed and retained for the analyses. When one antler was broken, the value of the remaining intact antler was retained. When both antlers were broken, the individual was removed from the dataset. Although a few captures occurred in October–November in the very first years of monitoring, we restricted our analyses to data collected from 1 December to avoid possible overlap between antlers growing during the current year and uncast antlers from the previous year. All males included in our analyses were of known age because they were caught within their first year of life, when age can

FIGURE 1 A fawn with visible buttons (photograph reproduced with permission from F. Débias)

be assigned without error from tooth eruption and wear patterns (Hewison et al., 1999).

In roe deer, the antler growth cycle is governed by complex interactions between the photoperiod and the endogenous rhythm of the hypothalamic–pituitary–gonadal axis (Sempéré & Lacroix, 1982). Therefore, for a given cohort, the date of capture provides a better predictor of antler size during antler growth than birth date (Vanpé et al., 2007). We analysed the relationship between antler length and the Julian date of capture (with 1 December as day 0). As antler growth is a nonlinear process (Goss, 1983), we fitted different functions to assess reliably the shape of the relationship between antler length (log-transformed) and date of capture in fawns (i.e. 8 months of age) and yearlings (i.e. 20 months of age): constant (i.e. no change in antler length with date of capture), linear, a threshold function (i.e. piecewise linear function) with one slope (i.e. antler length increasing linearly with date of capture until reaching a plateau at a given date) and a threshold function with two slopes (i.e. antler length increasing linearly with date of capture until a given date and then increasing further, but with a different slope). To estimate these threshold values, the deviance profile of the models including the slopes was used and the date of capture providing the lowest deviance was selected as the threshold value (Ulm & Cox, 1989). Model selection was based on the Akaike Information Criterion (AIC). We calculated AIC weights (AICw) to assess the relative likelihood that a given model was the best among all the fitted models (Burnham & Anderson, 2002). We selected the model with the lowest AIC except when the difference in AIC (ΔAIC) between two competing models was <2, in which case we retained the simplest model. Once the model that best captured the shape of the relationship was identified, we looked for potential differences between populations by including "population" as a fixed factor, either as an additive effect or in interaction with the Julian date.

As previously observed (Vanpé et al., 2007), the linear model best represented variation in antler length (log-transformed) with date of capture in fawns (Table S1; Figure 2a; slope of 0.022 ± 0.002, N = 224). Fawn antler length did not differ between populations (ΔAIC of + 1.93 for the model including "population"), and the daily growth rate did not differ between populations (ΔAIC of +3.57 for the model including the "population × date of capture" interaction term). We computed a *standardized fawn antler length* from the selected model by adjusting antler length to the median date of capture (i.e. 8 February) and pooling the data for both populations. The best model accounting for variation in yearling antler length in relation to capture date was the threshold model with a single slope (Table S2). Antler length increased linearly with date of capture until day 59 (i.e. 28 January) (Figure 2b; slope of 0.024 ± 0.002, N = 257) and remained constant thereafter. Antler length did not differ between populations (ΔAIC of +1.81 for the model including "population"), but the threshold date did (ΔAIC of −5.17 for the model including the "population x date of capture" interaction term). In both populations, antler length increased linearly with the date of capture (0.032 ± 0.004, N = 100, and 0.021 ± 0.002, N = 157 at Trois-Fontaines and Chizé, respectively), but the threshold date was earlier at Chizé (day 46 corresponding to 16 January; Figure 2d) than at Trois-Fontaines (day 72 corresponding to 11 February; Figure 2c), which matches the longer day length at Chizé than at Trois-Fontaines in January–February. We then computed a *standardized yearling antler length* by adjusting antler length to the median date of capture (i.e. 8 February) using the model selected for each population.

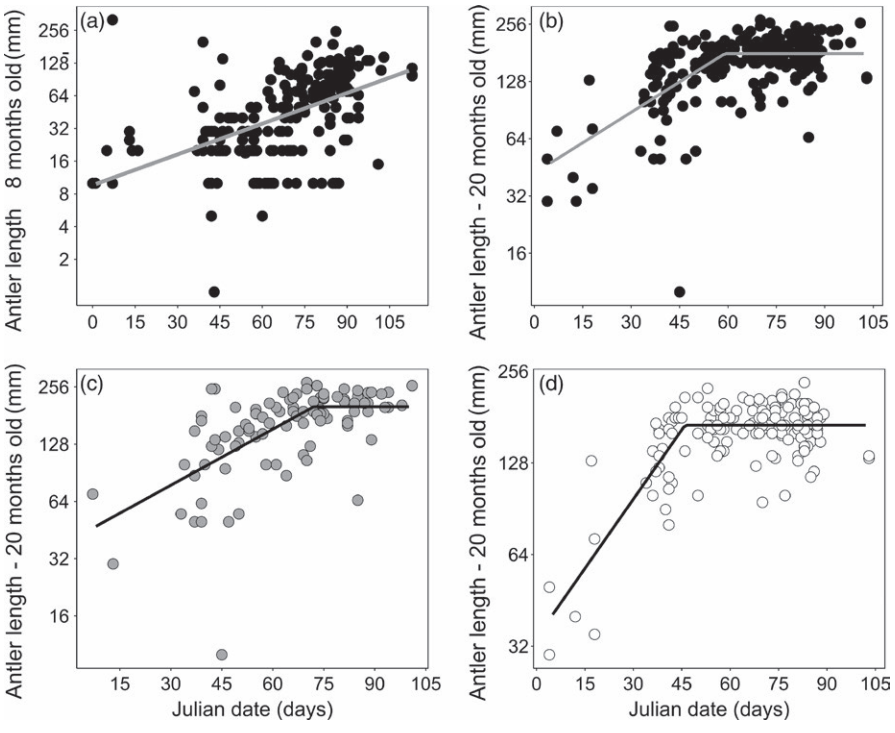

FIGURE 2 Relationship between antler length (log-transformed) during the first two years of life and Julian date of capture. Fawn antler length (at 8 months of age) for males at Chizé and Trois-Fontaines (a); yearling antler length (at 20 months of age) at Chizé and Trois-Fontaines (b); yearling antler length (at 20 months of age) at Trois-Fontaines (c); and yearling antler length (at 20 months of age) at Chizé (d)

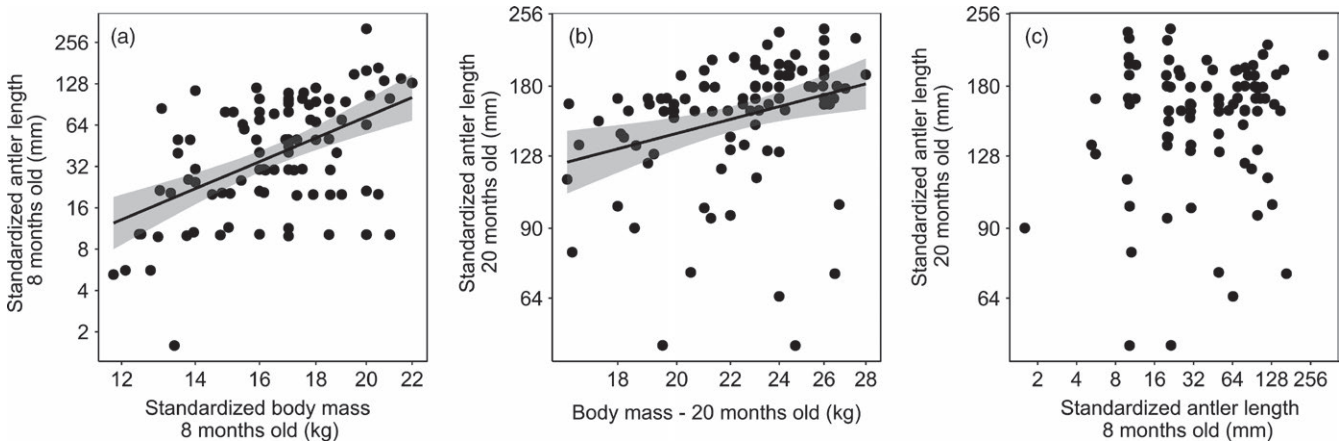

FIGURE 3 Relationship between fawn standardized antler length (log-transformed, measured at 8 months of age) and fawn standardized body mass (log-transformed) (a). Relationship between yearling standardized antler length (log-transformed, measured at 20 months of age) and yearling body mass (log-transformed, measured at 20 months of age) (b). Relationship between yearling standardized antler length (log-transformed and measured at 20 months of age) and fawn standardized antler (log-transformed, measured at 8 months of age)

We analysed the relationship between body mass (log-transformed) and date of capture during the first year of life. In roe deer, fawns consistently gain mass during their first year of life (Hewison, Gaillard, Angibault, Van Laere, & Vincent, 2002) and the date of capture explains a substantial part of the observed variation in body mass during the first winter (Douhard et al., 2017). In contrast, although the exact date of birth was unknown for most individuals, we expect such a factor to be negligible because roe deer births are highly synchronized (80% of births occur within a range of 20 days; Gaillard, Delorme, Julien, & Tatin, 1993b). As expected, we found that body mass increased with date of capture (slope of 0.0011 ± 0.0004, N = 224; Figure S1). To analyse the possible long-term effect of allocation to fawn *relative* antler length (see statistical analysis), we thus standardized body mass by the Julian date (8 February), as done previously (Vanpé et al., 2007). We followed the same procedure for yearling antler length. As expected, there was no detectable variation in body mass in relation to date at this age (Figure S2; slope of 0.0003 ± 0.0005, N = 257), and the yearling body mass was thus not corrected for date of capture.

2.3 | Covariation between fawn and yearling antler length

We used two metrics to describe the level of allocation to antler growth during early life: fawn and yearling *absolute* standardized antler length. As we expected heavier males to allocate more resources to antler growth in absolute terms than lighter males (Vanpé et al., 2007), we measured the effect of *relative* allocation to antlers during the first two years of life by adding body mass (i.e. fawn body mass standardized for the date of capture or yearling non-standardized body mass) as a covariate in the corresponding set of models (see below).

Yearling antler size could potentially be influenced by the allocation to antlers the year before, and we thus first tested whether fawn and yearling antler length could be considered as independent. As the physiological pathway governing antler growth is the same across all ages (Sempéré & Lacroix, 1982), we expected a positive relationship to occur between *absolute* antler length during the first and second years of life, and we thus tested for a positive correlation between allocation to fawn and yearling antler growth using a linear regression. We replicated the analyses on *relative* (to body mass) measures of antler length. To do this, we did not include fawn and yearling body mass (log-transformed) to avoid multicollinearity issues. We thus first computed fawn *relative* antler length as the residuals from the linear regression between *absolute* standardized antler length and fawn standardized body mass (slope of 1.98 ± 0.37, N = 224; Figure 3a). We followed the same procedure to compute yearling *relative* antler length (slope of 0.68 ± 0.17, N = 257; Figure 3b). We then computed yearling *relative* antler length as the residuals from this linear regression between yearling *relative* antler length and body mass. Both fawn and yearling antler length were measured on 93 males (76 in Chizé and 17 in Trois-Fontaines).

Measures of fawn and yearling antler length were independent (*absolute* metrics: slope of 0.043 ± 0.033; Figure 3c, Table S3; *relative* metrics: slope of 0.001 ± 0.04; Table S4).

2.4 | Early and late prime-age adulthood traits

To assess the consequences of male allocation to antler growth during early life on performance during early and late prime-age adulthood, we used three survival metrics: the probability of reaching 3 years of age (survival metric for short-term survival), the probability of reaching 6 years of age (survival metric for long-term survival) and longevity. We also used two phenotypic traits that are positively associated with fitness in roe deer: adult body mass (Gaillard, Festa-Bianchet, Delorme, & Jorgenson, 2000) and adult antler length (Vanpé, Gaillard, Kjellander, Delorme, & Hewison, 2010). For a given male, adult body mass and antler length were measured as the median values of measures recorded between 4 and 6 years of age (body mass) and 4 and 8 years of

age (antler length), as these traits subsequently show senescence (Douhard et al., 2017; Vanpé et al., 2007). Longevity (from 1.5 to 14 years) was measured as the exact number of years an individual lived, including only those individuals that were intensively monitored by observations and that died from natural causes (i.e. individuals that were removed from the population for translocation or that died from human-related incidents such as car collisions and capture-related mortality were right-censored).

2.5 | Analysis of the consequences of strong allocation to antlers during early life

We investigated potential trade-offs between allocation to antlers during early life and both condition and survival during adulthood and possible between-population differences in these trade-offs. For each combination of late-prime trait (dependent variable) and *absolute* antler length in early life, a set of competing models was fitted (e.g. Table S5a). When testing the possible long-term deleterious consequences of allocation to *relative* antler length early in life, body mass (i.e. fawn standardized body mass or yearling non-standardized body mass) was added as a covariate in all models of the set. All models included the cohort (year of birth) fitted as a random effect (32 cohorts between 1975 and 2006). When analysing adult antler length, we also included adult body mass as a covariate (e.g. Table S5b) to measure the consequences of early allocation to antler growth on both *absolute* and *relative* adult antler length. For analysis of longevity, adult body mass and adult antler length, we fitted linear mixed-effects models ("lmer" function in the R package LME4, Bates, Mächler, Bolker, & Walker, 2015). For survival beyond 3 and 6 years of age, we used generalized linear mixed-effects models with survival entered as a binomial variable. We tested the short-term survival costs (i.e. survival beyond 3 years of age) only for yearling antler length as our sample size was too limited to investigate short-term survival costs in fawns. All traits except survival were log-transformed. For each set of analyses, we used AIC for model selection (see above for a full description of the procedure). All analyses were performed using R version 3.4.0 (R Core Team, 2015), and all results are reported as *M* ± *SE*.

In our dataset, two individuals had particularly short antlers for their age (one fawn with antlers measuring 1 mm and one yearling with antlers measuring 10 mm). All analyses were performed with and without these males, and in most cases, results were qualitatively similar (Table S5 vs. Table S6 for fawn antler length; Table S7 vs. Table S8 for yearling antler length). As we did not have any biological reason to exclude these males, we decided to present the results obtained with the complete dataset. We explicitly specified in the text when including these outliers mattered. Population-specific sample sizes used for each analysis are provided in Table 2.

TABLE 2 Sample sizes used per analysis. Samples sizes in brackets are population-specific (Chizé; Trois-Fontaines)

	Independent variables	
Dependent variables	**Fawn antler length**	**Yearling antler length**
Longevity	137 (94; 43)	180 (101; 79)
Survival beyond 6 years of age	137 (94; 43)	190 (105; 85)
Survival beyond 2 years of age		190 (105; 85)
Adult body mass	121 (98; 23)	126 (78; 48)
Adult antler length	111 (89; 22)	110 (70; 40)
Antler length (20 months)	93 (76; 17)	

3 | RESULTS

3.1 | Late prime-age adulthood consequences of allocation to fawn antler growth

Absolute and *relative* antler length were not associated with longevity or survival beyond 6 years of age (longevity: slope of 0.04 ± 0.05 and slope of 0.05 ± 0.06; survival: slope of 0.07 ± 0.20 and slope of −0.71 ± 0.47 for *absolute* and *relative* antler length, respectively). In all cases, the constant model was selected (Table S5a and S5b). However, when analysing the effect of *absolute* antler length on longevity, the selected model contained population as a fixed factor. For a median value of antler length, males lived about one year longer at Chizé than at Trois-Fontaines (Trois-Fontaines: 4.49 years 95% CI [3.78–5.33], *N* = 43; Chizé: 5.39 years 95% CI [4.80–6.05], *N* = 94, estimates averaging all cohorts). The selected model of variation in adult body mass included additive effects of *absolute* antler length (slope of 0.022 ± 0.08, *N* = 121) and population (Table S5a,

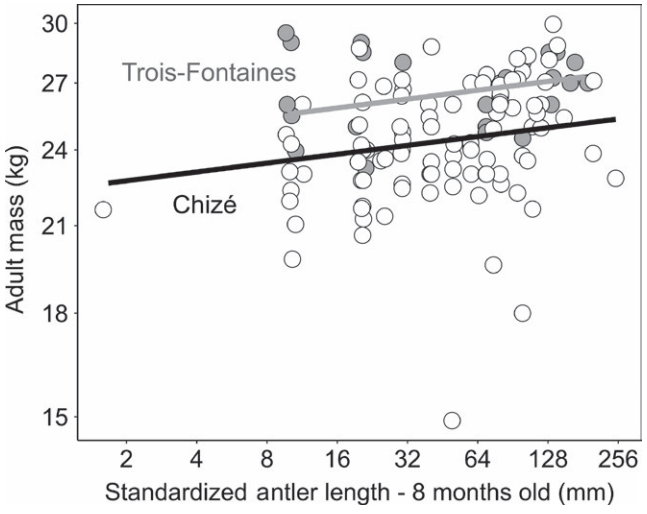

FIGURE 4 Relationship between adult body mass (log-transformed) and fawn *absolute* antler length (log-transformed and measured at 8 months of age). Chizé: dark line, open circle; Trois-Fontaines: grey line, grey points. The relationship and data are represented on a double logarithmic scale

Figure 4). For a median antler size, males were about 10% heavier at Trois-Fontaines than at Chizé (26.65 kg, 95% CI [25.59–27.76], *N* = 23 vs. 24.36 kg, 95% CI [23.89–24.85], *N* = 98, estimates averaging all cohorts). In the equivalent set of models for *relative* antler length (Table S5b), the selected model only included a positive effect of body mass (slope of 0.39 ± 0.06; Figure S3). The selected model for adult antler length only included a positive effect of adult body mass (slope of 0.61 ± 0.22, *N* = 111), as expected for this allometric relationship (Figure S4).

3.2 | Early and late prime-age adulthood consequences of yearling allocation to antler growth

No relationship occurred between allocation to *absolute* antler length and the probability of reaching 3 years of age (slope of −0.56 ± 0.48; Table S7a). On the other hand, yearling tended to suffer from carrying long antlers for their body mass (slope of −0.90 ± 0.55), even if the most parsimonious model included body mass only (Table S7b). Heavy individuals survived better (slope of 2.63 ± 1.29). Increasing by 1 kg the average body mass was associated with an increased survival of 2.5%.

Allocation to *absolute* antler length was not associated with any longevity or long-term survival cost in either roe deer population (slopes of −0.13 ± 0.11 and of −0.29 ± 0.35 for longevity and survival beyond six years, respectively; see Table S7a and S7b). Analyses of *relative* antler length suggested that antler length for a given mass was negatively associated with longevity (Table 3, Figure 5). Increasing the average antler size by 5 cm at a given mass led to a reduction in longevity by 4.84%. However, most models performed equally well (Table S7b). For survival beyond six years, the selected model included body mass and an interaction between population and antler length (Table S7b). At Chizé, a strong *relative* allocation to antler growth led to a decrease in the probability of surviving beyond six years of age (Table 4, Figure 6), while no relationship occurred at Trois-Fontaines (Table 4, Figure 5c,d). When the individual with 10-mm antlers was removed from the analysis, a negative effect of *relative* antler length was detected across both populations (Table S8b). Increasing the average relative antler size by 1 cm led survival to be reduced by 2.4%. Both *absolute* and *relative* antler length were independent of adult body mass (slope of 0.03 ± 0.02 and slope of −0.02 ± 0.01 for *absolute* and *relative* antler length, respectively; Table S7a and S7b). The selected model for *absolute* antler length only included population. Males were heavier at Trois-Fontaines than at Chizé, independently of antler growth. The selected model for *relative* antler revealed that body mass was positively associated with adult body mass (slope of 0.62 ± 0.05, *N* = 126). Finally, for adult antler length, the selected model contained both standardized antler length and body mass, revealing that relative antler length is positively associated with long antlers during adulthood (slope of 0.18 ± 0.08; Table S7a and S7b).

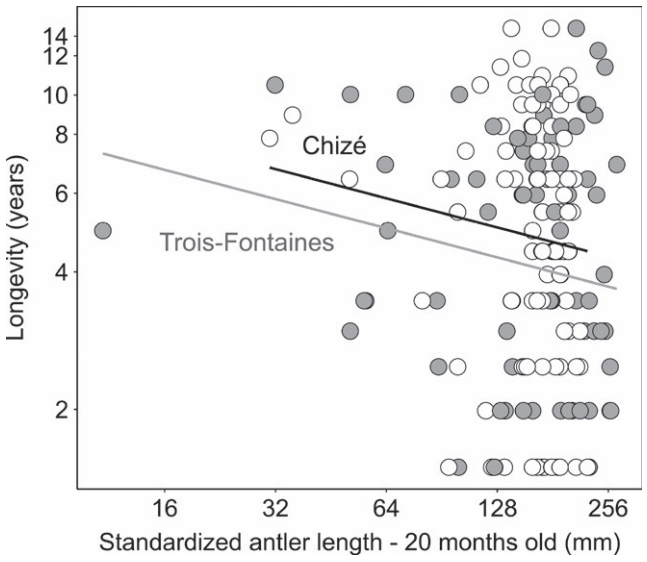

FIGURE 5 Relationship between the longevity and yearling *relative* antler length (at 20 months of age) for Chizé (dark line, open circle) and Trois-Fontaines (grey line, grey points). The relationship and data are represented on a double logarithmic scale

4 | DISCUSSION

Contrary to our predictions, we did not detect any evidence of delayed costs of allocation to antler growth during the first year of life in two roe deer populations living in highly different ecological contexts. Fawns with the longest (*absolute* or *relative*) antlers also had the highest body mass as adults, suggesting that the length of the first antlers reliably indicates individual performance during adulthood. This interpretation is supported by our finding that fawn antler length (but also yearling) was also positively associated with body mass, which indicates that antler length is a condition-dependent trait (Vanpé et al., 2007). In yearling, the growth of long *absolute* antlers has no influence on future male performance. However, for

TABLE 3 Parameter estimates from the model of variation in longevity as a function of *relative* allocation to antlers in yearlings (see Table S7b). Chizé is used as the reference population

	Estimates	*SE*	*t*
Intercept	−0.56	1.20	−0.47
Population	−0.15	0.10	−1.51
Yearling body mass	1.03	0.40	2.64**
Standardized yearling antler length	−0.21	0.11	−1.87

**$p < .01$.

TABLE 4 Parameter estimates from the selected models of variation in the probability of surviving beyond six years of age (on a logit scale) as a function of *relative* allocation to antlers in yearlings (see Table S7b). Chizé is used as the reference population

	Estimates	*SE*	*t*
Intercept	0.24	5.29	0.046
Population	−11.06	5.20	−2.13*
Standardized yearling antler length	−2.19	0.94	−2.33*
Yearling body mass	3.46	1.32	2.61**
Standardized yearling antler length × Population	2.11	1.02	2.08*

*$p < .05$; **$p < .01$.

a given body mass, yearlings with long antlers tend to be show a reduced longevity. The cost of growing large *relative* antlers as yearling was stronger on the probability of reaching 6 years of age, especially for males living in the poorest habitat (Chizé).

Although the exact physiological mechanisms regulating the full antler growth cycle are still debated (Bartos, Bubenik, & Kuzmova, 2012; Price & Allen, 2004), experimental manipulation of antler size has revealed that the annual growth and casting of antlers is governed by a complex interaction between photoperiod and several hormones, especially insulin-like growth factor 1 (IGF-1) and testosterone (Price & Allen, 2004). While the relative contribution of these hormones to antler size is difficult to decipher (Ditchkoff, Spicer, Masters, & Lochmiller, 2001), positive correlations between levels of testosterone and IGF-1 and antler length have been repeatedly reported (Bartos et al., 2012), notably in roe deer (Schams, Barth, Heinze-Mutz, Pflaum, & Karg, 1992; Sempéré & Boissin, 1981). An increasing number of studies have highlighted the potential deleterious effects of these hormones (Foo, Nakagawa, Rhodes, & Simmons, 2017), which are assumed to play a pivotal role in governing life-history trade-offs (Dantzer & Swanson, 2012), notably between allocation to sexual competition during early life and ageing (Brooks & Garratt, 2017). As yearlings with the longest *relative* antler length also carry long antlers as adults, repeated exposure to high hormonal levels might lead to reduced survival. The relationship between yearling and adult antler size suggests that yearlings with disproportionally large antlers will also have high competitive abilities over their entire life. Roe deer males are strongly territorial from early spring (March–April) to late August–early September (Andersen, Duncan, & Linnell, 1998), so that the costs of territory defence are likely higher than the costs associated with antler growth. Our results suggest that these costs might be higher when environmental conditions are poor, although the low sample size at Trois-Fontaines prevents to draw any firm conclusion. Overall, yearling males that grow long antlers for their mass might play a fast life-history tactic, involving the repeated defence of large territories, but impaired survival. Short antlers in yearlings should delay territoriality establishment (Vanpé et al., 2009), and thereby age at first mating. Delayed age at first

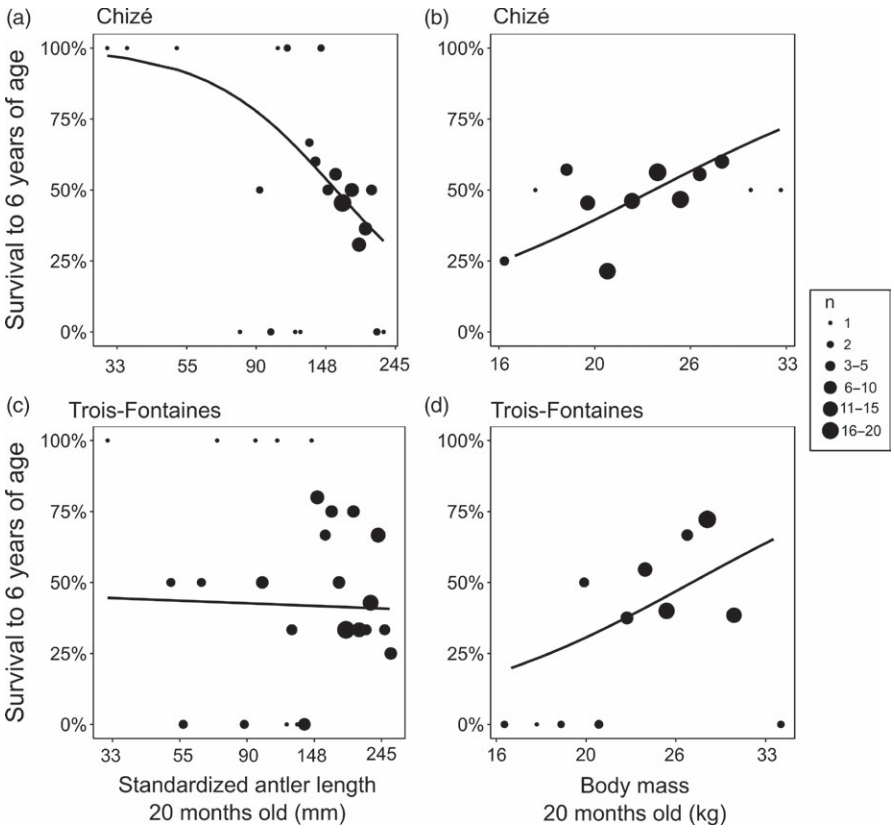

FIGURE 6 Relationship between the probability of surviving beyond 6 years of age and yearling *relative* antler length (at 20 months of age) in Chizé (a, b) and Trois-Fontaines (c, d). Points (of different size according to the sample size) indicate the average survival probabilities within each class of yearling *relative* antler length, or body mass. In both cases, the interval between two successive classes corresponds to an increment of 5%

reproduction is positively associated with longevity in mammalian females (e.g. Descamps, Boutin, Berteaux, & Gaillard, 2006), and our results suggest that this might also be the case in males. Yearlings that also grow *relatively* long antlers might suffer from more harassment by territorial males that are already established than those with smaller weapons (Wahlström, 1994), as suggested by the detected tendency for short-term survival costs.

A few recent studies have revealed that high levels of sexual competition can have long-term consequences in terms of age-specific decline in individual performance (Beirne, Delahay, & Young, 2015; Lemaître et al., 2014). More generally, one promising avenue of research linking functional traits and population performance would be to investigate the dynamics of antler length very early in life and its impact on population growth. Our study highlights that growing large secondary sexual traits early in life might be a reliable indicator of individual performance, suggesting that variation in the expression of sexual traits within populations might directly influence population demography. Integral projection models (IPMs) provide a powerful tool to investigate this question (Coulson, 2012), but so far, IPMs have mostly focused on body mass or size as the focal trait (Vindenes & Langangen, 2015) and most applications have been restricted to females. The recent development of two-sex models (Schindler et al., 2015) now offers a way to quantify how variation in the expression of sexual traits such as antler length influences the demography of a population, provided the required data (i.e. sex-specific relationships between demographic parameters [recruitment and survival] and antler length, father–offspring relationships and age-specific changes of antler length, pedigrees) will be available. As paternity analyses are becoming very common in wild vertebrates, we hope that our study will stimulate such studies that might reveal important insights on the role of sexual selection on population demography.

Finally, one striking paradox from our study is that the honest signal of individual performance conveyed by antler length is apparent in fawns when males do not compete for females. A possible explanation for the growth of long antlers in the pre-reproductive stage is the hormonal regulation of antler development that might be linked to physiological pathways involved in health and somatic maintenance. These constraints may have prevented natural selection from counterselecting the growth of substantial antlers in cervids well before they serve their purpose in male–male sexual contests (Sempéré & Lacroix, 1982). Future work should test whether long antlers in the first years of life reflect the quality of the ejaculate and thus, possibly, male breeding success (Malo, Roldan, Garde, Soler, & Gomendio, 2005). Whether this signal is already present in fawn antlers is unknown (but note that young roe deer with long antlers also have large testes; Wahlström, 1994). This is the missing piece of the jigsaw for our understanding of the relationships between age-specific functional traits, health and demographic parameters.

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AUTHORS' CONTRIBUTIONS

J.F.L. and J.M.G. conceived the ideas and designed methodology; all authors collected the data on both study sites; and J.F.L., F.D. and J.M.G. analysed the data. J.F.L. wrote the first draft of the manuscript with input from J.M.G. and A.J.M.H., and all authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

All data have been deposited on Dryad Digital Repository: https://doi.org/10.5061/dryad.vt169tn (Lemaître et al., 2018).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Appendix 1. *The influence of early-life allocation to antlers on male performance during adulthood: evidence from contrasted populations of a large herbivore.*

SUPPORTING INFORMATIONS

Table S1: Model selection for the relationship between fawn *absolute* antler length (log-transformed) and date of capture ($N = 224$) based on AIC and AICw (see methods). k is the number of parameters in the model, ΔAIC is the difference in AIC between the candidate model and the selected model (in bold) according to the rule of parsimony. Note that, in this analysis, no threshold was detected and the two threshold models (see methods) are similar to a linear model.

	k	AIC	ΔAIC	AICw
Constant	3	592.50	65.21	0.00
Linear	4	**527.29**	**0.00**	**0.33**
Threshold (1 slope)	4	527.29	0.00	0.33
Threshold (2 slopes)	4	527.29	0.00	0.33

Table S2: Model selection for the relationship between yearling *absolute* antler length (log-transformed) and date of capture ($N = 257$) based on AIC and AICw (see methods). k is the number of parameters in the model, ΔAIC is the difference in AIC between the candidate model and the selected model (in bold) according to the rule of parsimony.

	k	AIC	ΔAIC	AICw
Constant	3	268.33	128.32	0.00
Linear	4	178.43	38.42	0.00
Threshold (1 slope)	**4**	**140.01**	**0.00**	**0.72**
Threshold (2 slopes)	5	141.94	1.93	0.28

Table S3: Parameter estimates from the relationship between fawn and yearling *absolute* antler length ($N = 93$). This analysis was performed with a linear mixed-effects model with cohort fitted as a random effect.

	Estimate	SE	t	*p*
Intercept	4.89	0.13	38.88	< 0.001
Standardized fawn antler length	0.041	0.033	1.24	0.22

Table S4: Parameter estimates from the relationship between fawn and yearling *relative* antler length ($N = 93$). This analysis was performed with a linear mixed-effects model with cohort fitted as a random effect.

	Estimate	SE	t	*p*
Intercept	-0.004	0.03	-0.14	0.89
Relative fawn antler length at 8 months	0.001	0.04	0.03	0.98

Table S5: Model selection for the relationships between longevity (log-transformed), survival beyond 6 years of age, body mass (log-transformed), adult antler length and both *absolute* (a) and *relative* (b) fawn antler length (see methods for more details) based on AIC and AICw (see methods). k is the number of parameters in the model, ΔAIC is the difference in AIC between the candidate model and the selected model (in bold) according to the rule of parsimony. When the selected model differs from the model with the lowest AIC, this latter is given in italics.

		(a) Absolute Antler Length			(b) Relative Antler Length		
	k	AIC	ΔAIC	AICw	AIC	ΔAIC	AICw
Longevity (N = 137)							
Constant	**3**	**239.72**	**1.55**	**0.19**			
Standardized antler length	4	241.19	3.03	0.09			
Population	*4*	*238.16*	*0.00*	*0.41*			
Standardized antler length + Population	5	239.63	1.47	0.20			
Standardized antler length*Population	6	240.71	2.55	0.11			
Constant	**3**				**239.72**	**0.00**	**0.31**
Standardized body mass	4				241.41	1.70	0.13
Standardized antler length + Standardized body mass	5				242.68	2.96	0.07
Population + Standardized body mass	5				239.94	0.23	0.28
Standardized antler length + Population + Standardized body mass	6				241.54	1.82	0.13
Standardized antler length*Population + Standardized body mass	7				242.62	2.90	0.07
Survival beyond 6 years of age (N = 137)							
Constant	**2**	**192.10**	**0.00**	**0.43**			
Standardized antler length	3	193.96	1.86	0.17			
Population	3	193.17	1.07	0.25			
Standardized antler length + Population	4	195.01	2.91	0.10			
Standardized antler length*Population	5	196.38	4.28	0.05			
Constant	**2**				**192.10**	**0.00**	**0.40**
Standardized body mass	3				193.64	1.54	0.18
Standardized antler length + Standardized body mass	4				195.60	3.50	0.07
Population + Standardized body mass	4				193.27	1.17	0.22
Standardized antler length + Population + Standardized body mass	5				195.27	3.17	0.08

Standardized antler length*Population + Standardized body mass	6				196.55	4.45	0.04
Adult Body Mass (N = 121)							
Constant	3	-205.38	10.55	0.00			
Standardized antler length	4	-206.03	9.91	0.00			
Population	4	-213.39	2.54	0.14			
Standardized antler length + Population	**5**	**-215.93**	**0.00**	**0.48**			
Standardized antler length*Population	6	-215.41	0.52	0.37			
Constant	3				-205.38	38.81	0.00
Standardized body mass	**4**				**-244.19**	**0.00**	**0.43**
Standardized antler length + Standardized body mass	5				-242.21	1.99	0.16
Population + Standardized body mass	5				-243.12	1.07	0.25
Standardized antler length + Population + Standardized body mass	6				-241.30	2.90	0.10
Standardized antler length*Population + Standardized body mass	7				-240.51	3.69	0.07
Adult antler length (N = 111)							
Constant	3	1.90	5.71	0.02			
Standardized antler length	4	3.32	7.13	0.01			
Population	4	3.90	7.71	0.01			
Standardized antler length + Population	5	5.32	9.13	0.00			
Standardized antler length*Population	6	4.62	8.43	0.01			
Adult body mass	**4**	**-3.81**	**0.00**	**0.37**			
Standardized antler length + adult body mass	5	-1.83	1.98	0.14			
Population + adult body mass	5	-2.51	1.30	0.19			
Standardized antler length + Population + adult body mass	6	-0.52	3.29	0.07			
Standardized antler length*Population + adult body mass	7	-2.34	1.47	0.18			
Constant	3				1.90	6.90	0.01
Standardized body mass	4				3.90	8.89	0.00
Standardized antler length + Standardized body mass	5				5.28	10.27	0.00
Population + Standardized body mass	5				5.90	10.89	0.00
Standardized antler length + Population + Standardized body mass	6				7.27	12.26	0.00

Standardized antler length * Population + Standardized body mass	7		6.61	11.60	0.00
Adult body mass	**4**		**-3.81**	**1.18**	**0.19**
Adult body mass + Standardized body mass	*5*		*-4.99*	*0.00*	*0.34*
Standardized antler length + adult body mass + Standardized body mass	6		-3.28	1.71	0.14
Population + adult body mass + Standardized body mass	6		-3.05	1.94	0.13
Standardized antler length + Population + adult body mass + Standardized body mass	7		-1.30	3.69	0.05
Standardized antler length*Population + adult body mass + Standardized body mass	8		-3.07	1.92	0.13

Table S6: Model selection for the relationship between longevity (log-transformed), survival beyond 6 years of age, body mass (log-transformed), adult antler length and *absolute* (a) and relative (b) fawn antler length (see methods for more details) based on AIC and AICw (see methods). These analyses does not include the male with antlers of 1 mm. k is the number of parameters in the model, ΔAIC is the difference in AIC between the candidate model and the selected model (in bold) according to the rule of parsimony. When the selected model differs from the model with the lowest AIC, this latter is given in italics.

		(a) Absolute Antler Length			(b) Relative Antler Length		
	k	AIC	ΔAIC	AICw	AIC	ΔAIC	AICw
Longevity (N = 136)							
Constant	**3**	**238.79**	**1.46**	**0.19**			
Standardized antler length	4	239.95	2.62	0.10			
Population	*4*	*237.33*	*0.00*	*0.39*			
Standardized antler length + Population	5	238.53	1.20	0.21			
Standardized antler length*Population	6	239.87	2.54	0.11			
Constant	**3**				**238.79**	**0.00**	**0.31**
Standardized body mass	4				240.55	1.76	0.13
Standardized antler length + Standardized body mass	5				241.52	2.72	0.08
Population + Standardized body mass	5				239.06	0.27	0.27
Standardized antler length + Population + Standardized body mass	6				240.41	1.62	0.14
Standardized antler length*Population + Standardized body mass	7				241.76	2.96	0.07
Survival beyond 6 years of age (N = 136)							
Constant	**2**	**190.61**	**0.00**	**0.41**			
Standardized antler length	3	192.03	1.42	0.20			
Population	3	191.78	1.17	0.23			
Standardized antler length + Population	4	193.18	2.57	0.11			
Standardized antler length*Population	5	194.94	4.33	0.05			
Constant	**2**				**190.61**	**0.00**	**0.36**
Standardized body mass	3				191.89	1.28	0.19
Standardized antler length + Standardized body mass	4				193.56	2.95	0.08
Population + Standardized body mass	4				191.41	0.80	0.24

Standardized antler length + Population + Standardized body mass	5				193.28	2.67	0.09
Standardized antler length*Population + Standardized body mass	6				194.99	4.38	0.04
Adult Body Mass (N = 120)							
Constant	3	-203.59	9.58	0.00			
Standardized antler length	4	-203.49	9.68	0.00			
Population	**4**	**-211.55**	**1.62**	**0.20**			
Standardized antler length + Population	*5*	*-213.17*	*0.00*	*0.46*			
Standardized antler length*Population	6	-212.53	0.64	0.33			
Constant	3				-203.59	37.60	0.01
Standardized body mass	**4**				**-241.19**	**0.00**	**0.23**
Standardized antler length + Standardized body mass	5				-239.19	2.00	0.20
Population + Standardized body mass	5				-240.15	1.04	0.21
Standardized antler length + Population + Standardized body mass	6				-238.25	2.94	0.18
Standardized antler length*Population + Standardized body mass	7				-237.39	3.80	0.17
Adult antler length (N = 110)							
Constant	3	2.88	5.94	0.02			
Standardized antler length	4	4.06	7.12	0.01			
Population	4	4.88	7.94	0.01			
Standardized antler length + Population	5	6.06	9.12	0.00			
Standardized antler length*Population	6	5.68	8.74	0.00			
Adult body mass	**4**	**-3.06**	**0.00**	**0.38**			
Standardized antler length + adult body mass	5	-1.18	1.88	0.15			
Population + adult body mass	5	-1.75	1.31	0.20			
Standardized antler length + Population + adult body mass	6	0.17	3.23	0.08			
Standardized antler length*Population + adult body mass	7	-1.22	1.84	0.15			
Constant	3				2.88	6.96	0.01
Standardized body mass	4				4.88	8.96	0.00
Standardized antler length + Standardized body mass	5				6.02	10.10	0.00
Population + Standardized body mass	5				6.87	10.95	0.00

Standardized antler length + Population + Standardized body mass	6		8.01	12.09	0.00
Standardized antler length * Population + Standardized body mass	7		7.66	11.74	0.00
Adult body mass	**4**		**-3.06**	**1.02**	**0.20**
Adult body mass + Standardized body mass	*5*		*-4.08*	*0.00*	*0.33*
Standardized antler length + adult body mass + Standardized body mass	6		-2.59	1.49	0.16
Population + adult body mass + Standardized body mass	6		-2.14	1.94	0.13
Standardized antler length + Population + adult body mass + Standardized body mass	7		-0.61	3.47	0.06
Standardized antler length*Population + adult body mass + Standardized body mass	8		-1.94	2.14	0.11

Table S7: Model selection for the relationship between longevity (log-transformed), survival beyond 6 years of age, body mass (log-transformed), adult antler length and *absolute* (a) and relative (b) yearling antler length (see methods for more details) based on AIC and AICw (see methods). k is the number of parameters in the model, ΔAIC is the difference in AIC between the candidate model and the selected model (in bold) according to the rule of parsimony. When the selected model differs from the model with the lowest AIC, this latter is given in italics.

		(a) Absolute Antler Length			(b) Relative Antler Length		
	k	AIC	ΔAIC	AICw	AIC	ΔAIC	AICw
Longevity (N = 180)							
Constant	3	**359.72**	**0.00**	**0.37**			
Standardized antler length	4	360.32	0.59	0.27			
Population	4	361.30	1.57	0.17			
Standardized antler length + Population	5	361.80	2.08	0.13			
Standardized antler length*Population	6	363.32	3.60	0.06			
Constant	3				359.72	2.90	0.06
Body mass (20 months)	4				**358.02**	**1.19**	**0.15**
Standardized antler length + Body mass	5				357.14	0.31	0.24
Population + Body mass	5				358.38	1.55	0.13
Standardized antler length + Population + Body mass	6				*356.83*	*0.00*	*0.28*
Standardized antler length*Population + Body mass	7				358.16	1.33	0.14
Survival beyond 3 years of age (N = 190)							
Constant	2	**214.33**	**0.00**	**0.39**			
Standardized antler length	3	214.80	0.47	0.31			
Population	3	216.31	1.98	0.14			
Standardized antler length + Population	4	216.78	2.45	0.11			
Standardized antler length*Population	5	218.77	4.45	0.04			
Constant	2				214.33	3.62	0.06
Body mass (20 months)	3				**212.11**	**1.40**	**0.18**
Standardized antler length + Body mass	4				*210.71*	*0.00*	*0.36*
Population + Body mass	4				213.44	2.73	0.09

Standardized antler length + Population + Body mass	5				211.65	0.94	0.22
Standardized antler length*Population + Body mass	6				213.58	2.87	0.09
Survival beyond 6 years of age (N = 190)							
Constant	2	**266.36**	**0.00**	**0.38**			
Standardized antler length	3	267.66	1.30	0.20			
Population	3	268.35	1.99	0.14			
Standardized antler length + Population	4	269.65	3.28	0.07			
Standardized antler length*Population	5	267.48	1.12	0.21			
Constant	2				266.36	4.17	0.06
Body mass	3				264.36	2.16	0.16
Standardized antler length + Body mass	4				264.48	2.28	0.15
Population + Body mass	4				265.74	3.54	0.08
Standardized antler length + Population + Body mass	5				265.52	3.33	0.09
Standardized antler length*Population + Body mass	6				**262.20**	**0.00**	**0.47**
Adult Body Mass (N = 126)							
Constant	3	-219.81	6.85	0.02			
Standardized antler length	4	-220.00	6.66	0.02			
Population	4	**-225.81**	**0.85**	**0.31**			
Standardized antler length + Population	5	*-226.66*	*0.00*	*0.48*			
Standardized antler length*Population	6	-224.66	2.00	0.18			
Constant	3				-219.81	111.72	0.00
Body mass	4				**-331.53**	**0.00**	**0.34**
Standardized antler length + Body mass	5				-331.36	0.17	0.32
Population + Body mass	5				-329.97	1.56	0.16
Standardized antler length + Population + Body mass	6				-329.61	1.92	0.13
Standardized antler length*Population + Body mass	7				-327.62	3.91	0.05
Adult antler length (N = 110)							
Constant	3	58.95	3.85	0.03			

Standardized antler length	**4**	**55.10**	**0.00**	**0.20**			
Population	4	59.81	4.72	0.02			
Standardized antler length + Population	5	56.17	1.07	0.12			
Standardized antler length*Population	6	55.78	0.68	0.14			
Adult body mass	4	59.66	4.56	0.02			
Standardized antler length + adult body mass	5	56.03	0.93	0.13			
Population + adult body mass	5	59.51	4.41	0.02			
Standardized antler length + Population + adult body mass	6	56.25	1.15	0.11			
Standardized antler length*Population + adult body mass	7	55.13	0.03	0.20			
Constant	3				58.95	2.35	0.06
Body mass	4				60.36	3.77	0.03
Standardized antler length + Body mass	**5**				**56.98**	**0.38**	**0.15**
Population + Body mass	5				60.28	3.68	0.03
Standardized antler length + Population + Body mass	6				57.58	0.98	0.11
Standardized antler length * Population + Body mass	7				*56.60*	*0.00*	*0.19*
Adult body mass	4				59.66	3.06	0.04
Adult body mass + Body mass	5				61.62	5.02	0.02
Standardized antler length + adult body mass + Body mass	6				*57.44*	*0.84*	*0.12*
Population + adult body mass + Body mass	6				61.50	4.90	0.02
Standardized antler length + Population + adult body mass + Body mass	7				58.03	1.43	0.09
Standardized antler length*Population + adult body mass + Body mass	8				57.10	0.50	0.14

Table S8: Model selection for the relationship between longevity (log-transformed), survival beyond 6 years of age, body mass (log-transformed), adult antler length and *absolute* (a) and relative (b) yearling antler length (see methods for more details) based on AIC and AICw (see methods). These analyses do not include the male with antlers measuring 10 cm at 20 months of age. k is the number of parameters in the model, ΔAIC is the difference in AIC between the candidate model and the selected model (in bold) according to the rule of parsimony. When the selected model differs from the model with the lowest AIC, this latter is given in italics.

		(a) Absolute Antler Length			(b) Relative Antler Length		
	k	AIC	ΔAIC	AICw	AIC	ΔAIC	AICw
Longevity (N = 179)							
Constant	**3**	**358.74**	**0.00**	**0.35**			
Standardized antler length	4	359.15	0.41	0.29			
Population	4	360.29	1.56	0.16			
Standardized antler length + Population	5	360.68	1.94	0.13			
Standardized antler length*Population	6	362.33	3.60	0.06			
Constant	3				358.74	2.84	0.06
Body mass (20 months)	**4**				**356.80**	**0.90**	**0.16**
Standardized antler length + Body mass	5				356.15	0.25	0.23
Population + Body mass	5				356.92	1.03	0.15
Standardized antler length + Population + Body mass	6				*355.90*	*0.00*	*0.26*
Standardized antler length*Population + Body mass	7				357.20	1.30	0.13
Survival beyond 3 years of age (N = 189)							
Constant	**2**	**213.77**	**0.00**	**0.43**			
Standardized antler length	3	214.65	0.88	0.27			
Population	3	215.74	1.96	0.16			
Standardized antler length + Population	4	216.62	2.85	0.10			
Standardized antler length*Population	5	218.62	4.85	0.04			
Constant	2				213.77	3.24	0.06
Body mass	**3**				**210.96**	**0.42**	**0.24**
Standardized antler length + Body mass	4				*210.53*	*0.00*	*0.29*
Population + Body mass	4				211.98	1.45	0.14
Standardized antler length + Population + Body mass	5				211.41	0.88	0.19

Standardized antler length*Population + Body mass	6				213.26	2.72	0.08
Survival beyond 6 years of age (N = 189)							
Constant	**2**	**265.11**	**0.02**	**0.30**			
Standardized antler length	3	265.10	0.00	0.31			
Population	3	267.11	2.02	0.11			
Standardized antler length + Population	4	267.10	2.00	0.11			
Standardized antler length*Population	5	266.37	1.27	0.16			
Constant	2				265.11	3.49	0.06
Body mass	3				263.66	2.04	0.13
Standardized antler length + Body mass	**4**				**262.40**	**0.78**	**0.25**
Population + Body mass	4				265.17	3.54	0.06
Standardized antler length + Population + Body mass	5				263.69	2.07	0.13
Standardized antler length*Population + Body mass	6				*261.62*	*0.00*	*0.37*
Adult Body Mass (N = 125)							
Constant	3	-221.37	7.55	0.01			
Standardized antler length	4	-219.58	9.35	0.01			
Population	**4**	**-228.92**	**0.00**	**0.60**			
Standardized antler length + Population	5	-227.16	1.76	0.25			
Standardized antler length*Population	6	-225.86	3.06	0.13			
Constant	3				-221.37	106.81	0.00
Body mass	4				**-328.17**	**0.00**	**0.38**
Standardized antler length + Body mass	5				-327.67	0.51	0.29
Population + Body mass	5				-326.48	1.69	0.16
Standardized antler length + Population + Body mass	6				-325.93	2.24	0.12
Standardized antler length*Population + Body mass	7				-323.94	4.24	0.05

Fig. S1: Relationship between male body mass (log-transformed) measured at 8 months of age and the Julian date of capture (Day 0 = December 1st) (slope of the relationship: 0.001 ± 0.0005).

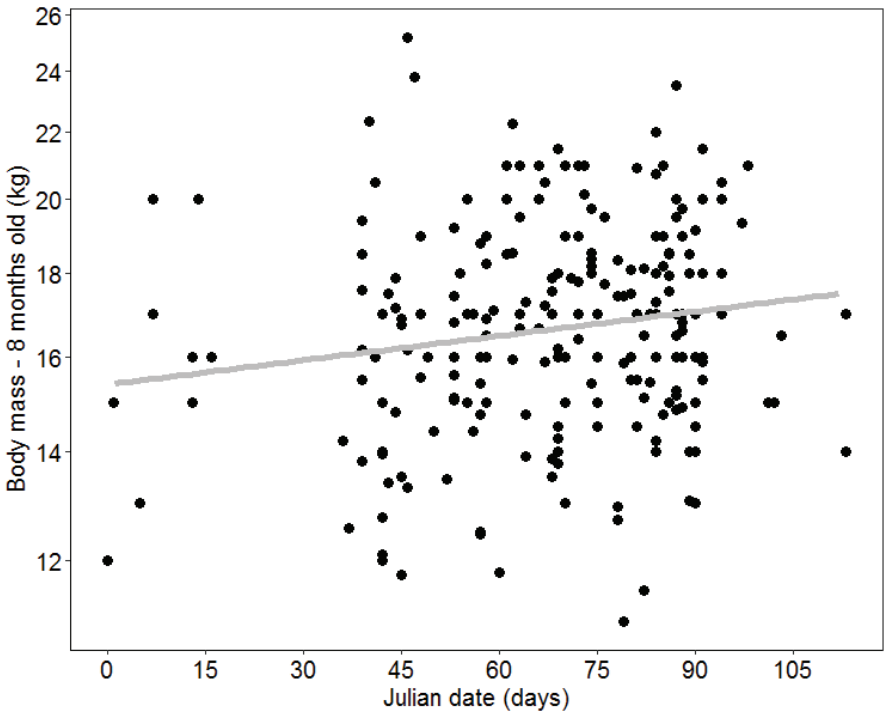


Fig. S2: Relationship between male body mass (log-transformed) measured at 20 months of age and the Julian date of capture (Day 0 = December 1st).

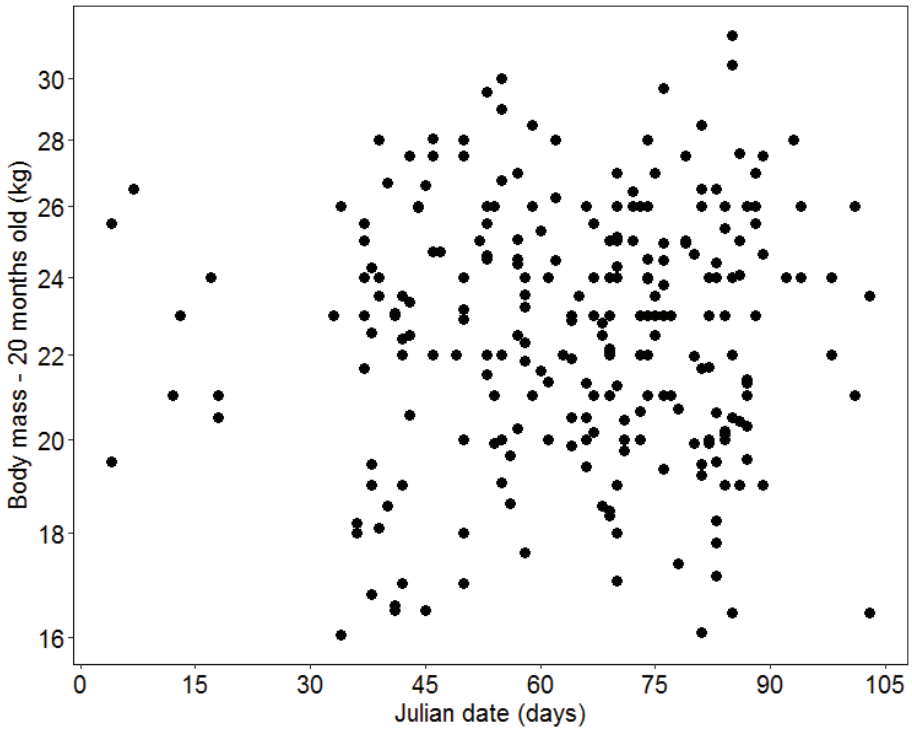


Fig. S3: Relationship between adult body mass (log-transformed) and body mass measured at 8 months of age (standardized to 8^{h} February) (slope of the relationship: 0.39 ± 0.06).

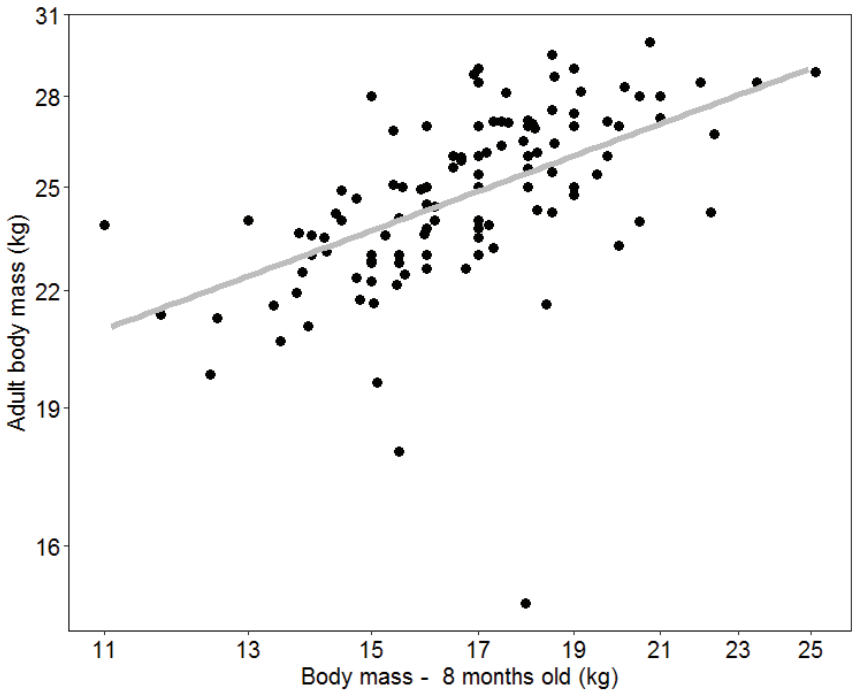
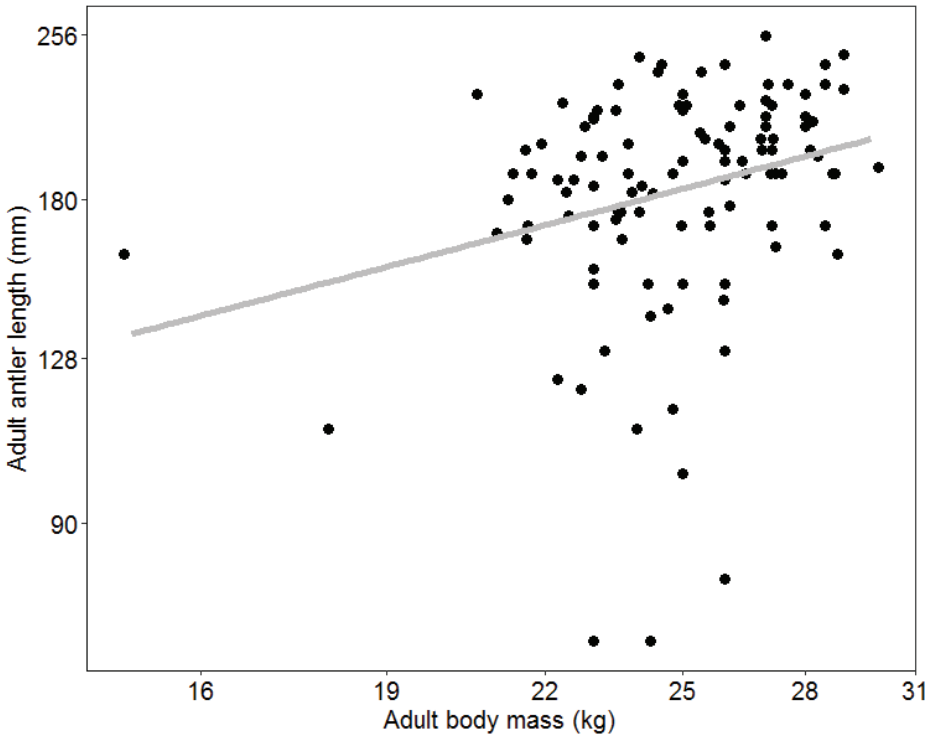
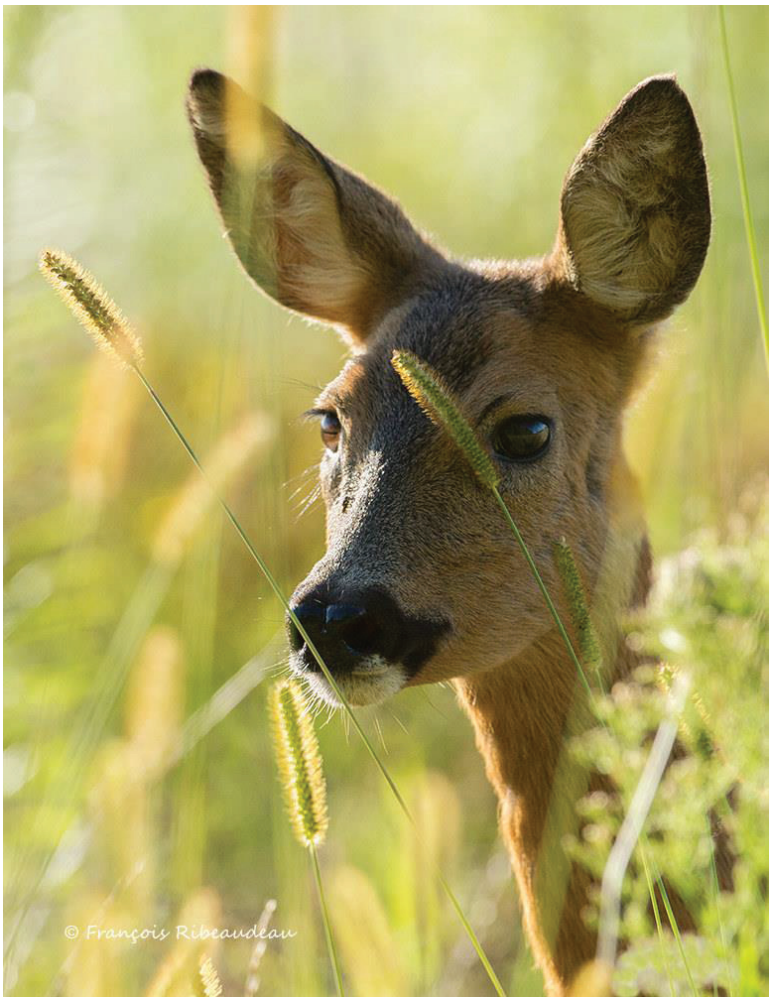


Fig. S4: Allometric relationship between adult antler length and adult body mass (on a log-scale) (slope of the relationship: 0.06 ± 0.22).



Appendix 2.

Glucocorticoids level predicts short-term telomere dynamics in wild roe deer.



Glucocorticoids level predicts short-term telomere dynamic in wild roe deer.

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- In preparation -

ABSTRACT

Among the biological mechanisms that underlie life-history trade-offs in vertebrates, the progressive loss of telomeres throughout life might play a key role. How environmental conditions influence the pace of telomere attrition in wild animal populations has thus become a crucial question. We investigated roe deer (*Capreolus capreolus*) in two different populations to test the hypothesis that high glucocorticoids in individuals facing environmental stressors accelerate telomere loss. Using two consecutive yearly sampling sessions, we found that roe deer displayed highly repeatable concentrations of faecal glucocorticoid metabolites, suggesting consistent between-individual differences in the activation of the stress response. As expected, a positive association linked the rate of telomere shortening in leukocytes between years and the concentration of glucocorticoids among roe deer. These findings were consistent between sexes and populations. Overall, our findings highlight the influence of stress in telomere dynamics and open the door for further studies of the link between physiological responses to environmental stressors and life-history trade-offs.

Keywords: Aging - *Capreolus capreolus* - Faecal Glucocorticoid Metabolite - Life-history - Stress.

Introduction

Telomeres are non-coding and repetitive DNA sequences enabling to maintain the integrity of eukaryotic linear chromosomes throughout cellular divisions [1]. Age-specific changes in telomere length play a pivotal role in life-history trade-offs [2,3] and negative relationship occur between telomere length and survival prospects across individuals (see [4] for a meta-analysis in wild vertebrates). We should now understand how environmental conditions and organismal biology interact to influence telomere dynamic [5].

In that context, stress level has been proposed to influence telomere dynamic [6,7]. Among other processes, environmental stressors (e.g. exposure to predators, food shortage) stimulate the hypothalamic-pituitary-adrenal axis triggering the release of glucocorticoids by the adrenal gland cortex [8]. Chronically elevated glucocorticoids have multiple adverse effects on growth, reproduction and immunity, and are expected to accelerate telomere shortening (see [6,7]). Although this chain reaction is well known [6,7,9], empirical studies testing the link between stress and telomere shortening are scarce and mostly relied on non-physiological measures of stress [6,9]. For instance, women with the highest level of life stress carry chromosomes with shorter telomeres (measured in peripheral blood mononuclear cells) [10].

In wild populations, differential activation of the stress axis is also a likely candidate for controlling the relationship between telomere length and habitat quality [7,9,11]. However, our current understanding of the link between stress level and telomere dynamics is weak in wild populations as measuring the level of stress in wild-living animals is challenging [12]. The few studies conducted so far focused on telomere length rather than telomere dynamics and have led to mixed results [7]. To fill this knowledge gap, we aimed to test whether glucocorticoid levels predict year-to-year telomere attrition rates in roe deer (*Capreolus capreolus*) in two populations living in different environments. We expected the relationship between glucocorticoid levels and telomere loss to be stronger in the more food-limited population.

Material and Methods

STUDY POPULATION AND LABORATORY ASSAYS

We studied two populations of roe deer living in enclosed forests. Trois-Fontaines forest (TF - 1,360 ha), located in north-eastern France (48°43'N, 4°55'E), has rich soils and provides high quality habitat for roe deer. In contrast, Chizé forest (CH - 2,614 ha), located in western France (46°50'N, 0°25'W), has low productivity due to poor quality soils and frequent summer droughts and thereby provides a poor habitat for roe deer relatively to the TF forest [13]. Every year and for each site, 10-12 days of

capture are organized between December and March since the mid-seventies as part of a long-term Capture-Mark-Recapture program [13]. Upon capture, individuals are sexed, weighed to within 50g and a basic clinical examination is performed.

In the 2016 and 2017, we collected blood samples on heparin (up to 1mL/kg) from the jugular vein. Buffy coat fractions containing leucocytes were isolated after centrifugation and immediately frozen at -80°C in a portable freezer (Telstar SF 8025) until DNA extraction. Relative telomere length (hereafter qRTL) was measured by quantitative PCR as previously described for this population (see [12] and supplementary information for details on qRTL measurements). As observed in many wild populations of vertebrates (e.g. [14] in Seychelles warbler, *Acrocephalus sechellensis*), individual qRTL did not consistently decrease during the year (figure S1). Instead, only 51.2% (22 out of 43 individuals) showed a decrease in telomere length between 2016 and 2017.

Baseline glucocorticoid levels by the time of captures were assessed by measuring fecal glucocorticoid metabolites (hereafter FGM). Feces were collected rectally and frozen immediately at -80°C in the field until assayed (except for TF samples from 2016 that were stored at -20°C). The FGM were extracted following a methanol-based procedure and assayed using a specific 11,17-dioxoandrostanes enzyme immunoassay as previously described and validated for roe deer [15]. Briefly, 500±5mg of homogenized fecal sample was vortexed for 30 min with 5ml of 80% methanol before being centrifuged (15 min at 2500*g*). FGM were then quantified in an aliquot of the supernatant diluted 1:10 with assay buffer. Measurements were carried out in duplicate (intra- and inter-assay coefficients of variation were: XX% and XX%, respectively) and the results expressed as nanograms per gram fecal wet weight. One male from the TF population with an extremely low (and non-explained) level of FGM in 2017 (only 8 ng/g) compared to other roe deer was removed from the analyses. We assessed the average level of stress across both years by computing the mean FGM value between 2016 and 2017 measures. Concentrations of FGM in 2016 and 2017 were correlated ($r = 0.42$, $p = 0.02$, $n = 30$, figure S2); with a repeatability of 0.22, which in the range of values generally observed for FGM [13]. However, this relationship was highly influenced by a male in CH having the highest level of FGM of the dataset in both 2016 and 2017 (without this individual: $r = 0.18$, $p = 0.34$, $n = 29$, repeatability of 0.32).

STATISTICAL ANALYSES

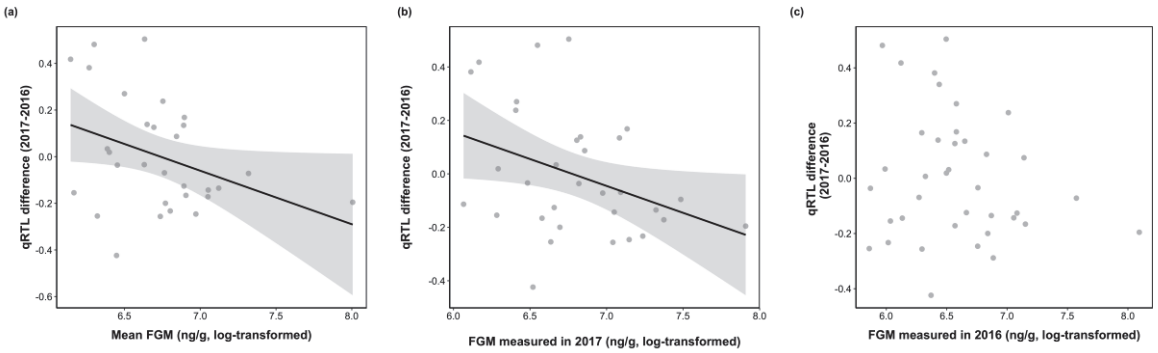
We performed a set of analyses seeking to understand whether qRTL changes (computed as the difference in qRTL between 2016 and 2017) could be explained by the stress level experienced during the period. We analyzed the influence of mean FGM and FGM measured in both 2016 and 2017 and separately (see Table S1 for sample sizes). For each FGM variable entered as the independent covariate, we performed a set of linear models that also included a possible additive or interactive effect of sex, population and log-transformed body mass (using body mass measured in 2016, 2017 or the mean body

mass for model with FGM in 2016, FGM in 2017 and mean FGM, respectively) on the change of qRTL. Finally, we also included age as covariate to control for any possible age-effect on telomere length [12] (see Table S2 for the full set of models). To avoid fitting over-parameterized models, we did not include more than two covariates simultaneously in the models and avoided three-way interaction (Table S2). We repeated the analyses without individuals sampled in their first year of life (i.e eight month, Table S3) because the dynamic of telomere attrition can be much faster in early life compared to adulthood in wild mammals (e.g. [16]). Since some roe deer were born in the same year, we repeated the analyses by including cohort (2003 to 2015) as a random effect (Table S4), using linear mixed effect models ('lmer' function in the R package lme4). In all cases, results were qualitatively unchanged (Table S3 to S4). Our results were also not impacted by inter-individual differences in number of days between the 2016 and 2017 capture session (mean number of days ± SE: 368.8 ± 2.26; Table S5). Model selection was based on the Akaike Information Criterion (AIC) [17] and we retained the model with the lowest AIC except when the difference in AIC (ΔAIC) between two competing models was less than 2, in which case we retained the simplest model.

Results

Our model selection procedure revealed that the change in qRTL between these two years was negatively associated with the average FGM across 2016 and 2017 (slope of -0.23 ± 0.11, *n*= 30, figure 2a). Likewise, FGM of roe deer captured in 2017 was also negatively associated with the change in qRTL (slope of -0.20 ± 0.09, *n*= 32, figure 2b). For FGM in 2017 and mean FGM the negative association remained significant when individuals captured at eight months old in 2016 were removed from the analyses (slope of -0.27 ± 0.12, *n*= 20 and slope of -0.29 ± 0.14, *n*= 19 respectively, Table S3). Population, sex and body mass had no detectable effect on short-term telomere dynamics. For FGM in 2016, the constant model was selected (Table S2 and Table S3, figure 2c).

Figure 1: Relationship between the differences in qRTL between 2017 and 2016 and the mean level of FGM across both years (a), the level of FGM measured in 2017 (b) or 2016 (c).



Discussion

A few studies reported that individuals facing harsh environmental conditions have shorter telomeres [11,18]. Results from our study support the hypothesis that activity of the stress axis could mediate the relationship between environmental conditions and telomere dynamics [6,7] as revealed by the influence of glucocorticoid levels on the pace of telomere attrition. Environmental conditions affect most life history traits through a cascade of physiological responses, some of which are mediated by glucocorticoids and contribute to accelerated telomere attrition. While an alteration of the redox homeostasis is often involved [6,7], evidence that oxidative stress causes increased telomere loss is equivocal in wild populations [19]. Future work should seek to integrate multiple measures of oxidative damages and antioxidant defenses together with measures of glucocorticoids and environmental conditions to decipher the physiological connections linking environment, stress hormones and telomere dynamics.

These results raise the question of what environmental factors could be responsible for elevated and repeatable levels of glucocorticoids in some animals, which ultimately undergo an accelerated telomere shortening. Since both study sites are enclosed forests with no predators, environmental harshness could at first glance constitutes a reasonable explanation. Everything else being equal, we thus expected a stronger concentration in fecal glucocorticoid metabolites in the poor-quality habitat (Chizé) than in the high-quality habitat (Trois-Fontaines), which was actually not the case (Chizé: 973.98 ng/g, 95% CI [717.93;1230.04]; Trois-Fontaines: 763.39ng/g, 95% CI [650.28;876.50]). This absence of population difference could be explained as an adaptive downregulation of the stress response in populations facing harsh environmental conditions (see [20] for similar observations in black-tailed deer, *Odocoileus hemionus sitkensis*). At the population level, the between-individual variability in glucocorticoids concentration might be a consequence of individuals living in contrasted habitats in terms of food access or parasite prevalence, or a consequence of individual differences in the way individuals respond to environmental conditions and metabolize cortisol [13]. Roe deer are highly sedentary animals that generally live in the same small home range (20-50 ha in forest) all along their life [21]. Individuals living in poor habitats, or highly reactive in terms of stress response, might thus repeatedly release high quantity of glucocorticoids and be at greater risk of chronic stress. If correct, this hypothesis would suggest that telomere dynamics might vary according to fine-scale differences in environmental conditions, which so far has never been investigated in free-ranging populations.

Ethics. The protocol of capture and blood sampling of roe deer under the authority of the Office National de la Chasse et de la Faune Sauvage (ONCFS) was approved by the Director of Food, Agriculture and Forest (Prefectoral order 2009–14 from Paris). The land manager of both sites, the Office National des Forêts (ONF), permitted the study of the populations (Partnership Convention ONCFS-ONF dated 2005-12-23). All experiments were performed in accordance with guidelines and regulations of the Ethical Committee of Lyon 1 University (project DR2014-09, June 5, 2014).

Data accessibility. Data available will be downloaded on dryad once the manuscript accepted.

Authors' contributions. JFL & EGF conceived and designed the study. JFL, BR, LC, JMG, AJM, HV, FD, JD, SP, MP, EGF performed fieldwork. BR, CR and LC extracted DNA. RP, JC, HV, BR ran FGM assays. HF, RW and DN ran telomere assays. JFL performed the statistical analysis, wrote the first draft of the paper and then received input from all other co-authors.

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1/ Supplementary tables

Table 1: Sex- and population-specific sample size of individuals used in the analyses. (a) Number of individuals with data on Relative Telomere Length (RTL) in 2016 and 2017, (b) Number of individuals with data on both fecal glucocorticoid metabolites (FGM) in 2016 and RTL in both 2016 and 2017, (c) Number of individuals with data on both FGM in 2017 and RTL in both 2016 and 2017, (d) Number of individuals with data on both FGM in 2016 and 2017 and RTL in both 2016 and 2017. The age range of individuals from a given subset is in brackets (for (a) and (d), the age range corresponds to the age range in 2016 and 2017 respectively).

(a) RTL (2016 and 2017)

	Males	Females	Both sexes
Trois-Fontaines	12 (age: 1-9)	13 (age: 1-13)	25 (age: 1-13)
Chizé	6 (age: 1-7)	12 (age: 2-13)	18 (age: 1-13)
Both populations	18 (age: 1-9)	25 (age: 1-13)	43 (age: 1-13)

(b) FGM (2016)

	Males	Females	Both sexes
Trois-Fontaines	11 (age: 1-9)	9 (age: 1-11)	20 (age: 1-11)
Chizé	6 (age: 1-7)	12 (age: 2-13)	18 (age: 1-13)
Both populations	17 (age: 1-9)	21 (age: 1-13)	38 (age: 1-13)

(c) FGM (2017)

	Males	Females	Both sexes
Trois-Fontaines	8 (age: 2-8)	6 (age: 2-14)	14 (age: 2-14)
Chizé	6 (age: 2-8)	12 (age: 3-14)	18 (age: 2-14)
Both populations	14 (age: 2-8)	18 (age: 2-14)	32 (age: 2-14)

(d) FGM (2016 and 2017)

	Males	Females	Both sexes
Trois-Fontaines	8 (age: 2-8)	4 (age: 2-9)	12 (age: 2-9)
Chizé	6 (age: 2-8)	12 (age: 2-14)	18 (age: 2-14)
Both populations	14 (age: 2-8)	16 (age: 2-14)	30 (age: 2-14)

Table S2: AIC summary of the different linear models testing the relationship between changes in relative telomere length (2017-2016) and the concentration of faecal glucocorticoid metabolites (FGM) measured in 2016 (a), 2017 (b) or across both years (c). The selected model is displayed with dark grey shading, K is the number of parameters in the model, ΔAIC is the difference in AIC between the candidate model and the selected model.

		(a) FGM (2016) - n=38			(b) FGM (2017) - n=32			(c) Mean FGM (2016-2017) - n = 30		
	k	AIC	ΔAIC	AICw	AIC	ΔAIC	AICw	AIC	ΔAIC	AICw
Constant	2	-1.11	0.00	0.11	0.86	2.64	0.05	2.60	2.26	0.05
FGM	3	-0.38	0.73	0.07	-1.78	0.00	0.18	0.34	0.00	0.15
Population	3	0.89	2.00	0.04	2.81	4.59	0.02	4.60	4.26	0.02
Sex	3	-0.39	0.72	0.07	1.90	3.68	0.03	3.91	3.57	0.03
Mass	3	0.22	1.33	0.05	2.85	4.63	0.02	4.35	4.01	0.02
Age	3	0.09	1.20	0.06	2.49	4.27	0.02	4.05	3.70	0.02
FGM + Population	4	1.55	2.66	0.03	0.07	1.86	0.07	2.11	1.77	0.06
FGM * Population	5	3.10	4.21	0.01	2.06	3.84	0.03	4.11	3.77	0.02
FGM + Sex	4	0.72	1.83	0.04	-0.09	1.69	0.08	2.28	1.94	0.06
FGM * Sex	5	1.75	2.86	0.03	1.70	3.49	0.03	3.25	2.91	0.04
FGM + Mass	4	0.31	1.42	0.05	0.11	1.89	0.07	1.60	1.26	0.08
FGM * Mass	5	1.98	3.09	0.02	1.71	3.49	0.03	3.39	3.05	0.03
FGM + Age	4	0.30	1.41	0.05	-0.87	0.91	0.11	0.83	0.49	0.12
Population + Age	4	2.03	3.14	0.02	4.48	6.27	0.01	5.95	5.61	0.01
Sex + Age	4	-0.24	0.87	0.07	2.77	4.55	0.02	4.53	4.18	0.02
Mass + Age	4	0.74	1.85	0.04	4.48	6.26	0.01	5.73	5.39	0.01
FGM + Population + Age	5	2.30	3.41	0.02	1.13	2.91	0.04	2.83	2.49	0.04
FGM * Population + Age	6	3.61	4.72	0.01	3.07	4.85	0.02	4.82	4.48	0.02
FGM + Sex + Age	5	0.38	1.49	0.05	0.14	1.93	0.07	2.28	1.94	0.06
FGM * Sex + Age	6	1.43	2.54	0.03	1.70	3.48	0.03	3.93	3.59	0.03
FGM + Mass + Age	5	-0.74	0.37	0.09	1.00	2.78	0.04	1.80	1.45	0.07
FGM * Mass + Age	6	1.18	2.29	0.03	2.33	4.11	0.02	3.48	3.14	0.03

Table S3: AIC summary of the different linear models testing the relationship between changes in relative telomere length (2017-2016) and the concentration of faecal glucocorticoid metabolites (FGM) measured in 2016 (a), 2017 (b) or across both years (c). The selected model is displayed with dark grey shading and when different the model with lowest AIC is displayed with a light grey shading. K is the number of parameters in the model. ΔAIC is the difference in AIC between the candidate model and the selected model. Individuals captured at 8 months of age in 2016 are removed from this analysis.

		(a) FGM (2016) - n=25			(b) FGM (2017) - n=20			(c) Mean FGM (2016-2017) - n =19		
		AIC	ΔAIC	AICw	AIC	ΔAIC	AICw	AIC	ΔAIC	AICw
Constant	2	5.22	1.94	0.06	5.84	2.81	0.04	6.55	2.51	0.04
FGM	3	4.72	1.44	0.08	3.03	0.00	0.17	4.05	0.00	0.16
Population	3	7.09	3.81	0.02	7.68	4.65	0.02	8.18	4.14	0.02
Sex	3	5.15	1.87	0.06	6.24	3.20	0.03	7.15	3.11	0.03
Mass	3	7.09	3.81	0.02	7.50	4.47	0.02	8.29	4.25	0.02
Age	3	5.83	2.55	0.05	7.56	4.53	0.02	7.87	3.82	0.02
FGM + Population	4	6.71	3.43	0.03	4.62	1.59	0.08	5.99	1.94	0.06
FGM * Population	5	8.51	5.23	0.01	6.16	3.13	0.04	7.65	3.60	0.03
FGM + Sex	4	4.88	1.60	0.07	3.73	0.70	0.12	4.74	0.70	0.11
FGM * Sex	5	6.85	3.57	0.03	5.72	2.69	0.04	6.42	2.37	0.05
FGM + Mass	4	6.56	3.28	0.03	4.55	1.51	0.08	6.00	1.96	0.06
FGM * Mass	5	8.44	5.16	0.01	6.39	3.36	0.03	7.84	3.80	0.02
FGM + Age	4	4.58	1.30	0.08	4.87	1.84	0.07	5.31	1.27	0.08
Population + Age	4	7.73	4.45	0.02	9.45	6.42	0.01	9.44	5.39	0.01
Sex + Age	4	4.49	1.21	0.09	6.91	3.88	0.02	7.21	3.16	0.03
Mass + Age	4	7.57	4.29	0.02	9.18	6.15	0.01	9.30	5.25	0.01
FGM + Population + Age	5	6.54	3.26	0.03	6.52	3.49	0.03	7.22	3.18	0.03
FGM * Population + Age	6	8.30	5.02	0.01	7.59	4.56	0.02	8.59	4.55	0.02
FGM + Sex + Age	5	3.28	0.00	0.16	4.79	1.76	0.07	4.73	0.69	0.11
FGM * Sex + Age	6	5.24	1.96	0.06	6.75	3.72	0.03	6.72	2.68	0.04
FGM + Mass + Age	5	6.49	3.20	0.03	6.34	3.31	0.03	7.31	3.27	0.03
FGM * Mass + Age	6	8.42	5.13	0.01	7.89	4.85	0.02	9.07	5.02	0.01

Table S4: AIC summary of the different linear mixed effect models testing the relationship between changes in relative telomere length (2017-2016) and the concentration of fecal glucocorticoid metabolites (FGM) measured in 2016 (a), 2017 (b) or across both years (c). The selected model is displayed with dark grey shading and when different the model with lowest AIC is displayed with a light grey shading. K is the number of parameters in the model. ΔAIC is the difference in AIC between the candidate model and the selected model. Contrary to Table S2, the cohort is fitted as a random effect.

		(a) FGM (2016) - n=38			(b) FGM (2017) - n=32			(c) Mean FGM (2016-2017) - n = 30		
	k									
Constant	3	0.89	0.00	0.11	2.86	2.64	0.05	4.60	2.26	0.05
FGM	4	1.62	0.73	0.07	0.22	0.00	0.18	2.34	0.00	0.15
Population	4	2.89	2.00	0.04	4.81	4.59	0.02	6.60	4.26	0.02
Sex	4	1.61	0.72	0.07	3.90	3.68	0.03	5.91	3.57	0.03
Mass	4	2.22	1.33	0.05	4.85	4.63	0.02	6.35	4.01	0.02
Age	4	2.09	1.20	0.06	4.49	4.27	0.02	6.05	3.70	0.02
FGM + Population	5	3.55	2.66	0.03	2.07	1.86	0.07	4.11	1.77	0.06
FGM * Population	6	5.10	4.21	0.01	4.06	3.84	0.03	6.11	3.77	0.02
FGM + Sex	5	2.72	1.83	0.04	1.91	1.69	0.08	4.28	1.94	0.06
FGM * Sex	6	3.75	2.86	0.03	3.70	3.49	0.03	5.25	2.91	0.04
FGM + Mass	5	2.31	1.42	0.05	2.11	1.89	0.07	3.60	1.26	0.08
FGM * Mass	6	3.98	3.09	0.02	3.71	3.49	0.03	5.39	3.05	0.03
FGM + Age	5	2.30	1.41	0.05	1.13	0.91	0.11	2.83	0.49	0.12
Population + Age	5	4.03	3.14	0.02	6.48	6.27	0.01	7.95	5.61	0.01
Sex + Age	5	1.76	0.87	0.07	4.77	4.55	0.02	6.53	4.18	0.02
Mass + Age	5	2.74	1.85	0.04	6.48	6.26	0.01	6.93	4.58	0.02
FGM + Population + Age	6	4.30	3.41	0.02	3.13	2.91	0.04	4.83	2.49	0.04
FGM * Population + Age	7	5.61	4.72	0.01	5.07	4.85	0.02	6.82	4.48	0.02
FGM + Sex + Age	6	2.38	1.49	0.05	2.14	1.93	0.07	4.28	1.94	0.06
FGM * Sex + Age	7	3.43	2.54	0.03	3.70	3.48	0.03	5.93	3.59	0.03
FGM + Mass + Age	6	1.26	0.37	0.09	3.00	2.78	0.04	3.80	1.45	0.07
FGM * Mass + Age	7	3.18	2.29	0.03	4.33	4.11	0.02	5.48	3.14	0.03

Table S5: Parameter estimates of the best model describing the decline in RTL as a function of the mean concentration in faecal glucocorticoid metabolites (FGM) (A) or concentration in faecal glucocorticoid metabolites (FGM) measured in 2017 (B) with the number of days between the two capture events entered as a covariate.

(A)	Estimate	SE	t	*p*
Intercept	-0.64	1.21	-0.53	0.60
Mean FGM	-0.24	0.09	-2.61	0.01
Number of days between captures	0.01	0.00	1.92	0.07

(B)	Estimate	SE	t	*p*
Intercept	-0.12	1.44	-0.08	0.94
FGM (2017)	-0.24	0.11	-2.15	0.04
Number of days between captures	0.00	0.00	1.35	0.19

2/ Supplementary figures

Figure S1: Histogram and density function of the differences in qRTL. (a) Data split according to the population (C=Chizé. TF=Trois-Fontaines) – (b) Data split according to sex (F=Female. M=Male). Dash lines correspond to the median value for each group.

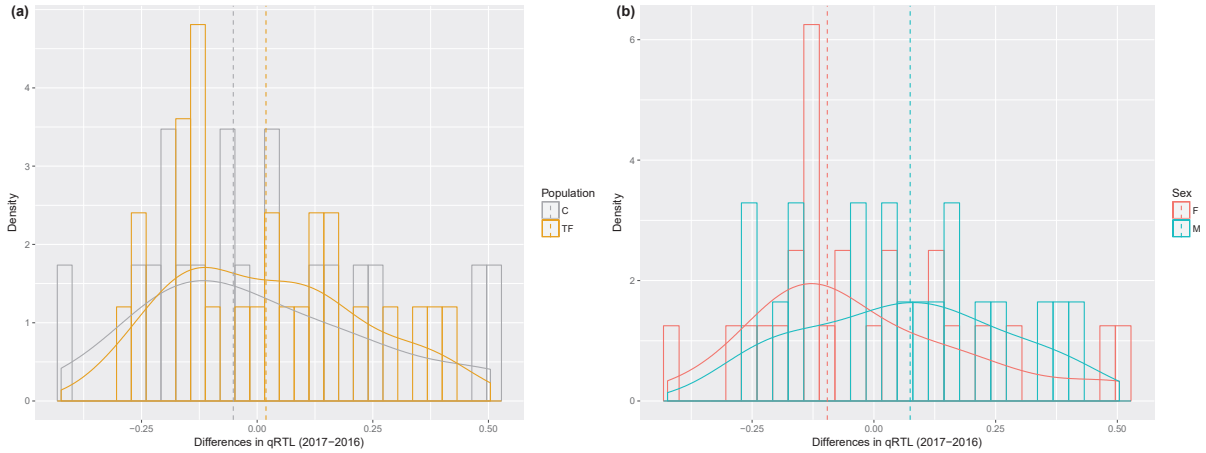
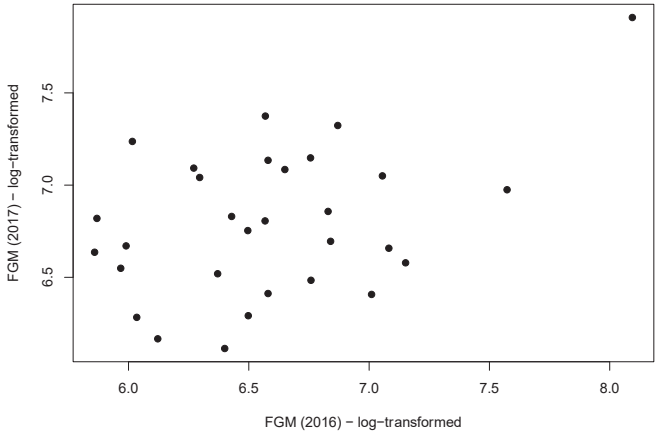


Figure S2: Relationship between the concentration in faecal glucocorticoid metabolites (FGM) measured in 2017 (ng/g, log-transformed) and the concentration in FGM measured in 2016 (ng/g, log-transformed).



3/ Supplementary methods for telomere assays

Buffy coat fractions. comprising mainly leucocytes. were prepared in the field and immediately frozen at -80°C until DNA extraction. Relative TL was measured by quantitative PCR as described previously [1]. During the 2016 and 2017 field seasons. blood samples were collected from the jugular vein of known-age individuals. Forty-four males were sampled during both field seasons. Within 30 min of sampling. whole blood was spun at approximately 3000 rpm for 10 min and the plasma layer drawn off and replaced by the same quantity of 0.9% w/v NaCl solution and spun again. The intermediate buffy coat layer. comprising mainly leukocytes (white blood cells) was collected into a 1.5-mL Eppendorf tube and stored at −80 °C until further use. The full protocol is provided in supplementary information.

We measured relative leukocyte telomere length (RTL) using a real-time quantitative PCR method (qPCR; [2] which has previously been optimized and validated in sheep and cattle [1]. This method measures the total amount of telomeric sequence present in a DNA sample. relative to the amount of a non-variable copy number reference gene (beta-2-microglobulin (B2M)). For telomere reactions we used the following HPLC purified primers. Tel 1b (5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3') and Tel 2b (5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3') (from [3]. For B2M reactions. primers were supplied by Primer Design (Catalogue number: HK-SY-Sh-900, Southampton, UK).

Using an automated liquid handling robot (Freedom Evo-2 150; Tecan) we were able to load both the DNA samples and qPCR master mix in 384 well plates; allowing us to run both telomeric and B2M reactions in separate wells but on a single plate. A separate master mix for each primer set was prepared containing 5 µl LightCycler 480SYBR Green I Master Mix (Cat # 04887352001, Roche, West Sussex, UK), 0.5 µl B2M (300 nm) primer or 0.6 µl each tel primer (900 nm), and 2 ng of sample DNA. DNA was amplified in 10 µl reactions. Each plate included a non-treated control (water; NTC), a calibrator sample (2ng) on each row to account for plate to plate variation and robot pipetting error, as well as a 1:4 serial dilution starting at 10ng/µl to visually inspect the qPCR curves. The calibrator sample is DNA that has been extracted from a large quantity of blood obtained from a single wild roe deer. In this case. the calibrator was extracted using the Qiagen DNeasy Blood and Tissue kit (Cat# 69581. Manchester. UK). pooled and quality controlled in the same way as our DNA samples of interest. All samples. calibrators and NTC's were run in triplicate and all qPCR performed using a Roche LC480 instrument using the following reaction protocol: 10 min at 95ºC (enzyme activation), followed by 50 cycles of 15 s at 95ºC (denaturation) and 30 s at 58ºC (primer annealing), then 30 s at 72ºC (signal acquisition). Melting curve protocol was 1 min at 95°C, followed by 30 s at 58°C, then 0.11 °C/s to 95°C followed by 10 s at 40°C.

We used the LinRegPCR software package (version 2016.0; [4] to correct for baseline fluorescence. set a window of linearity for each amplicon group and to calculate well-specific reaction efficiencies and Cq values. A constant fluorescence threshold was set within the window of linearity for each amplicon group, calculated using the average Cq across all three plates. The threshold values used were 0.140 and 0.203, and the average efficiency across all plates were 1.91 and 1.93 for the B2M and telomere amplicon groups. respectively. Samples were excluded from further analysis if the coefficient of variation (CV) across triplicate Cq values for either amplicon was > 5 %, or if at least one of their triplicate reactions had an efficiency that was 5 % higher or lower than the mean efficiency across all wells on that plate for the respective amplicon. Overall. thirteen samples failed quality control at either the DNA extraction or qPCR stage and were excluded from the study, leaving a total of 147 samples for further analyses.

We calculated relative telomere length (RTL) for each sample following [5] as follows:

$$RTL = (E_{TEL}^{(CqTEL[Calibrator] - CqTEL[Sample])}) / (E_{B2M}^{(CqB2M[Calibrator] - CqB2M[Sample])})$$

Where E_{TEL} and E_{B2M} are the mean reaction efficiencies for the respective amplicon group across all samples on a given plate; CqTEL[Calibrator] and CqB2M[Calibrator] are the average Cqs for the relevant amplicon across all calibrator samples on the plate; and CqTEL[Sample] and CqB2M[Sample] are the average of the triplicate Cqs for the sample for each amplicon.

DNA extraction protocol

1. Lyse blood samples

Pipette 25 µL Proteinase K and up to 150 µL blood (leucocytes) + 50 µL PBS into 1.5ML microcentrifuge tubes. Incubate at room temperature for 1 min. Add 200 µL Lysis Buffer BQ1 to the samples and vortex the mixture vigorously (10-20s). Incubate samples at 70°C for 10 minutes.

2. Adjust DNA binding conditions

Vortex again the mixture and centrifuge few seconds at 11.000 x g. Add 200µL ethanol (96-100%) to each samples, vortex again. centrifuge again few seconds at 11. 000 x g.

3. Bind DNA

Add the samples to the NucleoSpin Blood QuickPure Columns placed in a collection tubes and centrifuge 1 min at 11.000 x g. If the samples are not drawn through the matrix completely. repeat the centrifugation at higher g-force (up to 15.000 x g). Discard collection tube with flow-through.

4. Wash & dry silica membrane

Place the Nucleospin blood quickpure column into a new collection tube (2mL) and add 350 µL Buffer BQ2. Centrifuge 3 min at 11.000 x g. If the samples are not drawn through the matrix completely. repeat the centrifugation at higher g-force (up to 15.000 x g). Discard collection tube with flow-through.

5. Elute highly pure DNA

Place the NucleoSpin blood quickpure column in a 1.5 mL microcentrifuge tube and add 50 µL prewarmed Buffer BE (70°C). Dispense buffer directly onto the silicq membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11.000 x g.

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Appendix 3.

Mémoire réalisé dans le cadre du Diplôme Universitaire
« Biologie de l'évolution et médecine » à l'Université Claude Bernard Lyon 1.
(présenté en mai 2017)

Les maladies allergiques et l'hypothèse hygiéniste.

Diplôme Universitaire « Biologie de l'Évolution et Médecine »

Université Claude Bernard Lyon I

Les maladies allergiques & l'hypothèse hygiéniste

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SOMMAIRE

Introduction

Depuis la révolution industrielle et les grandes mesures de santé publiques prises dans nos pays occidentaux (vaccination contre les maladies infectieuses infantiles les plus communes, utilisation des antibiotiques, décontamination de l'eau, pasteurisation et stérilisation des aliments, respect de la chaîne de froid…), l'incidence des maladies infectieuses a drastiquement chuté (voir Fig. 1). Cependant, dans ces pays ayant éradiqué les infections les plus communes, on observe en parallèle une explosion des maladies allergiques et auto-immunes (voir Fig. 1). Les allergies et les maladies auto-immunes se caractérisent toutes les deux par une réponse inappropriée du système immunitaire. Mais les maladies auto-immunes sont caractérisées par une réponse dirigée contre des composants normaux de l'organisme (auto-antigènes) alors que les allergies sont déclenchées par des substances exogènes de l'environnement comme des pollens, des médicaments, des produits chimiques... Leurs mécanismes sont différents. Les maladies allergiques, dont les plus fréquentes sont le rhume des foins, les dermatites atopiques et l'asthme, affectent aujourd'hui environ 25 à 30% de la population de nos pays occidentaux, et représentent la première cause de maladies chroniques chez les enfants. L'Organisation mondiale de la santé (OMS) considère l'allergie comme étant la quatrième maladie mondiale en termes de morbidité après le cancer, les maladies cardiovasculaires et le sida.

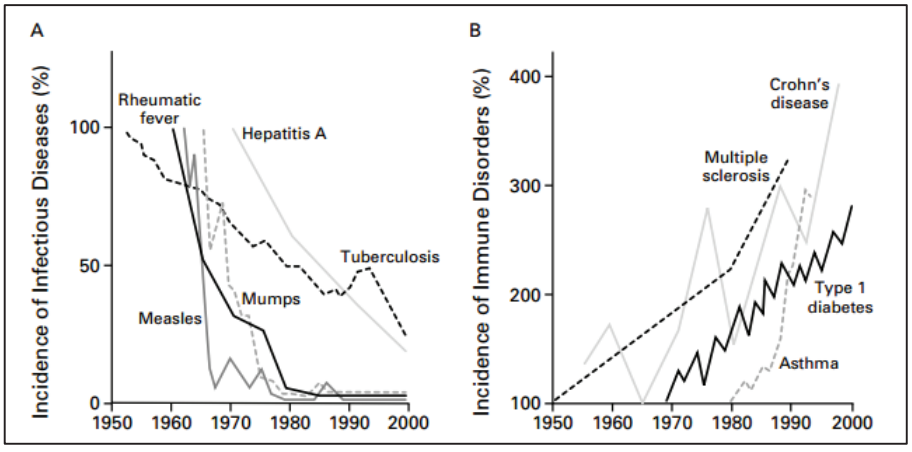
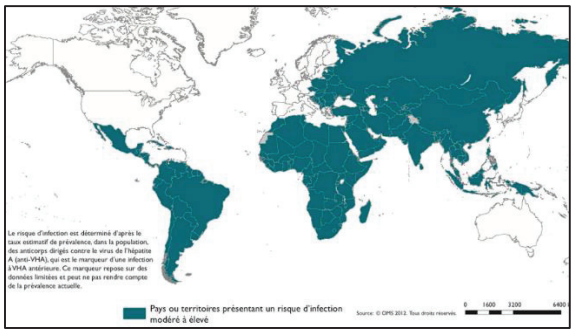


Figure 1. Relation opposée entre l'incidence des maladies infectieuses (graphe A) et l'incidence des désordres immunitaires (graphe B) entre 1950 et 2000 (figure tirée de Bach, 2002, The New England Journal of Medicine).

A l'échelle de la planète, il est frappant de voir que la distribution géographique de nombreuses maladies infectieuses (i.e. hépatite A, tuberculose… voir Fig. 2) peut être mise en miroir avec la distribution géographique des maladies allergiques et auto-immunes (i.e. asthme, diabète de type 1, sclérose en plaque… voir Fig. 3). Il y a globalement un gradient Nord-Sud des désordres immunitaires comme on le voit sur la Fig. 3 (à l'exception de l'asthme très présent en Amérique du Sud).



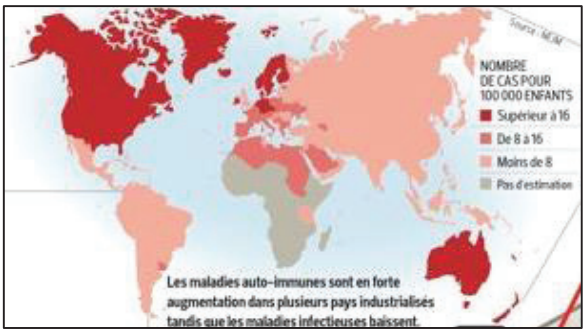


Figure 2: Répartition mondiale de l'hépatite A (OMS 2012) Figure 3: Incidence du diabète de type 1 chez des enfants de moins de 14 ans.

On peut aussi remarquer un gradient ouest-est en Europe par exemple, avec une incidence du diabète de type 1 ou de l'asthme plus bas en Europe de l'est qu'en Europe de l'ouest (voir Fig. 4), gradient qui tend cependant à s'estomper ces dernières années.

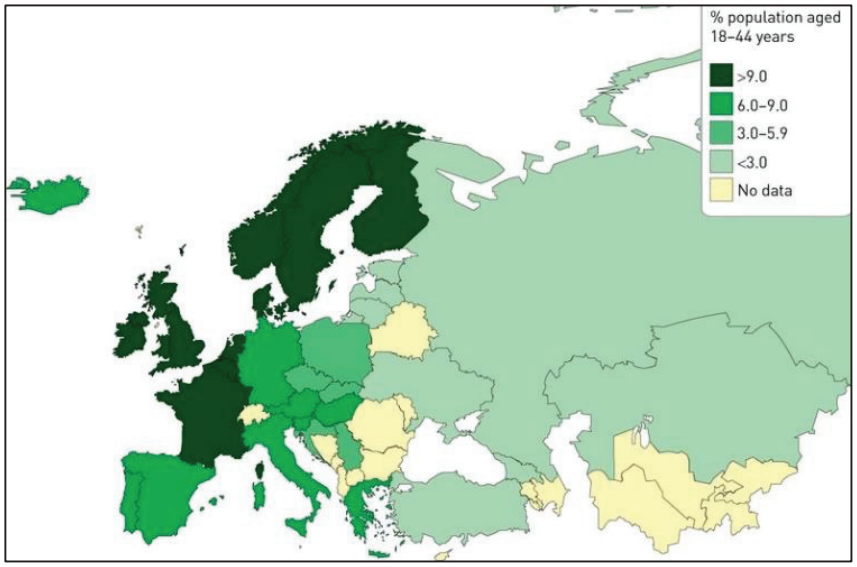


Figure 4 : Prévalence de l'asthme chez les adultes âgés de 18 à 44 ans (OMS 2002-2004).

Bien qu'une stabilisation de la prévalence de ces maladies semble se produire actuellement, une importante partie de la population souffre de troubles allergiques, donc mieux comprendre leur origine et leur évolution si rapide reste donc un enjeu actuel majeur pour la médecine (Grammatikos, 2008). Ce mémoire a pour but de retracer comment une pensée évolutionniste s'est imposée et a évolué pour comprendre l'explosion de ces maladies allergiques, et comment cette vision évolutionniste peut être un outil pour mettre au point des thérapies qui permettraient de diminuer l'incidence de ces maladies.

L’explosion récente des maladies allergiques

L’allergie est une réponse immunitaire disproportionnée, en réponse à un antigène *a priori* inoffensif, souvent étranger à l’organisme, appelé **allergène**. Les allergies peuvent avoir des manifestations cutanées (urticaire, dermatite), respiratoires (rhinite, asthme) ou généralisées (anaphylaxie). Les réactions allergiques les plus communes sont le rhume des foins, l’asthme, les dermatites atopiques, et les allergies alimentaires. Les réactions allergiques chroniques sont les plus fréquentes.

La condition sous-jacente et obligatoire aux maladies allergiques est l’**atopie** qui est héréditaire. Elle est caractérisée par des niveaux d’immunoglobuline E (IgE) élevés dans le sérum. Chez les individus sains, le taux d’IgE dans le sérum est le plus faible parmi toutes les classes d’immunoglobulines. Les individus sains génèrent des anticorps IgE seulement en réponse à une infection parasitaire. Au contraire, les personnes atopiques génèrent des IgE contre des antigènes environnementaux communs : des pollens de plante (ivraie vivace, ambroisie, fléole des prés, bouleau), des aliments (noix, fruits de mer, œufs, pois, haricots, lait), des médicaments (pénicilline, sulfonamides, anesthésiques locaux, salicylates), des produits dérivés d’insectes (venin d’abeille, de guêpe, de fourmi, acariens), des spores de moisissure, des poils et squames d’animaux, le latex, un sérum étranger ou encore des vaccins. Le développement d'une allergie se fait en plusieurs étapes : tout d’abord il y a sensibilisation à un allergène (condition nécessaire mais pas suffisante), puis lors d’une exposition suivante à cet allergène, cela déclenche une réaction allergique IgE dépendante, responsable des manifestations allergiques (voir Fig. 5). Une fois que la personne est sensibilisée, la réaction allergique s’aggrave à chaque exposition ultérieure à l’allergène.

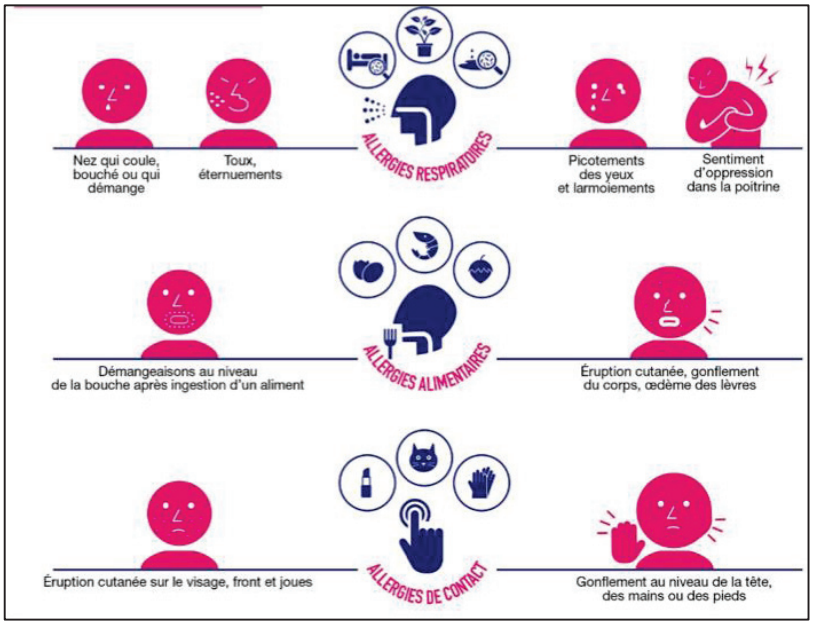


Figure 5: Les symptômes les plus fréquents en cas d'allergie (association Asthme & Allergies)

Les mécanismes précis de la réaction allergique n’étant pas le sujet principal de ce mémoire, ils ne seront pas détaillés ici.

La prévalence des maladies allergiques a considérablement augmenté au cours des 50 dernières années dans les pays industrialisés. Cette augmentation est réelle, et n'est pas le résultat d'un meilleur diagnostic ou d'un biais dû à des changements des critères dans le diagnostic de ces maladies (voir la méta-analyse de Eder *et al.*, 2006).

C'est le cas par exemple aux Etats-Unis, où la prévalence annuelle estimée de l'asthme augmente de 76% entre 1980 et 1995, avec une augmentation de 5,5% uniquement durant l'année 1995 (Mannino *et al.*, 2002). On estime aujourd'hui que 20 à 35% de la population des pays développés est concernée par une maladie allergique. En France, l'INSERM évalue actuellement la prévalence de la dermatite atopique à 15-20%, celle de l'asthme à 7-10 %, celle de la rhinite et de la conjonctivite allergique autour de 15-20%. La prévalence des allergies alimentaires oscillerait entre 2% chez l'adulte et 5% chez les enfants. Concernant les troubles respiratoires (wheezing), on voit qu'ils se développent chez des enfants de moins de 4 ans, et que c'est durant cette période que l'asthme commence chez 87% des patients (Grammatikos, 2008 ; Morgan *et al.*, 2005 ; Celedon *et al.*, 2002).

	Prevalence (%)	Proven IgE mediated (%)
Food allergy	7–8	40–60
Atopic dermatitis	15–20	20–70
Asthma/recurrent wheezing <5 yr	31–34	30–50
Asthma >5 yr	7–10	60–90

Frequencies of IgE-mediated reactions vary with age and selection. Different phenotypes, e.g. infectious asthma vs. atopic asthma.

Figure 6: Prévalence des maladies atopiques chez les enfants (Halken, 2004)

Mais si les allergies sont particulièrement fréquentes chez les enfants et les jeunes adultes, tout le monde peut en souffrir, avec des variations selon les pays et l'âge.

Le risque d'allergie dans la population générale est globalement de 10%, mais il est de 25% si vous avez un parent atopique, et de 50% si vos deux parents le sont. Il a aussi été observé qu'il y a un risque 4 fois plus élevé de transmission d'un trouble allergique par une mère affectée que par le père (Halonen *et al.,* 1992). Les jumeaux monozygotes présentent souvent le même type d'allergie, mais chez les jumeaux dizygotes la concordance n'est que de 50-60% (Barnes *et al.*, 1998). Il y a donc des facteurs génétiques qui participent au phénotype allergique, mais leur étude se révèle difficile, car il semblerait que la détermination génétique des maladies allergique soit complexe et n'obéisse pas aux lois mendéliennes classiques de dominance, récessivité et co-dominance (Grammatikos, 2008).

Ce qui rend le phénotype allergique clinique si difficile à comprendre et étudier est que non seulement il est déterminé par des interactions multiples entre divers gènes, mais aussi modulé par des facteurs non-génétiques comme l'environnement. Cependant certaines études ont tout de même réussi à mettre en évidence un nombre important de gènes candidats (Grammatikos, 2008). Mais si les gènes qui prédisposent à l'allergie commencent à être de mieux en mieux connus, il reste la question de leur rôle. Pourquoi sont-ils présents ? Peuvent-ils par ailleurs présenter avantage dans certains environnements, ou une protection contre certaines infections ? ont-ils un rôle lié à celui d'autres gènes ? Ou sont-ils simplement des inadaptations qui ne causaient pas de maladies avant d'interagir avec l'environnement moderne ?

L'hypothèse hygiéniste, première hypothèse évolutionniste

Le fait que la prévalence des troubles allergiques ait augmenté de manière si brutale durant les 50 dernières années, et que cette augmentation ait lieu principalement dans les pays développés a rendu évident l'implication de facteurs environnementaux dans ces pathologies.

David Strachan, un professeur d'épidémiologie propose une première hypothèse en 1989 dans un article du British Medical Journal, « Hay fever, hygiene and household size ». Il montre qu'il existe un lien fort entre l'exposition aux microbes durant la petite enfance et les allergies. Cette étude a suivi 17414 enfants anglais, tous nés en mars 1958, jusqu'à leurs 23 ans, sur 16 facteurs périnataux, sociaux et environnementaux. Cette étude montre qu'il y a un lien fort entre les pathologies allergiques, la taille de la famille et la position dans la fratrie de l'enfant. A 11 et 23 ans, le risque d'être atteint de rhume des foins diminue avec le nombre d'enfants du foyer, ainsi qu'avec nombre d'enfants plus âgés dans la fratrie de l'enfant concerné. C'est aussi le cas pour l'eczéma dans la première année de vie. Ces résultats ont été confirmés sur une autre cohorte d'enfants nés en 1970 à l'âge de 5 ans. Ces résultats ont permis de mettre en évidence que des contacts dans l'enfance avec des frères et sœurs, qui favorisent la transmission d'infections (ou ré-infection) entre les enfants, notamment dans les familles nombreuses, pourrait protéger du développement de maladies allergiques. Ces résultats suggèrent aussi qu'il pourrait y avoir une acquisition d'une certaine protection avant la naissance du fœtus, par une mère qui serait en contact avec d'autres enfants au moment de la grossesse. David Strachan fait donc l'hypothèse que de nos jours, l'excès d'hygiène, la diminution de la taille des fratries et des contacts entre les enfants, et donc la plus faible exposition aux microbes lors de la petite enfance, sont autant de facteurs responsables d'une immaturité du système immunitaire qui augmenterait le risque de développer des maladies allergiques. C'est la naissance de la « théorie hygiéniste ». Cette hypothèse a connu immédiatement un grand succès et de nombreuses autres études ont abondé dans ce sens. Il a été montré que l'exposition des jeunes enfants à d'autres à la maison (Cardoso *et al.*, 2004 ; von Mutius *et al.*, 1994) ou à la garderie (Celedon *et al.*, 2003 ; Ball *et al.*, 2000) peut protéger

du développement de l'asthme et du wheezing. De la même manière, vivre en milieu agricole pendant l'enfance semblent éviter le développement des allergies (Eduard *et al.*, 2004 ; Elliott *et al.*, 2004 ; Ernst *et al.*, 2000). Au niveau des mécanismes cellulaires sous-jacents, l'hypothèse proposée est celle d'un décalage de la **« l'équilibre Th1/Th2 »**. Lors d'une réaction immunitaire, les lymphocytes T se différencient en lymphocytes Th1 ou Th2 selon la nature des cytokines présentes dans l'environnement. Ces deux types de cellules diffèrent par le spectre des cytokines qu'elles sécrètent et leurs fonctions : traditionnellement, on associe les cellules Th1 à la réponse aux maladies infectieuses et les cellules Th2 à la réponse de type allergique. Les cytokines produites par les Th1 (IFNγ) inhibent les Th2, et inversement les cytokines produites par les Th2 (IL-4) inhibent les Th1. Grâce à leur potentiel inhibiteur des fonctions Th2, les maladies infectieuses (comme la tuberculose), associées à une forte réponse immunitaire de type Th1, seraient autant de facteurs de protection contre l'atopie. Ainsi la diminution de l'exposition à des infections déplacerait l'équilibre des réponses immunitaires Th1 vers Th2 chez les individus, et favoriserait donc les maladies allergiques.

Cependant, malgré l'engouement pour l'hypothèse hygiéniste, de nombreuses autres études ont aussi présenté des résultats en contradiction avec cette théorie. Tout d'abord, les infections dans l'enfance ne protègent pas forcément des allergies. Par exemple, contrairement autres infections, les infections respiratoires virales affectant les enfants tôt dans leur vie sont associées à un risque accru de problèmes respiratoires (Mallia *et al.*, 2002, Rusconi *et al.*, 1999). C'est notamment le cas du virus respiratoire syncytial (VRS, ou RSV pour *Respiratory Syncytial Virus*) qui est très associé au développement de l'asthme et à la sensibilité allergique (Sigurs *et al.*, 1995). De plus, les allergies ne sont pas toujours liées à un excès d'hygiène. Des études montrent en effet que dans des quartiers pauvres et assez sales de certaines villes des Etats-Unis, on retrouve une très forte occurrence de formes sévères d'asthme (Platts-Mills *et al.*, 2001). Ce n'est pas donc simplement un excès d'hygiène qui explique le développement de sensibilités allergiques. Pour finir, le mécanisme proposé de la modification de l'équilibre Th1/Th2 comme mécanisme de base à l'augmentation des allergies (décrit précédemment) a vite montré des contradictions. Tout d'abord, il a été observé qu'une infection par des vers parasitaires (helminthes) de l'organisme, qui provoque de fortes expressions d'IgE et reflète donc une forte réponse Th2, semble paradoxalement conférer une protection vis-à-vis des allergies. En effet, dans les pays où les infections par helminthes ont des prévalences élevées et que l'équilibre immunologique est déplacé vers les réponses de type Th2, les troubles allergiques sont les moins répandus, ce qui est contradictoire avec l'idée qu'un décalage vers des réponses Th2 serait responsable de l'augmentation des allergies. De plus, il semblerait que les allergies puissent aussi être liées à des réponses Th1. En effet, des cytokines Th1 comme les IFNγ sont retrouvés dans de nombreuses maladies allergiques comme l'asthme (Krug *et al.*, 1996) et dans les dermatites atopiques (Klunker *et al.*, 2003). Et une modification dans l'expression des voies IL-12 ou IFNγ (Th1) n'implique pas forcément une modification de l'incidence ou de la sévérité des maladies allergiques, ce qui montre bien que les réponses Th1 ne régulent pas les réponses Th2 (Lammas *et al.*, 2000).

Enfin, cette théorie de l'équilibre Th1/Th2 s'est trouvée réfutée lorsqu'il a été observé une augmentation simultanée des maladies chroniques inflammatoires médiées par des réponses Th1 (diabète de type 1, sclérose en plaque, maladies inflammatoires de l'intestin) et des maladies allergiques dans les mêmes pays. On observe effectivement une augmentation des pathologies liées à des mécanismes de régulation immunitaires, qui entraînent des réponses inflammatoires inappropriées, que ce soit Th1 ou Th2 (Rook *et al.*, 2008).

L'hypothèse des « vieux amis »

A la suite de l'hypothèse hygiéniste, Graham Rook a proposé la théorie des Vieux Amis (« Old Friends hypothesis » : Rook, 2012 ; Rook & Brunet, 2005), qui se place aussi dans une perspective évolutionniste de compréhension des maladies allergiques. La base de cette théorie est de prendre en compte l'histoire évolutive de l'homme et l'impact de tournants majeurs de son mode de vie sur son organisme, notamment au niveau du système immunitaire.

- L'homme a co-évolué avec des organismes de son environnement

Les populations humaines du paléolithique, des chasseurs-cueilleurs au mode de vie nomade, étaient les hôtes d'organismes hérités de leur ancêtre primate, un héritage constitué de nombreuses espèces qui incluent notamment des virus (Armelagos *et al.*, 2005 ; Van Blerkom, 2003). A cette époque, les hommes étaient aussi en contact avec des carcasses d'animaux, et étaient donc exposés à de nombreuses bactéries, virus, parasites ou champignons microscopiques transmissibles de l'animal à l'homme (Armelagos *et al.*, 2005). Enfin, chaque jour, ces populations étaient amenées à consommer plusieurs milligrammes de saprophytes environnementaux (« pseudo-commensaux ») ubiquistes dans les sols et l'eau. Ainsi on retrouvait dans l'organisme humain à cette époque des helminthes, des *Mycobacteria* saprophytes, la tuberculose, le virus de l'Hépatite A, un microbiote intestinal varié, *Helicobacter pylori, Salmonella, Toxoplasma*, des lactobacilles… Les relations entre une grande partie de ces organismes et l'homme peuvent être décrites comme un commensalisme, une relation entre deux espèces dans laquelle une obtient des substances (nutritionnelles ou autres) sans épuiser ou être néfaste pour l'autre espèce. Tous ces organismes ont accompagné l'évolution des mammifères et donc des hominidés, on parle de co-évolution. Comme ils étaient obligatoirement présents chez l'homme, car présents dans l'environnement avec lequel il était en contact quotidiennement, il était nécessaire que ces organismes soient tolérés par le système immunitaire humain. Par exemple, dans le cas des helminthes, bien qu'ils ne soient pas toujours inoffensifs, une fois établis dans leur hôte, le système immunitaire est incapable de s'en débarrasser. Le système immunitaire a donc évolué pour les tolérer, *via* une réponse inflammatoire abaissée, afin d'éviter une réaction immunitaire constante mais inutile pouvant entraîner une destruction excessive des tissus

(Babu *et al.*, 2006). Chez des individus infectés par des helminthes, on peut observer par exemple une augmentation de l'expression de molécules anti-inflammatoires comme IL-10 (Yazdanbakhsh *et al.*, 2002). Ainsi, malgré le challenge immunitaire persistent que l'hébergement de certains organismes peut représenter, le système immunitaire a évolué pour mettre en place des mécanismes permettant de réguler ses réponses (voir Fig. 7).

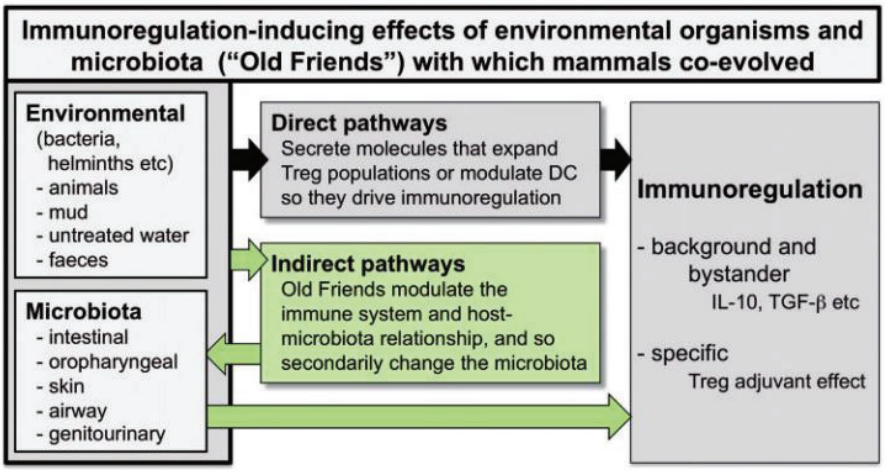


Figure 7: Immuno-régulation microbienne (figure tirée de Rook et al., 2013)

- Des transitions épidémiologiques

Il y a 10.000 ans, la transition vers un mode de vie agricole et d'élevage a représenté pour l'homme ce que l'on appelle la **première transition épidémiologique du néolithique** (Armelagos *et al.*, 2005). Les hommes vivent désormais au sein de plus grands groupes sociaux et en grande proximité avec de nombreuses espèces animales, avec la domestication de certaines espèces (chiens, chats) et l'élevage d'autres (animaux de la ferme). 97% des hommes vivent alors en milieu rural. Cette première transition a eu peu d'impacts au niveau de l'exposition aux pseudo-commensaux, toujours très présents dans l'eau et les sols et donc toujours largement consommés, et sur les espèces héritées. Par contre, cette vie plus sédentaire a fortement augmenté la transmission féco-orale d'organismes, c'est-à-dire la transmission d'organismes présents dans les fécès d'un hôte via la bouche d'un autre hôte. Cela se produit par exemple lorsque les eaux bues sont souillées par des fécès (situation favorisée par la sédentarité des grands groupes), par des contacts de mains non lavées, par contamination des aliments... Ce mode de vie basé sur l'élevage a aussi induit des contacts plus prolongés avec les animaux et leurs fécès. Tous ces changements se traduisent par un plus fort parasitisme par les helminthes, de nouvelles adaptations à des virus animaux et l'apparition de nouvelles d'infections (choléra, typhus, variole, rougeole…). L'exposition de l'homme à divers organismes a donc beaucoup changé durant cette première transition épidémiologique, mais ces modifications n'ont pas représenté une perte d'exposition à des organismes.

Au contraire, au XIXème siècle, de grands bouleversements de mode de vie ont eu un fort impact sur cet équilibre entre l'homme et les organismes avec lesquels il a évolué : on parle de **deuxième transition épidémiologique**. En effet, dans de nombreux pays en cours d'industrialisation, l'exode rural a fait exploser les populations urbaines, qui vont représenter 95% de la population au XXème siècle dans les pays développés. Dans ces sociétés se sont mises en place des mesures de santé publique (traitement des eaux, décontamination des aliments, apparition des traitements antibiotiques…). Ces changements de mode de vie ont drastiquement diminué l'exposition à de nombreux organismes qui ont été présents tout au long de l'histoire humaine, et de nombreuses espèces hébergées par l'homme ont été perdues. Or, comme expliqué précédemment, des voies de régulation immunitaire ont été mises en place en réponse à la présence constante de ces organismes. Dans les pays où ces « vieux amis » immuno-régulateurs sont abondants, la régulation immunitaire est efficace : la réponse inflammatoire est vigoureuse pendant une infection, mais s'arrête rapidement dès qu'elle n'est plus nécessaire, avec notamment des taux de base de protéine C-réactive (CRP) proches de zéro (McDade *et al.*, 2012). La perte des organismes qui induisent ces circuits immuno-régulateurs va alors entraîner une immuno-régulation défectueuse. Les variants génétiques qui ont été sélectionnés pour induire des mécanismes permettant de les tolérer ou de les éliminer, vont alors continuer à induire des réponses sans cible. On observe alors chez de nombreux individus un taux d'inflammation de base constant et élevé (CRP, IL-6) en absence de stimulus clinique apparent. La perte de ces organismes devient donc un facteur de risque de maladies inflammatoires chroniques comme les allergies ou les maladies auto-immunes (Fumagalli *et al.*, 2009 ; Moller *et al.*, 2007 ; Barnes *et al.*, 2005). « We realized humans beings coevolved with a whole host of organisms, and it was fare more likely what was going on was that we were being deprived of organisms on which we are dependent » Graham Rook. Ainsi l'exposition à une grande diversité d'organismes permettait au système immunitaire d'identifier et d'être capable de réagir aux menaces de manière appropriée, apprentissage perturbé par la perte de ces organismes. « Ce n'est pas juste apprendre ce qu'il faut attaquer, mais aussi apprendre ce qu'il faut tolérer. Le problème vient quand notre système immunitaire rencontre un allergène comme du pollen ou des cacahuètes et ne sait pas que c'est inoffensif » Sally F. Bloomfield.

- Les « vieux amis »

Ces organismes, ces « vieux amis » (définis par Rook, 2012) sont donc des organismes qui ont été abondants et qui ont co-évolué avec l'homme depuis longtemps, c'est-à-dire bien avant le début de l'agriculture. Ils sont maintenant pratiquement absents de notre environnement, et ceci notamment depuis le siècle dernier. Et ce sont des organismes dont il a été montré que leur présence a un effet thérapeutique sur des modèles animaux de pathologies inflammatoires chroniques et/ou dont des preuves existent que leur présence a un effet thérapeutique dans des essais cliniques chez l'homme.

Dans le cas des maladies allergiques, il pourrait y avoir un effet protecteur de la présence d'organismes liés à la transmission oro-fécale comme *Helicobacter pylori* (Matriarci *et al.,* 2000), *Salmonella* (Pelosi *et al.*, 2005), *Toxoplasma* (Matriarci *et al.*, 2000, 2002), des entérovirus (Seiskari *et al.*, 2007), le virus de l'hépatite A (Matriarci *et al.*, 2002). C'est le cas aussi de nombreuses espèces d'helminthes dont le rôle immuno-régulateur est très étudié (Yazdanbakhsh & Wahyuni, 2005). Il y a encore une cinquantaine d'années, environ 41% de la population européenne était porteuse d'helminthes tels que *Enterobicus vermicularis, Trichuris tricuria* ou *Ascaris lumbricoides* (Stoll *et al.*, 1947), alors qu'actuellement ils sont bien plus rares. De nombreuses études ont montré la corrélation inverse entre la présence d'helminthes dans l'organisme et la sensibilité allergiques aux allergènes de l'environnement (voir par exemple Cooper *et al.*, 2003 ; Nyan *et al.*, 2001). Par exemple, l'infection à *Schistosoma mansoni* a été associé à des formes moins graves d'asthmes (Medeiros *et al.*, 2003). Ou encore, une étude en Ethiopie a montré que les risque de wheezing sont réduits chez les individus porteur d'ankylostome – i.e. *Necator americanus* (Scrivener *et al.*, 2001). Enfin, on compte aussi dans ces « vieux amis » protecteurs vis-à-vis des allergies des microbiotes variés et commensaux comme celui de l'intestin (Maslowski & Mackay, 2011) ou des poumons (Huang *et al.*, 2011) ; ou encore des saprophytes environnementaux, comme *Mycobacterium vaccae* (Zuany-Amorim *et al.*, 2002).

Cette théorie explique bien la distribution géographique des maladies allergiques, où l'on voit que dans des pays où les gens ont encore un mode de vie rural et traditionnel (plutôt dans certains pays du Sud), avec des expositions journalières aux animaux, à leurs déchets, et donc toujours en contact avec de nombreux organismes tels que ceux rencontrés tout au long de l'histoire humaine, le phénotype atopique est assez rare. Au contraire, dans les pays en pleine industrialisation, les maladies allergiques explosent, comme cela s'est produit chez nous à partir du XIX^{ème} siècle. D'ailleurs, cette augmentation des maladies allergiques en Europe à cette époque, corrélée avec les développement économique et l'urbanisation, a été décrite tout d'abord de manière caractéristique chez les riches urbains, et était à ce moment là encore très rare chez les fermiers (Mackenzie, 1887).

Il est aussi intéressant de regarder l'effet de migrations entre des pays « traditionnels » et des pays plus industrialisés, donc de changements environnementaux majeurs, chez des populations. Une étude chez des migrants originaires du Mexique installés aux USA a montré que la prévalence de l'asthme était plus importante pour ceux nés aux USA que chez ceux nés au Mexique. De plus, chez ceux nés au Mexique, plus la migration vers les Etats-Unis s'est faite tôt dans leur vie, plus la prévalence de l'asthme augmente (Eldeirawi *et al.*, 2009). De manière semblable, une étude sur des enfants adoptés en Suède venant de pays relativement pauvres et ruraux montre que la prévalence de l'asthme, du rhume des foins et de l'eczéma était plus importante chez ceux qui étaient adoptés avant l'âge de 2 ans que chez ceux adoptés à des âges plus avancés (Hjern *et al.*, 1999). Ces études mettent bien en lumière l'importance et l'influence des expositions environnementales à certains organismes sur les

risques de développer des maladies allergiques, et notamment de manière précoce dans la vie (exposition pré-natale et exposition dans la petite enfance).

Et bien d'autres facteurs…

Des changements de plusieurs facteurs de l'environnement en même temps sont susceptibles d'avoir un impact encore plus fort, surtout lorsqu'ils impactent des stades précoces de la vie. « On parle d'une multitude de facteurs [N.D.L.R. pour expliquer l'augmentation des allergies], pas seulement d'un. C'est le régime alimentaire, l'hygiène publique, l'utilisation d'antibiotiques, les parasites et autres. On a modifié tous ces facteurs en même temps et la capacité à moduler de manière adéquate son système immunitaire en a été perturbée » Marsha Wills-Karp.

Les récents changements de mode de vie ont par exemple considérablement modifié le régime alimentaire de l'homme. L'augmentation de la consommation de produits très gras et sucrés augmente la perméabilité intestinale, ce qui peut augmenter l'absorption d'endotoxines et mener à des situations d'inflammation (Cani & Delzenne, 2011). Cela modifie aussi considérablement la flore intestinale, avec la prolifération d'espèces microbiennes inappropriées. Ces régimes alimentaires qui manquent de diversité tout en étant trop riches qui entraînent dans les pays développés des problèmes d'obésité, sont associés à un microbiote intestinal altéré et à une inflammation locale augmentée (voir la review de Tremaroli & Backhed, 2012). Ces changements du microbiote intestinal sont bien visibles lorsqu'on compare le microbiote intestinal des Européens et celui de populations de villages ruraux traditionnels du Burkina Faso : ils sont extrêmement différents (De Filippo *et al.*, 2010). Or, il est de plus en plus reconnu que la nature du microbiote intestinal affecte profondément la régulation immunitaire (Maslowski & Mackay, 2011), par exemple en sécrétant des molécules qui dirigent l'expansion de populations de cellules Treg (lymphocytes T régulateurs). Avec en plus la perte des « vieux amis » comme les helminthes qui agissent aussi sur ce microbiote intestinal, cela pourrait exacerber les problèmes immuno-régulateurs. Par son rôle dans la régulation immunitaire, le microbiote instestinal fait donc partie des « vieux amis » avec lesquels nous avons co-évolué. Des auteurs ont aussi proposé que la diversification actuelle de l'alimentation, avec la mondialisation des échanges alimentaires, mais aussi les migrations des populations, nous exposeraient à un flot important de nouveaux antigènes qui pourraient induire une plus grande réactivité de l'organisme. Enfin, il faut aussi prendre en compte le fait que durant les 10 dernières années, la prescription d'un large spectre d'antibiotiques a doublé (notamment chez les enfants), antibiotiques bien connus pour fortement perturber cette flore intestinale (Modi *et al.*, 2014). Cette utilisation d'antibiotiques durant la petite enfance semble être un facteur de risque dans le développement de l'asthme (Wickens *et al.*, 1999) et des allergies alimentaires (Hirsch *et al.*, 2017).

Un autre facteur qui jouerait un rôle crucial dans l'augmentation récente des maladies allergiques serait l'augmentation massive des émissions de polluants atmosphériques, augmentation due à la croissance économique et industrielle au siècle dernier. La qualité de l'air un problème environnemental de premier ordre dans un grand nombre de pays d'Europe et d'Amérique du Nord et c'est maintenant un problème émergent dans d'autres régions du monde. De nombreuses études ont montré que la **pollution de l'air** affecte la fonction pulmonaire, et notamment le développement des poumons chez les enfants (Gauderman *et al.,* 2004). Une étude sur près de 4000 enfants durant les 8 premières années de leur vie a montré que des niveaux de pollution de l'air élevés étaient associés à de fortes prévalence de l'asthme chez ces enfants (Gehring *et al*., 2010). Avec l'augmentation de la pollution de l'air et le réchauffement climatique qui va s'accentuer dans les années à venir, on peut s'attendre à une augmentation de la prévalence des maladies allergiques respiratoires (voir la review de D'Amato *et al.*, 2013).

Pour finir, on peut penser que **l'accouchement par césarienne,** qui a fortement augmenté au cours des dernières années dans de nombreux pays (voir Fig. 8) pourrait avoir un impact sur l'augmentation de la prévalence de certaines maladies allergiques. En effet, les enfants nés par césarienne auraient davantage de risque d'avoir de l'asthme que ceux nés par voie vaginale. Une méta-analyse récente (basée sur 26 études de cohortes) a montré qu'il y avait une augmentation de presque 20% du risque de souffrir d'asthme chez des enfants nés par césarienne par rapport à ceux nés par voie vaginale (Huang *et al.*, 2015).

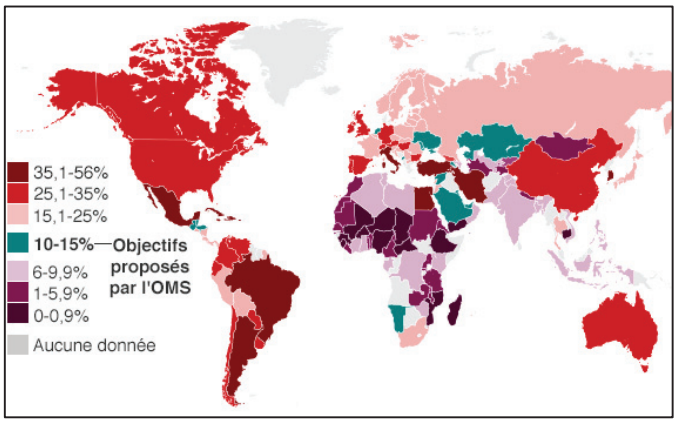


Figure 8: Taux de naissance par césariennes par pays (source OMS, 2015)

Quand il s'agit de césariennes programmées (i.e. > à 37 semaines, non liée à une situation d'urgence), elles sont souvent faites plus tôt que les césariennes « d'urgence », et le poids de naissance des enfants est par conséquent généralement inférieur à ceux nés par césarienne « d'urgence » (Boutsikou & Malamitsi-Puchner, 2011). Aux Etats-Unis, 1/3 de ces césariennes programmées sont faites avant 39 semaines de grossesse. Cela a des conséquences sur les poumons du nouveau-né, par toujours complètement développés anatomiquement ou immunologiquement. Cette immaturité pourrait les rendre plus

sensibles à des expositions futures pouvant causer de l'asthme (Jaakkola *et al.*, 2006). De plus, la naissance par césarienne empêche le contact et la colonisation du nouveau-né par la flore vaginale de sa mère. Sans cette exposition précoce à la flore vaginale de la mère, on observe chez ces enfants une flore bactérienne intestinale différente de ceux nés par voie basse, notamment moins de *Bacteriodes* et de *Bifidobacterium* (Penders *et al.*, 2006). Or il a été montré que les *Bacteriodes* pouvaient avoir des propriétés anti-inflammatoires (Mazmanian *et al*., 2008). Et une diversité intestinale plus faible durant le premier mois de la vie a été associé à un risque accru d'être atteint par la suite d'une maladie atopique (Abrahamsson *et al.*, 2011). Cela montre à nouveau l'importance des facteurs qui influence la maturation précoce du système immunitaire dans le développement de maladies allergiques.

Applications thérapeutiques et perspectives actuelles

Les « vieux amis » stimulent des voies de régulation du système immunitaire. Ce n'est donc pas simplement un excès d'hygiène qui serait une cause des allergies, comme cela a pu être perçu après les premières théories proposées dans les années 80-90. Il n'est en effet pas question de supprimer les standards actuels d'hygiène, qui ne feraient pas revenir nos « vieux amis » et ne diminuerait pas les allergies mais nous exposeraient surtout à de « nouveaux ennemis » comme *E. coli* O104 (Scudellari, 2017). "We know an awful lot now about our immune system's regulation is not in terribly good shape, and it's got absolutely nothing to do with hygiene" Graham Rook. En février 2016, Graham Rook, Sally F. Bloomfield et d'autres experts des maladies infectieuses et allergiques se sont réunis pour faire le point sur l'hypothèse hygiéniste proposée il y a 27 ans et l'avancée des connaissances depuis, et ont décidé que ce nom devait impérativement être changé (Bloomfield *et al.*, 2016). « Le problème est que dès qu'on utilise les mots « d'hypothèse hygiéniste », le mot hygiène laisse penser que c'est la cause principale. Pour le public « hygiène » est interprété comme la propreté : se laver les mains, garder de la nourriture propre et fraiche, nettoyer sa maison. Or diminuer les standards d'hygiène ne ferait qu'augmenter le risque d'attraper des maladies infectieuses. Ce terme d'hypothèse hygiéniste échoue à donner une vision d'ensemble des causes de l'augmentation des allergies et autres dérèglements immunitaires. » Mais cet appel à changer de terminologie n'a pas eu grand écho dans la communauté scientifique, et on a pas trouvé le nom qui allait pouvoir la remplacer : hypothèse de la perte du microbiome, l'hypothèse de la diversité microbienne, l'hypothèse des vieux amis ? Pour l'instant le terme « hypothèse hygiéniste » reste donc encore le plus utilisé (Scudellari, 2017).

Au niveau thérapeutique, le but actuel est d'identifier quelles sont les voies régulatrices qui modulent l'activité du système immunitaire pour trouver des médicaments qui permettraient de les activer et re-réguler le système immunitaire. Le grand challenge serait d'arriver à des traitements personnalisés pour ne pas fatiguer le système immunitaire en l'activant de manière non spécifique, avec des mélanges microbiens personnalisés qui

stimuleraient des cellules immunitaires régulatrices. On en est encore loin, car actuellement il y a un manque d'études qui réussissent effectivement à diminuer les allergies *via* des traitements. Bien qu'il y ait eu des centaines d'études épidémiologiques et d'observations, il y a peu d'études randomisées, des études prospectives contrôlées, testant des moyens de re-réguler le système immunitaire (Scudellari, 2017).

- Thérapies helminthiques

Comme il a été montré chez les souris et les rats de laboratoire que des infections induites aux helminthes réduisent leur réactivité allergique (voir la review de Helmby, 2009), la « thérapie helminthique » a été suggérée comme un traitement possible pour les maladies allergiques chez l'homme. Pour l'instant, les essais cliniques utilisent notamment deux espèces d'helminthes : les œufs d'un nématode parasite du porc, *Trichuris suis* ou des larves d'ankylostome humain, *Necator*. Après l'ingestion des œufs de *T. suis*, les larves éclosent et colonisent le caecum et le colon. Cette colonisation ne tient que quelques semaines, ce qui signifie que le traitement doit être répété à intervalles régulières. Le fait que l'infection ne devienne pas chronique présente l'avantage que ce traitement est facilement réversible. Les larves de *Necator* sont administrées par voie percutanée et vont migrer à travers le système vasculaire et les poumons jusqu'à l'instestin grêle. Elles se fixent à la muqueuse intestinale où elles se nourrissent de sang jusqu'à maturité, ce qui peut entraîner des pertes de sang au niveau de l'intestin et donc une anémie ferriprive si elles sont administrées à trop haute dose. Et elles sont responsables d'infections de longue durées, qui peuvent se maintenir des années, ce qui peut être problématique dans le cas d'infections sévères.

Les résultats des études sont pour l'instant souvent peu concluants au niveau de l'amélioration des symptômes des maladies allergiques. Par exemple, une étude a suivi 100 patients souffrant de rhinite allergique induite par le pollen, à qui on a administré 8 doses de 2500 œufs de *Trichuris suis* à 21 jours d'intervalle (Bager *et al.*, 2010). Les patients ont effectivement été infectés par les helminthes (vérifiée par détection d'anticorps et par les symptômes intestinaux induits) mais cette infection n'a montré aucun effet significatif sur les symptômes de la rhinite ou sur la réactivité à des tests allergiques cutanés. Une autre étude a montré de la même manière que des individus souffrant de rhinite-conjonctivite allergique traités par des larves de *Necator* et suivis pendant 12 semaines ne montraient pas d'améliorations significatives de leurs symptômes allergiques ou de leur fonction pulmonaire, ni de diminution de la réactivité à des tests allergiques cutanés, malgré la présence de symptômes prouvant l'installation du parasite (Feary *et al.*, 2009).

Il faut tout de même bien noter que dans la majorité des études sur des modèles animaux ayant des résultats positifs, l'exposition aux helminthes permettait de prévenir le développement ultérieur d'une réactivité allergique. Par contre, il n'y a que quelques exemple d'études montrant que des infections aux helminthes peuvent avoir un impact sur une

réactivité allergique déjà établie. Il semblerait en effet qu'une fois la sensibilité allergique installée, les infections aux helminthes ne pourraient pas avoir un réel impact dessus (Helmby, 2015). Dans le cas de thérapies chez l'homme, cela suggère qu'il faudrait les exposer à des infections helminthiques à des âges précoces, afin que le système immunitaire se développe avec ces parasites, ce qui éviterait de développer une sensibilité allergique ultérieure. Des études sont encore nécessaires sur ces thérapies, car il se pose de nombreuses questions, comme la dose à administrer, s'il faut le faire de manière transitoire ou chronique, et prendre en compte si l'infection est systémique ou non. La dose de *Necator* utilisée dans l'étude présentée précédemment (10 larves) n'est aussi peut être pas suffisante pour avoir un effet (Feary *et al.*, 2009), car les doses utilisées dans les thérapies sur les modèles animaux étaient bien supérieures. Et les oeufs de *Trichuris suis* restent dans les intestins, alors que les larves de *Necator* peuvent migrer jusqu'aux poumons dans des stades d'infection précoces, ce qui peut être irritant pour les voies respiratoires.

Une autre piste de recherche intéressante pour l'avenir serait l'utilisation des helminthes pour la production de nouveaux médicaments. En effet, les helminthes sécrètent de nombreux mélanges de protéines, carbohydrates, lipides dans leur environnement que l'on appelle des produits d'excrétion-sécrétion. Beaucoup de ces produits ont des propriétés immuno-régulatrices (Harnett, 2014). Par exemple la glyco-protéine ES-62 produite par le nématode *Acanthocheilonema vitae* semble capable d'inhiber l'activation de mastocytes, d'induire la sécrétion d'IL-10 (cytokine anti-inflammatoire) par les lymphocytes B… Chez la souris, des études ont montré que certains de ces produits d'excrétion-sécrétion pouvaient protéger des hypersensibilités aux allergènes des voies respiratoires (Harnett, 2014). L'idée de pouvoir caractériser puis synthétiser des molécules de ce type pour créer de nouveaux médicament est donc une nouvelle piste excitante pour les chercheurs (Helmby, 2015).

- Traitement probiotique préventif

Il a été montré que les enfants qui développement des maladies allergiques montrent des différences dans la composition et la diversité de leur microbiote intestinal pendant les premiers mois de leur vie (Arrieta *et al.,* 2015 ; Abrahamsson *et al*., 2014 ; Penders *et al.,* 2013 ; Azad *et al*., 2015). C'est pourquoi des traitements intervenant sur le microbiote intestinal (via des probiotiques) ont suscité un grand intérêt comme stratégies préventives au développement des allergies (West *et al*., 2016 ; Cuello-Garcia *et al*., 2015 ; Osborn & Sinn, 2013). Les probiotiques sont définis comme des micro-organismes vivants, qui quand ils sont ingérés dans certaines proportions, peuvent avoir des effets bénéfiques sur la santé. Il y a maintenant des preuves que certaines souches de *Lactobacilli* ou *Bifidobacteria* qui peuvent moduler la fonction immunitaire par de nombreuses voies (voir la review de Prescott & Björkstén, 2007).

Les études sur des adultes avec des pathologies respiratoires établies n'ont pas montré de résultats très significatifs sur l'asthme (Wheeler *et al*., 1997) ou la rhinite allergique (Helin *et*

al., 2002), ce qui est en accord avec l'idée que les effets positifs sont significatifs quand les réponses immunitaires sont encore en développement, et pas quand la sensibilité allergique est établie (Prescott & Björkstén, 2007).

Deux méta-analyses publiées en 2015 ont conclu qu'il y avait un effet bénéfique chez les enfants d'un traitement préventif de probiotiques pour l'eczéma, mais pas pour les autres manifestations allergiques comme l'asthme ou la rhino-conjonctivite (Cuello-Garcia *et al*., 2015 ; Zuccotti *et al*., 2015). Cette réduction du risque d'eczéma est notamment observable lorsque les probiotiques sont pris par la mère lors du dernier trimestre de la grossesse ou pendant l'allaitement (Cuello-Garcia *et al.*, 2015). Il reste la question de savoir à partir de quel moment de la grossesse ce traitement est-il efficace, et combien de temps après la naissance il peut être utile (Forsberg *et al.*, 2016). Et ces résultats nécessitent d'être confirmés par d'autres études. Pour les allergies autres que l'eczéma (allergies alimentaires et respiratoires), les preuves d'une efficacité de ce type de traitement restent donc faibles et considérées comme insuffisantes. Des directives récentes de la World Allergy Organization recommandent l'utilisation de probiotiques pour la prévention de l'eczéma chez les femmes enceintes et allaitantes dont les nourrissons présentent un risque très élevé de développer une allergie (Forsberg *et al*., 2016). Il est toutefois bien souligné que ces recommandations sont conditionnelles, basées sur de faibles preuves d'efficacité, et une grande hétérogénéité entre les études. Cela rend difficile les conseils pour les patients sur les traitements les plus efficaces (le choix des souches probiotiques, le moment et la durée de l'administration). Dans ce domaine aussi, des études sont encore nécessaires.

Conclusion

La vitesse de l'évolution culturelle dans notre espèce est supérieure à celle de son évolution biologique, nos changements de mode de vie sont extrêmement récents comparativement aux millions d'années qui ont développé les caractéristiques des homininés. L'homme se trouve donc actuellement dans une situation d'inadéquation entre l'adaptation de son organisme à un environnement « traditionnel » et son mode de vie actuel. Cette inadéquation serait responsable de l'explosion de nouvelles pathologies telles que les allergies. Il est devenu évident qu'il est nécessaire de prendre en considération cette histoire évolutive et les changements environnementaux, qu'ils soient de nature biologique, physique ou socioculturelle, pour mieux comprendre développement de certaines pathologies. L'exemple des allergies illustre parfaitement comment une vision évolutionniste permet d'améliorer notre compréhension de certaines maladies et permet ensuite de proposer des pistes de recherches de traitements pour les soulager.

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Appendices of the main text of the manuscript

Appendix 2.1. Fieldwork form for roe deer captures in Trois-Fontaines and Chizé.

Fiche de renseignement capture chevreuil

OFFICE NATIONAL DE LA CHASSE ET DE LA FAUNE SAUVAGE
VETAGRO SUP
ANIMAUX d'AGE CONNU

Étiquette kit	N°ordre ONCFS : .....

DATE: ...../...../..... matin / après-midi

LIEU: Chizé Trois-Fontaines **SECTEUR :** .....

Numéro d'individu :.....

Boucle (n°/couleur): 1. ..... 2. .....

N° collier (plaque, GPS…): .....

Chevrillard non capturé faon (pas d'hémato-bioch) : ✷

Tiques: nombre de tiques fixées sur la tête :
0 / 1-4 / 5 – 20 / > 20

Autres ectoparasites (ex :*Lipoptena* (non fixés),…) : oui non

Heure de la prise de sang : .....

Heure de la centrifugation du plasma : .....

N° kit: …… N° ordre ONCFS : ……..……

Mesure du stress

Dans le sabot: N° sabot:

(entourer les scores donnés ci-dessous)

Sur la table:

Score	Comportement sur la table Mesure subjective des réactions de l'animal sur la table de manipulation
0	Calme - Sans résistance - Pas de cri - Pas de coup de pattes
1	Calme - Pas plus de deux cris - Presque aucun coups de pattes
2	Animal qui crie et donne des coups de pattes mais qui reste relativement calme entre ces événements
3	Animal stressé, beaucoup de cris et de coups de pattes mais la manipulation reste possible
4	Animal extrement stressé. Très difficile à manipuler / impossibilité de prendre des mesures précises
5	Animal exténué/ Aucune résistance, facile à manipuler. Ce score diffère de 0 car ici l'animal est stressé

Lâché:

Heure : ……

De nuit - de jour

Nombre de personnes présentes : ……

Animal sorti spontanément de la boîte : oui / non

Score	Comportement au lâché Mesure subjective du comportement de l'animal lors du lâcher
0	Quitte le site lentement. S'arrête plusieurs fois
1	Quitte le site en courant mais s'arrête après une courte distance
2	Quitte le site en courant et ne s'arrête que lorsqu'il ne peut plus être vu
3	Animal qui tombe ou saute en quittant le site / qui essaye d'arracher son collier / ou de s'échapper
4	Animal qui reste allongé et qui ne peut plus tenir debout seul

Nom de la personne ayant mesuré le stress :

Table : …… Lâché : ……..

Appendix 3.1. Supplementary methods and results for the paper *Does body growth impair immune functions in a large herbivore?*

Electronic Supplemental Material for:
Does body growth impair immune functions in a large herbivore? *Oecologia*
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Online Resource 1. Detailed protocol and repeatability of the 12 immune measurements of our study assessed with the rptR package.

DETAILED PROTOCOL

In this study, as in two previous studies [1,2], we measured eleven immune traits using haematological parameters and specific assays. These multiple measures are analysed simultaneously because they reflect both the innate and the adaptive responses, which are represented by both humoral and cell-mediated components [3, 4]. Concerning cellular immunity, we measured neutrophil, monocyte, lymphocyte, eosinophil and basophil counts. Neutrophils and monocytes counts reflect acute and chronic inflammatory responses, respectively, and both may increase after infection. Eosinophils are specifically induced by Th2 responses, but may be also present in other contexts like hypersensitivity [4]. Basophils, which are quite rare, play a key role against macroparasites such as ticks. Lymphocytes counts include both T and B cells, the latter being particularly involved in the production of antibodies and thus in adaptive responses [4]. We manually estimated the proportion of the various leucocytes forms by counting the 100 first cells on blood smears with a microscope under 200x magnification (in %). Blood smears are stained with May-Grünwald's (#T863.2, Carl Roth GmbH) and Giemsa (#T862.1, Carl Roth GmbH) solution, a method that has been used before in studies on mammalian immunology [5]. A complete blood count was also performed using an ABC Vet automaton (Horiba Medical, Montpellier, France). It measured the total white blood cells (10^3 cells/mL) by impedance technology, considering parameters for bovine samples, since the size of blood cells is comparable between the two species [6]. With both the proportion of each leucocyte forms (in %) and the total white blood cells measurement (10^3 cells/mL), we obtained the count of each leucocyte (10^3 cells/mL). Humoral aspects were first assessed using the levels of alpha1-,alpha-2, beta- and gamma-globulins (mg/mL). Alpha1-, alpha2- and beta-globulins fractions including several acute phase proteins (APPs), a group of proteins which concentration changes following external or internal challenges such as trauma, inflammation or infection [7]. We also measured the specific level of haptoglobin (in mg/mL) which belongs to alpha2-globulin fraction. Total protein content (in mg/mL) was first assessed by refractometry followed by automatic agarose gel electrophoresis (HYDRASYS, Sebia, Evry, France) that separates albumin and the 4 fractions of globulins (α1, α2, β, and γ). Haptoglobin analyses were performed on a Konelab 30i automaton (Fisher Thermo Scientific, Cergy-Pontoise, France) using phase Haptoglobin assay (Tridelta Development LTD, County Kildare, Ireland) chromogenic kit. Finally, we used the level of gamma-globulins (mg/mL) derived from the protein analysis

described above as an estimator of total antibodies. Gamma-globulins are indeed essentially constituted of circulating antibodies [8], produced during adaptive response and often used as a measurement of allocation to long-term immunity [9]. Humoral innate immunity was also assessed by the levels of natural antibodies and complement. Natural antibodies are circulating antibodies that are present in the absence of any previous exposure to antigens. Their level is thus independent from the exposure of individuals to infection, but they are correlated to the ability to produce antibodies after a challenge [10]. Their presence is revealed by hemagglutination (HA, titre score), which measures the ability of samples to agglutinate exogenous cells. The complement is a group of proteins that acts through chain reactions and causes the lysis of exogenous cells in the presence of an antigen-antibody complex. They can thus be revealed by their ability to cause hemolysis (HL, titer score) [10]. We used the hemagglutination-hemolysis protocol defined in [10] and reported below, but modified using chicken red blood cells as target cells. The assay is carried out in 96-well (eight rows by 12 columns) round (U) bottom assay plates. Twenty-five microliters of eight plasma samples are pipetted into columns 1 and 2 of the plate, and 25 µl of 0.01M phosphate buffered saline (PBS; Sigma #P3813, St Louis, MO) are added to the columns 2–12. Using a multi-channel pipetter the contents of the column 2 wells are serially diluted (1:2) through column 11. This results in dilutions ranging from 1 to 1/1024 and 25 ml in every well. The column 12 with 25 µl of PBS serves as a negative control. For the assay itself, 25 µl of a 1% chicken blood cell suspension is added to all wells, effectively halving all plasma dilutions. Each plate is then sealed with Parafilm M (Pechiney Plastic Packaging, Neenah, WI) and covered with a polystyrene plate lid. Plates are gently vortexed for 10 s prior to incubation during which they are floated in a 37°C water bath for 90 min. Upon completion of the incubation, the long axis of each plate is tilted to a 458 angle for 20 min at room temperature in order to enhance visualization of HA. Plates are then scanned (full size image at 300 dpi) using the positive transparency (top-lit) setting of a flatbed scanner. Afterward, plates are kept at room temperature for an additional 70 min and scanned for a second time to record maximum lytic activity. From the digitized images, lysis and agglutination are scored for each sample. Lysis reflects the interaction of complement and NAbs, whereas agglutination results from NAbs only. Both variables are recorded as the negative log2 of the last plasma dilution exhibiting each behavior, i.e. column 9 is a score of 9 (see the figure below). Half scores between two titres are recorded when the termination of lysis or agglutination is intermediate or is ambiguous.

Figure 1: An example of scan . Ly = lysis, Ag = agglutination, No = no lysis or agglutination

To conclude, our main indicators of innate immunity were the neutrophil, monocyte and basophil counts, globulins levels (alpha1-,alpha2-,beta-), haptoglobin and hemolysis, while hemagglutination and gamma- globulins were more reflecting adaptive response. Eosinophil and lymphocyte counts may reflect both aspects. Haematological and biochemical assays were performed at the Biochemical and Endocrinological laboratory, VetAgro-Sup, France, while the hemagglutination-hemolysis assay was per- formed at the UMR 5558, Villeurbanne, France.

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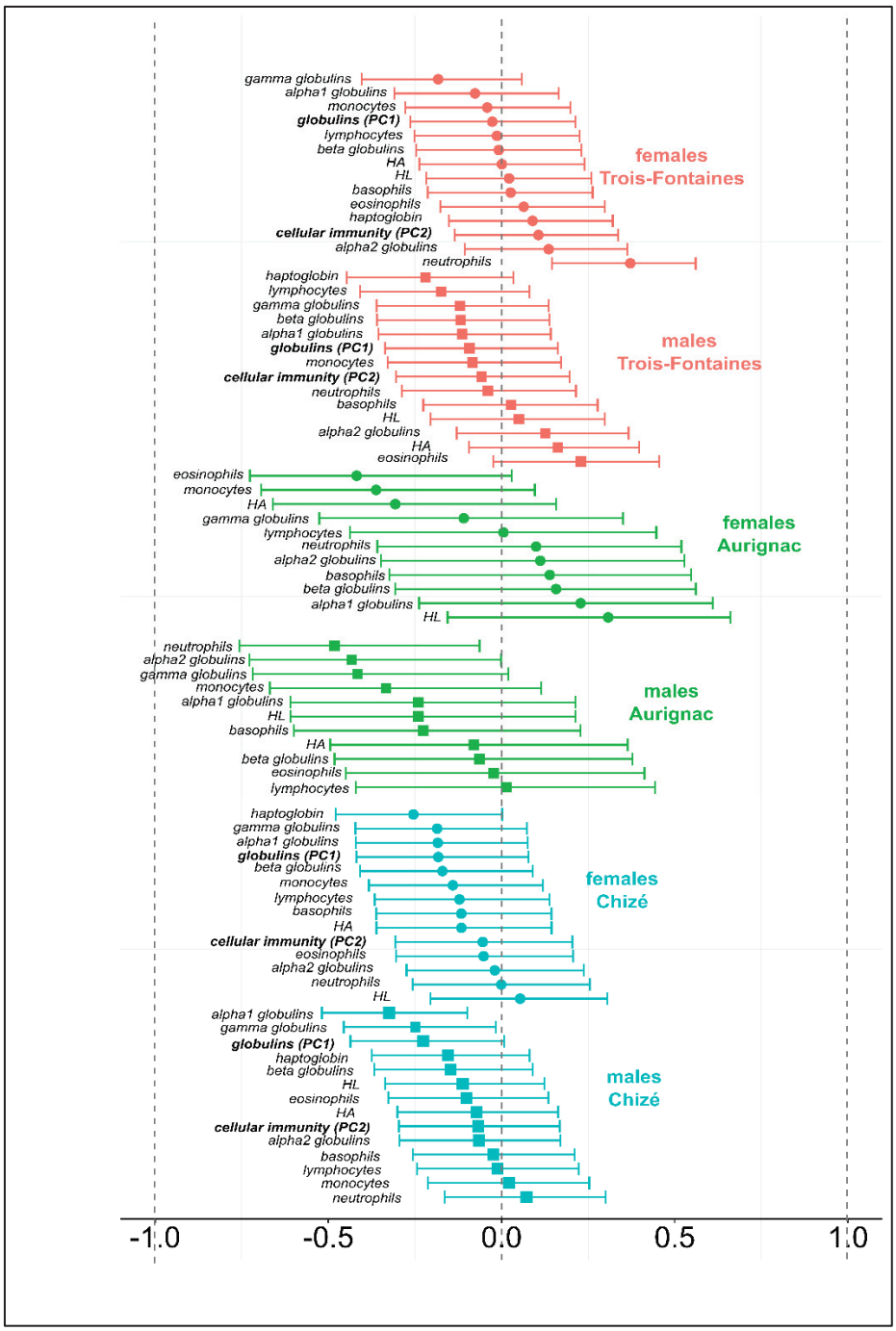
REPEATABILITY

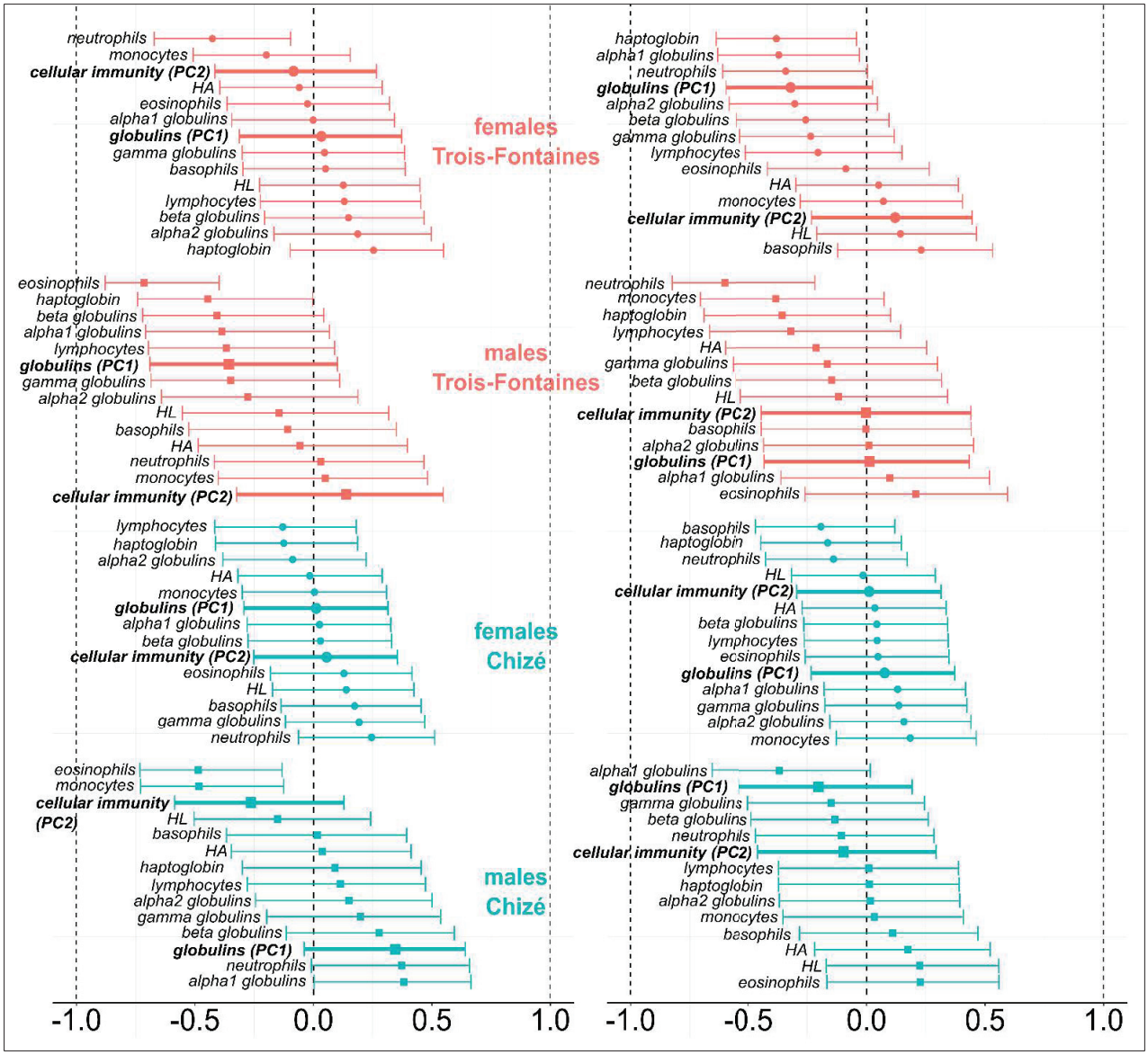
Immune trait	R	SE	CI
Neutrophil count	0.41	0.072	[0.26-0.55]
Monocyte count	0.29	0.140	[0.00-0.55]
Basophil count	0.22	0.154	[0.00-0.54]
Eosinophil count	0.23	0.160	[0.00-0.55]
Lymphocyte count	0.33	0.080	[0.17-0.48]
Hemagglutination	0.07	0.066	[0.00-0.22]
Hemolysis	0.42	0.093	[0.24-0.61]
Alpha1-gloublin	0.03	0.057	[0.00-0.20]
Alpha2-globulin	0.04	0.060	[0.00-0.19]
Haptoglobin	0.10	0.073	[0.00-0.26]
Beta-globulin	0.35	0.080	[0.18-0.50]
Gamma-globulin	0.46	0.070	[0.31-0.58]

Online Resource 2. Effect size of *early* growth on the immune traits measured in growing (8 months of age) at Chizé, Trois-Fontaines and Aurignac ; and effect size of *early* (left) and *late* growth (right) in adult roe deer at Chizé and Trois-Fontaines. Effect sizes (symbols) are reported together with associated 95%-confidence interval.

GROWING ROE DEER



ADULT ROE DEER



Online Resource 3. Supplementary methods (additional models testing the effect of *early* and *late* growth on roe deer immunity) and model selection table for immune traits of growing and adult roe deer. The best fitting models among the set of candidate models considering the inclusion of 'early growth' (EG), and late growth (LG) for adults, sex, population (Pop.), their interaction, and average cohort fawn mass (ACF mass) are reported. The selected model is indicated in grey.

SUPPLEMENTARY METHODS

We performed complementary analyses by testing the effect of *early* and *late* growth on immunity using linear mixed-effect models. Results of model selection in growing and adult roe deer are reported in Online Resource 3. Parameter estimates of the selected models in growing and adult roe deer are reported in Online Resource 4, respectively. Each immune trait (and the two first PCS measuring their covariation) during the growing period or at adulthood, was analysed as a response variable. When individuals had repeated measures during a given growth period, the median of these individual measures was retained to avoid any pseudo-replication problems (*sensu* Hurlbert 1984). *Early* growth, *late* growth, sex and population, as well as all possible interactions were included as fixed effects in the full model. All models included cohort as a random effect and average cohort fawn mass (calculated in each population from the total number of fawn mass records in a given year) as a fixed effect, to account for cohort variation in immunity that is not directly related to body growth (Gaillard et al. 1997). The different models accounting for the possible effects of *early* and *late* growth were ranked based on the Akaike Information Criterion corrected for small sample size (AICc, Burnham, Anderson 2002) in the R package MuMIn (Barton 2016). We selected the model with the lowest AICc. When the difference in AIC (denoted ΔAICc) of two competing models was less than 2, we retained the model with the lowest number of parameters by following parsimony rules. In addition, we calculated the AIC weights (ωi) to measure the relative likelihood of each model to be the best among the set of fitted models.

MODEL SELECTION FOR GROWING ROE DEER

Trait	Model	df	ΔAICc	ω_i
PC1	EG + Pop. + (EG × Pop.)	6	0.00	0.225
	EG + Pop.	5	1.03	0.134
	EG + Pop. + (EG × Pop.) + ACFmass	7	1.94	0.085
	Null	3	18.22	0.000
Alpha1-globulin	EG + Pop. + (EG × Pop.) + ACFmass.	9	0.00	0.470
	EG + Pop. + (EG × Pop.) + Sex + ACFmass	10	1.99	0.174
Alpha2-globulin	Pop. + Sex + (Sex × Pop.) + ACFmass	9	0.00	0.155
	Pop. + Sex + (Sex × Pop.)	8	0.77	0.106
	Pop.	5	1.43	0.076
	EG + pop. + Sex + (Pop. × Sex) + ACFmass	10	1.47	0.074
Haptoglobin	ACFmass + Pop. + EG	6	0.00	0.227
	ACFmass + Pop.	5	0.17	0.209
	ACFmass + Pop. + EG + Sex	7	1.54	0.105
	Null	3	9.33	0.002
Beta-globulin	Pop.	5	0.00	0.105
	EG + Pop. + (EG × Pop.)	8	0.06	0.102
	ACFmass	4	0.16	0.097
	EG + Pop.	6	0.49	0.082
Gamma-globulin	EG + Pop. + (EG × Pop.) + ACFmass	9	0.00	0.171
	EG + Pop. + (EG × Pop.)	8	0.18	0.156
	EG + Pop. + ACFmass	7	0.84	0.112
	EG + Pop.	6	0.88	0.110
PC2	Pop. + ACFmass	5	0.00	0.200
	Pop.	4	1.51	0.094
	Pop. + ACFmass + EG	6	1.82	0.081
	Null	3	8.94	0.002
Neutrophil count	Pop.	5	0.00	0.177
	EG + Sex + Pop. + (EG × Sex)	8	0.82	0.117
	EG + Pop.	6	1.51	0.082
	Pop. + Sex	6	1.70	0.075
Monocyte count	Pop.	5	0.00	0.229
	Pop. + ACFmass	6	0.68	0.163
	EG + Pop.	6	1.66	0.099
Basophil count	Sex	4	0.00	0.240
	Sex + ACFmass	5	1.12	0.137
	Null	3	1.50	0.113
	EG + Sex	5	1.50	0.088
Eosinophil count	Null	3	0.00	0.214
	Sex	4	1.03	0.128
	EG	4	1.30	0.111
	ACFmass	4	1.95	0.080
Lymphocyte count	Sex + Pop.	6	0.00	0.243
	EG + Sex + Pop.	7	1.24	0.131
	Pop. + Sex + ACFmass	7	1.77	0.101
Hemagglutination	ACFmass + Pop.	6	0.00	0.334
	ACFmass + Pop. + Sex	7	1.06	0.197
Hemolysis	ACFmass + Pop. + Sex	7	0.00	0.281
	ACFmass + Pop.	6	0.06	0.273
	EG + ACFmass + Pop.	7	1.87	0.110
	EG + ACFmass + Pop. + Sex	9	1.94	0.107

MODEL SELECTION FOR ADULT ROE DEER

Trait	Model	df	ΔAICc	ω_i
PC1	Pop. + Sex + (Sex × Pop.)	6	0.00	0.167
	Pop. + Sex + (Sex × Pop.) + LG	7	1.43	0.052
	Pop. + Sex + (Sex × Pop.) + ACFmass	7	2.13	0.058
	Null	3	30.92	0.000
Alpha1-globulin	Pop. + Sex + (Sex × Pop.)	6	0.00	0.071
	Pop. + Sex + (Sex × Pop.) + LG	7	0.54	0.055
	Sex	4	1.43	0.035
	Null	3	3.91	0.010
Alpha2-globulin	Null	3	0.00	0.059
	Pop.	4	0.39	0.049
	ACFmass	4	0.62	0.043
Haptoglobin	Pop. + Sex + (Sex × Pop.)	6	0.00	0.193
	Pop. + Sex + (Sex × Pop.) + LG	7	1.76	0.080
	Pop. + Sex + (Sex × Pop.) + EG	7	1.95	0.073
	Null	3	14.4	0.000
Beta-globulin	Pop. + Sex + (Sex × Pop.)	6	0.00	0.163
	Pop. + Sex + (Sex × Pop.) + LG	7	1.25	0.087
	Pop. + Sex + (Sex × Pop.) + ACFmass	7	1.71	0.069
	Null	3	25.46	0.000
Gamma-globulin	Pop.	4	0.00	0.105
	Pop. + EG + (EG × Pop.)	6	1.23	0.057
	Pop. + EG	5	1.61	0.047
	Null	3	62.28	0.000
PC2	Pop. + ACFmass	5	0.00	0.110
	Pop.	4	1.42	0.089
	Pop. + ACFmass + Sex + (Sex × Pop.)	7	1.89	0.043
	Null	3	7.62	0.002
Neutrophil count	EG + LG + Pop. + Sex + (EG × Pop.) + (EG × Sex.)	9	0.00	0.057
	EG + LG + Pop. + (EG × Pop.)	7	0.86	0.037
	EG + LG + Pop. + Sex + (EG × Pop.) + (EG × Sex.) + (LG × Pop.)	10	0.89	0.037
	Null	3	22.58	0.000
Monocyte count	LG + Sex + (LG × Sex.)	6	0.00	0.057
	LG + Sex + (LG × Sex.) + ACFmass	7	0.67	0.040
	Null	3	1.07	0.033
Basophil count	Null	3	0.00	0.123
	EG	4	1.23	0.066
	Pop.	4	1.51	0.058
Eosinophil count	Sex + EG + (EG × Sex.)	6	0.00	0.142
	Sex + EG + (EG × Sex.) + Pop.	7	1.97	0.053
	Sex + EG + (EG × Sex.) + LG	7	2.03	0.052
	Null	3	3.22	0.028
Lymphocyte count	Pop.	4	0.00	0.114
	Pop. + Sex	5	0.99	0.070
	Pop. + LG	5	1.50	0.054
	Null	3	20.58	0.000
Hemagglutination	Null	3	0.00	0.164
	Sex	4	1.33	0.084
	LG	4	2.00	0.064
Hemolysis	Pop.	5	0.00	0.055
	Pop. + ACFmass	4	0.03	0.054
	Null	3	0.79	0.037

Online Resource 4

- Parameter estimates of the selected linear mixed-effect model of immune traits in **growing roe deer** (at 8 months of age). Potential fixed effect are *early* growth (EG), population (Pop.; Chizé (CH), Aurignac (AURI) minus Trois-Fontaines), sex (males (M) minus females), their interaction, and average cohort fawn mass (ACFmass). All models included the cohort of individuals as random effects. Estimates are presented ± standard error (SE).Statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001. R^2m and R^2c are the marginal and conditional variance of the model, respectively.

Immune trait	Selected model	Estimate ± SE	t-value	p	R^2m	R^2c
PC1	**Intercept**	**-0.22 ± 0.38**	**-0.59**	**-**	**0.06**	**0.38**
	EG	**-0.11 ± 0.04**	**-2.55**	*****		
	Pop. (CH)	**0.73 ± 0.17**	**4.30**	*****		
Alpha1-globulin	Intercept	-0.91 ± 1.43	-0.636	-	0.08	0.31
	EG	-0.01 ± 0.03	-0.385	-		
	Pop. (CH)	1.11 ± 0.33	3.81	***		
	Pop. (AURI)	-0.05 ±0.12	-0.47	-		
	EG * Pop. (CH)	-0.11 ± 0.04	-2.90	**		
	EG * Pop. (AURI)	-0.01 ± 0.05	-0.21	-		
	ACFmass	0.27 ± 0.09	3.18	**		
Alpha2-globulin	Intercept	6.09 ± 0.33	18.51	***	0.03	0.11
	Pop. (CH)	0.03 ± 0.29	0.10	-		
	Pop. (AURI)	-1.49 ± 0.45	-3.33	***		
Haptoglobin	Intercept	1.83 ± 0.46	3.95	***	0.06	0.07
	ACFmass	-0.10 ± 0.03	-3.60	**		
	Pop. (CH)	-0.42 ± 0.11	-3.72	**		
Beta-globulin	Intercept	7.78 ± 0.59	13.15	***	0.04	0.26
	AFCmass	-0.13 ± 0.04	-3.62	***		
Gamma-globulin	Intercept	14.02 ± 0.87	16.11	***	0.33	0.51
	EG	-0.36 ± 0.09	-3.63	***		
	Pop. (CH)	4.57 ± 0.43	10.56	***		
	Pop. (AURI)	-3.36 ± 0.66	-5.11	***		
PC2	**Intercept**	**0.12 ± 0.37**	**0.56**	**-**	**0.02**	**0.52**
	Pop. (CH)	**-0.37 ± 0.12**	**-3.10**	**		
Neutrophil count	Intercept	5.80 ± 0.18	32.02	***	0.14	0.16
	Pop. (CH)	-1.57 ± 0.23	-6.86	***		
	Pop (AURI)	-0.10 ±0.34	-0.30	-		
Monocyte count	Intercept	0.32 ± 0.12	2.76	*	0.05	0.58
	Pop. (CH)	0.07 ±0.03	2.11	*		
	Pop. (AURI)	-0.24 ±0.05	-4.73	***		
Basophil count	Intercept	0.06 ± 0.02	3.85	-	0.00	0.16
Eosinophil count	Intercept	0.06 ± 0.01	6.79	-	0.00	0.05
Lymphocyte count	Intercept	2.66 ± 0.12	16.28	***	0.07	0.11
	Pop. (CH)	-0.36 ± 0.12	-2.98	**		
	Pop. (AURI)	0.30 ±0.18	1.70	-		
	Sex (M)	-0.27 ± 0.11	-2.43	*		
Hemagglutination	Intercept	11.41 ± 2.46	4.63	***	0.10	0.35
	AFCmass	-0.44 ± 0.15	-2.97	**		
	Pop. (CH)	-1.28 ± 0.57	-2.25	*		

	Pop. (AURI)	-0.49 ± 0.21	-2.35	*		
Hemolysis	Intercept	10.88 ± 1.95	5.57	***	0.07	0.61
	ACFmass	-0.53 ± 0.12	-4.58	***		
	Pop. (CH)	-2.15 ± 0.44	-4.87	***		
	Pop. (AURI)	0.59 ± 0.16	3.73	***		

- Parameter estimates of the selected linear mixed-effect model of immune traits in **adult roe deer**. Potential fixed effect are *early* growth (EG), *late* growth (LG), population (Pop.; Chizé (CH) minus Trois-Fontaines), sex (males (M) minus females), their interaction, and average cohort fawn mass (ACFmass). All models included the cohort of individuals as random effects. Estimates are presented ± standard error (SE).Statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001. R^2m and R^2c are the marginal and conditional variance of the model, respectively.

Immune trait	Selected model	Estimate ± SE	t-value	p	R^2m	R^2c
PC1	**Intercept**	**-0.37 ± 0.25**	**-1.47**	**-**	**0.26**	**0.26**
	Pop. (CH)	**0.81 ± 0.34**	**2.38**	*****		
	Sex (M)	**-0.38 ± 0.41**	**-0.92**	**-**		
	Pop. (CH) * Sex (M)	**1.64 ± 0.55**	**3.01**	******		
Alpha1-globulin	Intercept	3.14 ± 0.08	39.99	***	0.04	0.12
	Sex (M)	0.21 ± 0.10	2.23	*		
Alpha2-globulin	Intercept	5.77 ± 0.10	2.23	*	0.00	0.06
Haptoglobin	Intercept	0.12 ± 0.12	1.06	-	0.15	0.15
	Pop. (CH)	0.11 ± 0.16	0.71	-		
	Sex (M)	0.03 ± 0.19	0.15	-		
	Pop. (CH) * Sex (M)	0.60 ± 0.25	2.36	*		
Beta-globulin	Intercept	6.78 ± 0.24	28.17	***	0.23	0.23
	Pop. (CH)	0.65 ± 0.32	2.01	*		
	Sex (M)	-0.15 ± 0.40	-0.38	-		
	Pop. (CH) * Sex (M)	1.40 ± 0.52	2.69	**		
Gamma-globulin	Intercept	15.19 ± 0.52	28.96	***	0.41	0.41
	Pop. (CH)	6.45 ± 0.70	9.24	***		
PC2	**Intercept**	**0.43 ± 0.20**	**2.10**	*****	**0.07**	**0.15**
	Pop. (CH)	**-0.70 ± 0.23**	**-3.08**	******		
Neutrophil count	Intercept	6.11 ± 0.21	29.39	***	0.22	0.22
	EG	-0.24 ± 0.11	-2.11	*		
	Pop. (CH)	-1.38 ± 0.29	-4.81	***		
	LG	-0.22 ± 0.09	-2.42	*		
	EG* Pop. (CH)	0.52 ± 0.17	3.08	***		
Monocyte count	Intercept	0.30 ± 0.04	6.83	-	0.00	0.08
Basophil count	Intercept	0.08 ± 0.01	7.86	-	0.00	0.06
Eosinophil count	Intercept	0.10 ± 0.01	8.49	***	0.08	0.08
	EG	0.01 ± 0.01	1.14	-		
	Sex (M)	-0.01 ± 0.02	-0.32	-		
	EG * Sex (M)	-0.03 ± 0.01	-2.72	**		
Lymphocyte count	Intercept	2.24 ± 0.10	22.24	***	0.17	0.18
	Pop. (CH)	-0.64 ± 0.13	-4.95	***		
Hemagglutination	Intercept	4.15 ± 0.13	32.64	-	0.00	0.06
Hemolysis	Intercept	2.17 ± 0.19	11.68	-	0.00	0.17

Appendix 3.2. Supporting information for the paper *Maternal effects shape offspring condition and immunity in a wild mammal.*

L. Cheynel[1,*], E. Gilot-Fromont[1,2], B. Rey[1], F. Débias[1], S. Pardonnet[1], M. Pellerin[3], J-M. Gaillard[1] and J-F. Lemaître[1].

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Table S1. Distribution of the juvenile roe deer sampled according to sex, maternal age class and population (CH: Chizé, TF: Trois-Fontaines).

Maternal age (years)	**OFFSPRING**				
	MALES		FEMALES		
	CH	TF	CH	TF	**TOTAL**
3	0	4	3	4	**11**
4	2	2	1	1	**6**
5	2	1	2	4	**9**
6	0	3	2	2	**7**
7	3	2	4	0	**9**
8	1	1	0	3	**5**
9	0	0	1	1	**2**
10	0	1	0	0	**1**
11	0	0	0	3	**3**
12	0	1	0	0	**1**

Table S2. Comparison of the best explanatory models for each offspring trait (i.e. difference of AICs ≤ 2). "k" is the number of terms in the model. All models included the cohort of the offspring and maternal identity as random effects. The model with the lowest AIC is shown in boldface type; models with very similar explanatory power (ΔAIC ≤ 2.0) that included fewer terms (i.e. models selected) are highlighted in grey.

	Models	k	AIC	ΔAIC
HEMATOLOGICAL TRAITS				
ALBUMIN	**Population + maternal body mass**	**6**	**295.36**	**0.00**
	Population + maternal body mass + maternal age (classes)	7	296.92	1.55
	Population + maternal body mass * maternal age (classes)	8	296.95	1.59
	Population + offspring body mass	6	297.23	1.87
	Population	5	297.24	1.88
	Population + maternal body mass + maternal age (linear)	7	297.36	2.00
	Population + maternal body mass * maternal age (classes) + offspring body mass	9	297.36	2.00
FRUCTOSAMINE	**Population + maternal body mass**	**6**	**423.67**	**0.00**
	Population + maternal body mass + maternal age (linear)	7	424.71	1.04
	Population + maternal body mass + maternal age (linear) + offspring body mass	8	425.47	1.80
	Population + maternal body mass + maternal age (classes)	7	425.57	1.90
HEMATOCRIT	**Offspring body mass**	**5**	**321.36**	**0.00**
	Offspring body mass + maternal body mass	6	321.61	0.24
	Maternal body mass + population	6	322.45	1.09
	Offspring body mass + maternal age (classes)	6	322.96	1.60
	Offspring body mass + population	6	323.05	1.68
	Offspring body mass + offspring sex	6	323.05	1.69
	Offspring body mass + maternal age (linear)	6	323.14	1.78
	Offspring body mass + maternal body mass + maternal age (classes)	7	323.32	1.96
HEMOGLOBIN	**Population + maternal body mass**	**6**	**186.18**	**0.00**
	Population + maternal body mass * maternal age (linear)	8	187.29	1.11
	Population + maternal body mass + maternal age (linear)	7	187.98	1.80
	Population + maternal body mass + maternal age (classes)	7	188.17	1.99
IMMUNE TRAITS				
NEUTROPHIL COUNT	**Maternal body mass + population**	**6**	**203.07**	**0.00**
	Maternal body mass + maternal age (linear) + population	7	204.99	1.93
	Maternal body mass + maternal age (classes) + population	7	205.05	1.99
LYMPHOCYTE COUNT	**Maternal body mass**	**5**	**138.72**	**0.00**
	Maternal body mass + offspring sex	6	139.07	0.35
	Maternal body mass + population	6	139.37	0.65
	Maternal body mass + offspring body mass	6	140.54	1.82
	Maternal body mass + maternal age (linear)	6	140.62	1.90
	Maternal body mass + materna age (classes)	6	140.72	2.00
GAMMA-GLOBULIN	**Population**	**5**	**292.94**	**0.00**
	Population + offspring body mass	6	293.87	0.93
	Population + maternal age (linear)	6	294.30	1.36
	Population + maternal body mass	6	294.42	1.48
	Population + maternal age (classes)	6	294.67	1.73
	Population + offspring sex	6	294.92	1.99
HAPTOGLOBIN	**Null**	**4**	**-90.28**	**0.00**
	Population	5	-88.49	1.79
	Maternal age (linear)	5	-88.35	1.93
	Offspring sex	5	-88.33	1.95
	Maternal age (classes)	5	-88.33	1.95
	Maternal body mass	5	-88.30	1.98
	Offspring body mass	5	-88.28	2.00

Appendix 4.1. Supporting information for the paper *Immunosenescence patterns differ between populations but not between sexes in a long-lived mammal*.

L. Cheynel[1], J.-F. Lemaître[1], J.-M. Gaillard[1], B. Rey[1], G. Bourgoin[1,2], H. Ferté[3], M. Jégo[1], F. Débias[1], M. Pellerin[4], L. Jacob[1] & E. Gilot-Fromont[1,2].

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Supporting information

Immunosenescence patterns differ between populations but not between sexes in a long-lived mammal.

AUTHORS: L. Cheynel, J-F. Lemaître, J-M. Gaillard, B. Rey, G. Bourgoin, H. Ferté, M. Jégo, F. Débias, M. Pellerin, L. Jacob and E. Gilot-Fromont.

Table S1. Composition of age classes in both sexes of the two populations of roe deer.

Table S2. Best models selected to describe senescence patterns of 12 immune and 4 parasitic traits, when including the two oldest males in Chizé (see Methods). We tested the effect of population ("pop"), sex ("sex") and 4 age functions (linear, factor, threshold and quadratic). Model comparison was based on AIC, "k" is the number of parameters, "weight" is the AIC weight of each model. All models included individual identity, the year of capture and the cohort of individuals as random effects. r2m and r2c are the marginal and conditional variance of the model, respectively.

Table S3. Pearson correlation matrix for the immune traits used in this study.

Table S4. Linear mixed effect model selected for WBC. The effect of 4 different age functions (factor, linear, threshold, quadratic), of sex (F: Female, M: Male) and of population (TF: Trois-Fontaines, CH: Chizé) were tested. All models included individual identity, the year of capture and the cohort of individuals as random effects. Statistical significance of age or body mass function are represented by * for p=0.05, ** for p=0.01 and *** for p=0.001. R2m and r2c are the marginal and conditional variance of the model, respectively. Values are presented ± Standard Error.

Table S5. Set of models fitted to assess senescence patterns of 12 immune parameters. We tested the effect of population ("P"), sex ("S), 4 age functions (linear "AL", factor "AF", threshold "AT" and quadratic "I(AL^2)") with all two and three-way interactions between them. "I" is the Intercept, "df" is the number of parameters, "delta" is the difference of AIC between the candidate model and the model having the lowest AIC, and "weight" the AIC weight of each model. We selected the model with the lowest AIC, and when some models had very similar explanatory power (delta<2), we selected the one which included fewer terms (shown in bold).

Table S6. Set of models fitted to assess senescence patterns of 4 parasitic traits. We tested the effect of population ("P"), sex ("S), 4 age functions (linear "AL", factor "AF", threshold "AT" and quadratic "I(AL^2)") with all two and three-way interactions between them. "I" is the Intercept, "df" is the number of parameters, "delta" is the difference of AIC between the candidate model and the model having the lowest AIC, and "weight" the AIC weight of each model. We selected the model with the lowest AIC, and when some models had very similar explanatory power (delta<2), we selected the one which included fewer terms (shown in bold).

Table S7. Linear mixed effect models selected for 12 immune parameters and 4 parasitic traits, analysed separately according to sex and roe deer populations (TF: Trois-Fontaines, CH: Chizé). The effect of different age functions (factor, linear, threshold, quadratic) was tested. All models included individual identity, the year of capture and the cohort of individuals as random effects; and were tested with and without body mass (BM) as a covariate. When the age threshold model was selected, "Age of change" represents the age at which the parameter begins to vary, and the "Parameter estimate" of the age function is the slope of the variation with age after the threshold age. Statistical significance of age or body mass function are represented by * for p=0.05, ** for p=0.01 and *** for p=0.001. R2m and R2c are the marginal and conditional variance of the model, respectively. Values are presented ± Standard Error.

Table S8. Linear mixed models describing the relationships between the 12 immune traits and the 4 parasitic traits of the study. Each immune trait was analysed as a function of parasite load, population and the interaction parasite load*population, considering 4 different parasite groups. Models included individual identity as a random effect. The "estimate" for the population or the parasitic trait is the slope of the variation. Statistical significance is represented by . for p=0.1, * for p=0.05, ** for p=0.01 and *** for p=0.001. Values are presented ± 1 SE.

Table S9. Set of models fitted to assess senescence patterns of immune traits including a fixed effect of the longevity of individuals, in males and females at Trois-Fontaines, and in females at Chizé. We do not have enough data on Chizé males to test these models. Model comparison was based on AIC, k is the number of parameters, ΔAIC is the difference of AIC between the candidate model and the model having the lowest AIC, and wi the AIC weight of each model. All models included individual identity, the year of capture and the cohort of individuals as random effects. The model with the lowest AIC is shown in italics; and models with very similar explanatory power (ΔAIC < 2), which included fewer terms, are shown in bold.

Fig. S1. Selection of the best threshold for the "threshold model" by maximum likelihood (see Methods). We used the deviance profiles of a continuous age model with a varying threshold and selected the age leading to the lowest deviance as the threshold age.

Table S1. Composition of age classes in both sexes of the two populations of roe deer.

	TROIS-FONTAINES		CHIZE	
Age (years)	Males	Females	Males	Females
2	29	38	29	24
3	18	29	25	20
4	17	24	16	19
5	22	14	11	23
6	20	23	9	16
7	12	9	7	14
8	8	12	8	27
9	8	7	4	11
10	3	10	2	13
11	3	5	1	5
12	2	4	0	5
13	1	4	0	1
14	0	0	1	0
15	0	1	0	0
16	0	1	0	0
total	**143**	**181**	**113**	**178**

Table S2. Best models selected to describe senescence patterns of 12 immune and 4 parasitic traits, when including the two oldest males in Chizé (see Methods). We tested the effect of population ("pop"), sex ("sex") and 4 age functions (linear, factor, threshold and quadratic). Model comparison was based on AIC, "k" is the number of parameters, "weight" is the AIC weight of each model. All models included individual identity, the year of capture and the cohort of individuals as random effects. r^2m and r^2c are the marginal and conditional variance of the model, respectively.

Trait	Best model selected	Age function	k	AIC	weight	r2m	r2c
Neutrophil count	age : pop : sex	Threshold (9 years)	12	2609.15	0.74	0.10	0.50
Monocyte count	constant	-	5	202.52	0.51	0.00	0.46
Basophil count	constant	-	5	-1017.03	0.41	0.00	0.28
Eosinophil count	sex	-	6	-690.54	0.12	0.02	0.15
Hemagglutination	age : pop	Threshold (8 years)	8	2043.18	0.43	0.01	0.44
Hemolysis	age + pop	Threshold (8 years)	7	1771.51	0.14	0.01	0.61
Alpha1-globulin	age + pop + sex	Threshold (8 years)	8	1093.19	0.09	0.03	0.50
Alpha2-globulin	constant	-	5	2278.83	0.07	0.00	0.26
Beta-globulin	age + pop + sex	Linear	8	2656.85	0.23	0.12	0.42
Haptoglobin	age : pop + sex	Threshold (9 years)	9	1839.66	0.31	0.08	0.15
Gamma-globulin	age + pop	Threshold (4 years)	7	3653.52	0.36	0.22	0.65
Lymphocyte count	Age + I(age^2) + pop	Quadratic	8	1509.26	0.14	0.14	0.37
Gastro-intestinal strongyles	age : sex	Threshold (9 years)	8	6105.00	0.32	0.10	0.75
Trichuris sp.	age : sex : pop	Factor	48	6991.74	0.99	0.34	0.39
Protostrongylids	age : sex : pop	Threshold (11 years)	12	2936.80	0.99	0.50	0.50
Coccidia	pop	-	6	10756.79	0.38	0.00	0.99

Table S3. Pearson correlation matrix for the immune traits used in this study.

	WBC	Neutrophil	Eosinophil	Basophil	Monocyte	Lymphocyte	Alpha-1-globulin	Alpha-2-globulin	Beta-globulin	Gamma-globulin	Haptoglobin	Hemag-glutination	Hemo-lysis
WBC	1.00	0.91	0.08	0.08	0.07	0.48	0.04	-0.01	-0.01	-0.09	0.04	-0.01	0.10
Neutrophil	0.91	1.00	-0.02	-0.03	-0.07	0.12	0.05	0.05	0.07	-0.05	0.08	-0.03	0.13
Eosinophil	0.08	-0.02	1.00	0.27	0.09	0.07	-0.07	0.03	0.03	0.07	-0.03	-0.05	-0.04
Basophil	0.08	-0.03	0.27	1.00	0.22	0.05	0.00	-0.05	0.04	0.11	0.10	0.04	-0.05
Monocyte	0.07	-0.07	0.09	0.22	1.00	-0.09	-0.13	-0.12	-0.06	0.01	0.05	0.01	-0.24
Lymphocyte	0.48	0.12	0.07	0.05	-0.09	1.00	0.04	-0.09	-0.15	-0.13	-0.07	0.03	0.07
Alpha-1-globulin	0.04	0.05	-0.07	0.00	-0.13	0.04	1.00	0.39	0.52	0.44	0.24	-0.04	-0.05
Alpha-2-globulin	-0.01	0.05	0.03	-0.05	-0.12	-0.09	0.39	1.00	0.17	0.26	-0.04	-0.06	0.03
Beta-globulin	-0.01	0.07	0.03	0.04	-0.06	-0.15	0.52	0.17	1.00	0.65	0.63	-0.07	-0.02
Gamma-globulin	-0.09	-0.05	0.07	0.11	0.01	-0.13	0.44	0.26	0.65	1.00	0.32	0.04	-0.08
Haptoglobin	0.04	0.08	-0.03	0.10	0.05	-0.07	0.24	-0.04	0.63	0.32	1.00	0.02	0.00
Hemagglutination	-0.01	-0.03	-0.05	0.04	0.01	0.03	-0.04	-0.06	-0.07	0.04	0.02	1.00	0.45
Hemolysis	0.10	0.13	-0.04	-0.05	-0.24	0.07	-0.05	0.03	-0.02	-0.08	0.00	0.45	1.00

Table S4. Linear mixed effect model selected for WBC. The effect of 4 different age functions (factor, linear, threshold, quadratic), of sex (F: Female, M: Male) and of population (TF: Trois-Fontaines, CH: Chizé) were tested. All models included individual identity, the year of capture and the cohort of individuals as random effects. Statistical significance of age or body mass function are represented by * for p=0.05, ** for p=0.01 and *** for p=0.001. R2m and r2c are the marginal and conditional variance of the model, respectively. Values are presented ± Standard Error.

Immune trait	Best model selected	Age function	Variable	Parameter estimate ± SE	t-value	p	r2m	r2c
White Blood Cells count	Age +I(age^2)*sex + pop	quadratic	Intercept	9.87 ±0.45	21.91	***	0.13	0.52
			Age	-0.36 ±0.15	-2.39	*		
			I(age^2)	0.04 ±0.01	3.17	**		
			Sex (M)	0.35 ±0.33	1.05	-		
			Pop (CH)	-1.74 ±0.33	-6.98	***		
			I(age^2) : sex (M)	-0.02 ±0.01	-2.76	**		

Predicted value at two years			
♂ TF	♀TF	♂ CH	♀ CH
8.93 ±0.28	8.90 ±0.39	7.50 ±0.41	7.49 ±0.23

Table S5. Set of models fitted to assess senescence patterns of 12 immune parameters. We tested the effect of population ("P"), sex ("S), 4 age functions (linear "AL", factor "AF", threshold "AT" and quadratic "I(AL^2)") with all two and three-way interactions between them. "I" is the Intercept, "df" is the number of parameters, "delta" is the difference of AIC between the candidate model and the model having the lowest AIC, and "weight" the AIC weight of each model. We selected the model with the lowest AIC, and when some models had very similar explanatory power (delta<2), we selected the one which included fewer terms (shown in bold).

NEUTROPHIL COUNT																
	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
24	5.71	0.15	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	2588.20	0.00	0.50
56	5.75	0.15	+	+	NA	+	+	NA	NA	NA	NA	NA	10	2590.11	1.90	0.19
32	5.69	0.16	+	+	+	+	NA	NA	NA	NA	NA	NA	10	2590.25	2.05	0.18
64	5.73	0.16	+	+	+	+	+	NA	NA	NA	NA	NA	11	2592.17	3.97	0.07
128	5.62	0.18	+	+	+	+	+	+	NA	NA	NA	NA	12	2593.44	5.24	0.04
4	6.11	0.06	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	2597.30	9.09	0.01
3	6.41	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2597.92	9.72	0.00
8	6.18	0.06	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	2598.97	10.76	0.00
7	6.49	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2599.31	11.11	0.00
12	6.14	0.05	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	2599.31	11.11	0.00
40	6.23	0.06	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	2600.67	12.46	0.00
16	6.20	0.05	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	2601.01	12.81	0.00
39	6.53	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	2601.17	12.97	0.00
48	6.26	0.05	+	+	+	NA	+	NA	NA	NA	NA	NA	10	2602.69	14.49	0.00
22	5.21	0.14	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	2612.32	24.11	0.00
2	5.59	0.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2617.69	29.49	0.00
1	5.90	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2618.46	30.25	0.00
6	5.66	0.06	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2619.44	31.24	0.00
5	5.97	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2619.95	31.75	0.00
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
3	6.41	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2597.92	0.00	0.32
4	6.43	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	2598.09	0.17	0.29
7	6.49	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2599.31	1.39	0.16
8	6.48	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	2599.98	2.05	0.11
39	6.53	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	2601.17	3.25	0.06
40	6.51	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	2601.94	4.01	0.04
12	6.53	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	2606.04	8.12	0.01
24	6.34	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	2607.86	9.94	0.00
16	6.59	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	2607.95	10.03	0.00
48	6.63	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	2609.94	12.02	0.00
56	6.36	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	2610.00	12.08	0.00
32	6.43	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	2616.20	18.27	0.00
2	5.95	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	2616.48	18.55	0.00
64	6.45	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	2618.40	20.48	0.00
6	6.00	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	2618.42	20.50	0.00
1	5.90	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2618.46	20.53	0.00
5	5.97	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2619.95	22.03	0.00
128	6.34	+	+	+	+	+	+	+	NA	NA	NA	NA	46	2627.36	29.44	0.00
22	5.88	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	2629.50	31.58	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
4	2.02	0.48	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	2590.51	0.00	0.25
24	0.67	0.63	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	2590.65	0.14	0.23
12	2.69	0.40	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	2592.21	1.69	0.11
8	2.15	0.47	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	2592.32	1.81	0.10
56	0.72	0.63	+	+	NA	+	+	NA	NA	NA	NA	NA	10	2592.64	2.13	0.08
32	0.52	0.64	+	+	+	+	NA	NA	NA	NA	NA	NA	10	2592.71	2.20	0.08
16	2.80	0.40	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	2594.04	3.53	0.04
40	2.18	0.47	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	2594.16	3.64	0.04
64	0.63	0.64	+	+	+	+	+	NA	NA	NA	NA	NA	11	2594.72	4.20	0.03
48	2.90	0.39	+	+	+	NA	+	NA	NA	NA	NA	NA	10	2595.82	5.30	0.02
128	0.74	0.62	+	+	+	+	+	+	NA	NA	NA	NA	12	2596.70	6.18	0.01
3	6.41	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2597.92	7.41	0.01
7	6.49	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2599.31	8.80	0.00
39	6.53	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	2601.17	10.66	0.00
2	1.06	0.53	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2609.19	18.68	0.00
22	-0.16	0.66	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	2610.34	19.82	0.00
6	1.16	0.52	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2611.08	20.56	0.00
1	5.90	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2618.46	27.94	0.00
5	5.97	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2619.95	29.44	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
144	6.61	-0.24	0.03	+	+	NA	NA	NA	+	NA	NA	NA	10	2583.83	0.00	0.15
143	**6.00**	**NA**	**0.01**	**+**	**+**	**NA**	**NA**	**NA**	**+**	**NA**	**NA**	**NA**	**9**	**2584.89**	**1.05**	**0.09**
48	6.44	-0.16	0.03	+	+	NA	+	NA	NA	NA	NA	NA	10	2585.09	1.25	0.08
176	6.69	-0.27	0.03	+	+	NA	+	NA	+	NA	NA	NA	11	2585.81	1.98	0.06
400	6.63	-0.24	0.03	+	+	NA	NA	NA	+	+	NA	NA	11	2585.85	2.02	0.06
208	6.59	-0.24	0.03	+	+	NA	NA	+	+	NA	NA	NA	11	2585.89	2.06	0.05
160	6.60	-0.24	0.03	+	+	+	NA	NA	+	NA	NA	NA	11	2585.90	2.07	0.05
399	6.04	NA	0.01	+	+	NA	NA	NA	+	+	NA	NA	10	2586.82	2.99	0.03
207	5.98	NA	0.01	+	+	NA	NA	+	+	NA	NA	NA	10	2586.87	3.04	0.03
304	6.46	-0.16	0.02	+	+	NA	+	NA	NA	+	NA	NA	11	2587.07	3.23	0.03
64	6.44	-0.16	0.03	+	+	+	+	NA	NA	NA	NA	NA	11	2587.16	3.33	0.03
112	6.44	-0.16	0.03	+	+	NA	+	+	NA	NA	NA	NA	11	2587.16	3.33	0.03
432	6.70	-0.27	0.03	+	+	NA	+	NA	+	+	NA	NA	12	2587.84	4.01	0.02
240	6.68	-0.28	0.03	+	+	NA	+	+	+	NA	NA	NA	12	2587.86	4.03	0.02
192	6.68	-0.27	0.03	+	+	+	+	NA	+	NA	NA	NA	12	2587.89	4.05	0.02
224	6.67	-0.27	0.03	+	+	+	NA	+	+	NA	NA	NA	12	2587.90	4.06	0.02
464	6.61	-0.24	0.03	+	+	NA	NA	+	+	+	NA	NA	12	2587.92	4.09	0.02
416	6.62	-0.24	0.03	+	+	+	NA	NA	+	+	NA	NA	12	2587.93	4.10	0.02
46	5.71	0.15	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	2588.20	4.37	0.02
463	6.01	NA	0.01	+	+	NA	NA	+	+	+	NA	NA	11	2588.84	5.01	0.01
320	6.47	-0.16	0.03	+	+	+	+	NA	NA	+	NA	NA	12	2589.15	5.31	0.01
368	6.46	-0.16	0.02	+	+	NA	+	+	NA	+	NA	NA	12	2589.15	5.32	0.01
128	6.47	-0.18	0.03	+	+	+	+	+	NA	NA	NA	NA	12	2589.23	5.40	0.01
1488	6.62	-0.26	0.03	+	+	NA	NA	+	+	+	NA	+	13	2589.32	5.49	0.01
832	6.38	-0.16	0.03	+	+	+	+	NA	NA	+	+	NA	13	2589.79	5.96	0.01
256	6.78	-0.32	0.04	+	+	+	+	+	+	NA	NA	NA	13	2589.85	6.01	0.01
496	6.69	-0.27	0.03	+	+	NA	+	+	+	+	NA	NA	13	2589.91	6.08	0.01
448	6.69	-0.27	0.03	+	+	+	+	NA	+	+	NA	NA	13	2589.93	6.10	0.01
480	6.69	-0.27	0.03	+	+	+	NA	+	+	+	NA	NA	13	2589.94	6.10	0.01
302	5.75	0.15	NA	+	+	NA	+	NA	NA	+	NA	NA	10	2590.11	6.27	0.01
62	5.69	0.16	NA	+	+	+	+	NA	NA	NA	NA	NA	10	2590.25	6.42	0.01
8	6.98	-0.33	0.03	+	NA	NA	NA	NA	NA	NA	NA	NA	8	2590.51	6.67	0.01
1487	5.98	NA	0.02	+	+	NA	NA	+	+	+	NA	+	12	2590.67	6.83	0.01
960	6.58	-0.25	0.03	+	+	+	+	NA	+	+	+	NA	14	2590.96	7.13	0.00
384	6.50	-0.18	0.03	+	+	+	+	+	NA	+	NA	NA	13	2591.22	7.39	0.00
1520	6.65	-0.28	0.04	+	+	NA	+	+	+	+	NA	+	14	2591.39	7.56	0.00
1504	6.63	-0.27	0.04	+	+	+	NA	+	+	+	NA	+	14	2591.42	7.58	0.00
896	6.33	-0.13	0.02	+	+	+	+	+	NA	+	+	NA	14	2591.85	8.02	0.00
512	6.79	-0.32	0.04	+	+	+	+	+	+	+	NA	NA	14	2591.91	8.07	0.00
318	5.73	0.16	NA	+	+	+	+	NA	NA	+	NA	NA	11	2592.17	8.34	0.00
16	7.03	-0.33	0.03	+	+	NA	NA	NA	NA	NA	NA	NA	9	2592.31	8.48	0.00
72	7.02	-0.33	0.03	+	NA	NA	NA	+	NA	NA	NA	NA	9	2592.41	8.57	0.00
24	7.04	-0.35	0.03	+	NA	+	NA	NA	NA	NA	NA	NA	9	2592.43	8.60	0.00
1024	6.60	-0.26	0.03	+	+	+	+	+	+	+	+	NA	15	2593.06	9.23	0.00
830	5.62	0.18	NA	+	+	+	+	NA	NA	+	+	NA	12	2593.44	9.61	0.00
1536	6.69	-0.29	0.04	+	+	+	+	+	+	+	NA	+	15	2593.49	9.66	0.00
272	7.06	-0.33	0.03	+	+	NA	NA	NA	NA	+	NA	NA	10	2594.19	10.36	0.00
80	7.06	-0.33	0.03	+	+	NA	NA	+	NA	NA	NA	NA	10	2594.26	10.42	0.00
32	7.08	-0.34	0.03	+	+	+	NA	NA	NA	NA	NA	NA	10	2594.28	10.45	0.00
7	6.17	NA	0.01	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2594.43	10.60	0.00
2048	6.44	-0.18	0.03	+	+	+	+	+	+	+	+	+	16	2594.43	10.60	0.00
88	6.99	-0.32	0.03	+	NA	+	NA	+	NA	NA	NA	NA	10	2594.47	10.64	0.00
336	7.10	-0.33	0.03	+	+	NA	NA	+	NA	+	NA	NA	11	2596.09	12.26	0.00
288	7.12	-0.34	0.03	+	+	+	NA	NA	NA	+	NA	NA	11	2596.13	12.29	0.00
15	6.23	NA	0.01	+	+	NA	NA	NA	NA	NA	NA	NA	8	2596.21	12.38	0.00
96	7.03	-0.32	0.03	+	+	+	NA	+	NA	NA	NA	NA	11	2596.32	12.49	0.00
71	6.20	NA	0.01	+	NA	NA	NA	+	NA	NA	NA	NA	8	2596.41	12.58	0.00
6	6.11	0.06	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2597.30	13.46	0.00
271	6.29	NA	0.01	+	+	NA	NA	NA	NA	+	NA	NA	9	2597.88	14.04	0.00
5	6.41	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2597.92	14.09	0.00
352	7.06	-0.31	0.03	+	+	+	NA	+	NA	+	NA	NA	12	2598.16	14.33	0.00
79	6.25	NA	0.01	+	+	NA	NA	+	NA	NA	NA	NA	9	2598.22	14.39	0.00
14	6.18	0.06	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	2598.97	15.13	0.00
13	6.49	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	2599.31	15.48	0.00
22	6.14	0.05	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	2599.31	15.48	0.00
335	6.31	NA	0.01	+	+	NA	NA	+	NA	+	NA	NA	10	2599.85	16.01	0.00
270	6.23	0.06	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	2600.67	16.83	0.00
30	6.20	0.05	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	2601.01	17.18	0.00
269	6.53	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	2601.17	17.34	0.00
286	6.26	0.05	NA	+	+	+	NA	NA	NA	+	NA	NA	10	2602.69	18.86	0.00
140	6.21	-0.29	0.03	NA	+	NA	NA	NA	+	NA	NA	NA	9	2605.95	22.12	0.00
44	6.08	-0.23	0.03	NA	+	NA	+	NA	NA	NA	NA	NA	9	2607.35	23.51	0.00
172	6.38	-0.36	0.04	NA	+	NA	+	NA	+	NA	NA	NA	10	2607.62	23.79	0.00
139	5.47	NA	0.01	NA	+	NA	NA	NA	+	NA	NA	NA	8	2608.23	24.40	0.00
4	6.55	-0.36	0.04	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	2609.45	25.62	0.00
12	6.60	-0.36	0.04	NA	+	NA	NA	NA	NA	NA	NA	NA	8	2611.33	27.49	0.00
42	5.21	0.14	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	2612.32	28.48	0.00
3	5.66	NA	0.01	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2614.43	30.59	0.00
11	5.71	NA	0.01	NA	+	NA	NA	NA	NA	NA	NA	NA	7	2616.28	32.45	0.00
2	5.59	0.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2617.69	33.86	0.00
1	5.90	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2618.46	34.62	0.00
10	5.66	0.06	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	2619.44	35.61	0.00

9	5.97	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	2619.95	36.12	0.00

MONOCYTE COUNT

	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
1	**0.29**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**5**	**203.82**	**0.00**	**0.28**
2	0.31	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	204.87	1.05	0.17
3	0.30	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	205.70	1.88	0.11
5	0.29	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	205.79	1.97	0.11
6	0.32	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	206.74	2.91	0.07
4	0.32	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	206.76	2.94	0.07
7	0.30	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	207.67	3.85	0.04
22	0.33	-0.01	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	208.24	4.42	0.03
12	0.31	0.00	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	208.59	4.77	0.03
8	0.32	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	208.61	4.79	0.03
39	0.29	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	209.34	5.51	0.02
40	0.32	0.00	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	210.11	6.29	0.01
24	0.34	-0.01	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	210.19	6.36	0.01
16	0.31	0.00	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	210.40	6.58	0.01
48	0.31	0.00	+	+	+	NA	+	NA	NA	NA	NA	NA	10	211.74	7.92	0.01
56	0.33	-0.01	+	+	NA	+	+	NA	NA	NA	NA	NA	10	211.76	7.94	0.01
32	0.33	0.00	+	+	+	+	NA	NA	NA	NA	NA	NA	10	212.02	8.20	0.00
64	0.32	0.00	+	+	+	+	+	NA	NA	NA	NA	NA	11	213.46	9.64	0.00
128	0.31	0.00	+	+	+	+	+	+	NA	NA	NA	NA	12	215.38	11.56	0.00
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
1	0.29	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	203.82	0.00	0.51
3	0.30	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	205.70	1.88	0.20
5	0.29	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	205.79	1.97	0.19
7	0.30	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	207.67	3.85	0.07
39	0.29	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	209.34	5.51	0.03
2	0.33	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	218.18	14.36	0.00
4	0.34	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	220.11	16.29	0.00
6	0.34	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	220.19	16.36	0.00
8	0.34	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	222.10	18.28	0.00
40	0.33	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	223.51	19.69	0.00
22	0.38	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	233.03	29.21	0.00
12	0.34	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	233.53	29.71	0.00
24	0.38	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	235.18	31.36	0.00
16	0.34	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	235.59	31.77	0.00
56	0.37	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	236.53	32.71	0.00
48	0.33	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	237.04	33.22	0.00
32	0.38	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	246.79	42.97	0.00
64	0.37	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	248.04	44.22	0.00
128	0.38	+	+	+	+	+	+	+	NA	NA	NA	NA	46	259.75	55.93	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
1	0.29	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	203.82	0.00	0.32
2	0.31	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	205.38	1.56	0.15
3	0.30	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	205.70	1.88	0.12
5	0.29	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	205.79	1.97	0.12
4	0.32	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	207.27	3.45	0.06
6	0.32	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	207.28	3.46	0.06
7	0.30	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	207.67	3.85	0.05
12	0.30	0.00	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	208.96	5.14	0.02
8	0.32	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	209.16	5.34	0.02
22	0.32	-0.01	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	209.24	5.42	0.02
39	0.29	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	209.34	5.51	0.02
40	0.32	0.00	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	210.73	6.91	0.01
16	0.31	0.00	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	210.81	6.99	0.01
24	0.33	0.00	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	211.15	7.33	0.01
48	0.30	0.00	+	+	+	NA	+	NA	NA	NA	NA	NA	10	212.20	8.38	0.00
56	0.32	-0.01	+	+	NA	+	+	NA	NA	NA	NA	NA	10	212.75	8.93	0.00
32	0.31	0.00	+	+	+	+	NA	NA	NA	NA	NA	NA	10	212.83	9.01	0.00
64	0.30	0.00	+	+	+	+	+	NA	NA	NA	NA	NA	11	214.26	10.43	0.00
128	0.29	0.00	+	+	+	+	+	+	NA	NA	NA	NA	12	216.10	12.28	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
1	0.29	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	203.82	0.00	0.16
2	0.31	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	204.87	1.05	0.09
3	0.30	NA	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	205.33	1.51	0.07
5	0.30	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	205.70	1.88	0.06
4	0.36	-0.02	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	205.76	1.94	0.06
9	0.29	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	205.79	1.97	0.06
10	0.32	0.00	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	206.74	2.91	0.04
6	0.32	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	206.76	2.94	0.04
172	0.45	-0.06	0.00	NA	+	NA	+	NA	+	NA	NA	NA	10	207.07	3.25	0.03
7	0.30	NA	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	7	207.22	3.40	0.03
11	0.31	NA	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	7	207.23	3.41	0.03
13	0.30	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	207.67	3.85	0.02
12	0.36	-0.02	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	8	207.67	3.85	0.02
8	0.36	-0.02	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	8	207.69	3.87	0.02
42	0.33	-0.01	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	208.24	4.42	0.02
22	0.31	0.00	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	208.59	4.77	0.01
14	0.32	0.00	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	208.61	4.79	0.01
44	0.39	-0.03	0.00	NA	+	NA	+	NA	NA	NA	NA	NA	9	208.75	4.93	0.01
71	0.30	NA	0.00	+	NA	NA	NA	+	NA	NA	NA	NA	8	208.82	5.00	0.01
15	0.31	NA	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	8	209.11	5.29	0.01
176	0.45	-0.05	0.00	+	+	NA	+	NA	+	NA	NA	NA	11	209.13	5.31	0.01
139	0.31	NA	0.00	NA	+	NA	NA	NA	+	NA	NA	NA	8	209.23	5.41	0.01
269	0.29	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	209.34	5.51	0.01
140	0.37	-0.03	0.00	NA	+	NA	NA	NA	+	NA	NA	NA	9	209.48	5.66	0.01
72	0.35	-0.02	0.00	+	NA	NA	NA	+	NA	NA	NA	NA	9	209.48	5.66	0.01
16	0.37	-0.02	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	9	209.60	5.78	0.01
24	0.35	-0.02	0.00	+	NA	+	NA	NA	NA	NA	NA	NA	9	209.63	5.81	0.01
270	0.32	0.00	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	210.11	6.29	0.01
46	0.34	-0.01	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	210.19	6.36	0.01
432	0.45	-0.06	0.00	+	+	NA	+	NA	+	+	NA	NA	12	210.39	6.56	0.01
30	0.31	0.00	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	210.40	6.58	0.01
79	0.30	NA	0.00	+	+	NA	NA	+	NA	NA	NA	NA	9	210.65	6.83	0.01
271	0.30	NA	0.00	+	+	NA	NA	NA	NA	+	NA	NA	9	210.68	6.86	0.01
240	0.44	-0.05	0.00	+	+	NA	+	+	+	NA	NA	NA	12	210.75	6.92	0.00
48	0.39	-0.03	0.00	+	+	NA	+	NA	NA	NA	NA	NA	10	210.75	6.93	0.00
272	0.36	-0.02	0.00	+	+	NA	NA	NA	NA	+	NA	NA	10	210.97	7.14	0.00
192	0.44	-0.05	0.00	+	+	+	+	NA	+	NA	NA	NA	12	210.98	7.16	0.00
88	0.38	-0.03	0.00	+	NA	+	NA	+	NA	NA	NA	NA	10	211.08	7.26	0.00
143	0.31	NA	0.00	+	+	NA	NA	NA	+	NA	NA	NA	9	211.13	7.31	0.00
80	0.36	-0.02	0.00	+	+	NA	NA	+	NA	NA	NA	NA	10	211.34	7.52	0.00
144	0.38	-0.02	0.00	+	+	NA	NA	NA	+	NA	NA	NA	10	211.45	7.63	0.00
32	0.36	-0.02	0.00	+	+	+	NA	NA	NA	NA	NA	NA	10	211.50	7.68	0.00
496	0.43	-0.06	0.00	+	+	NA	+	+	+	+	NA	NA	13	211.73	7.91	0.00
286	0.31	0.00	NA	+	+	+	NA	NA	NA	+	NA	NA	10	211.74	7.92	0.00
302	0.33	-0.01	NA	+	+	NA	+	NA	NA	+	NA	NA	10	211.76	7.94	0.00
335	0.30	NA	0.00	+	+	NA	NA	+	NA	+	NA	NA	10	212.01	8.19	0.00
62	0.33	0.00	NA	+	+	+	+	NA	NA	NA	NA	NA	10	212.02	8.20	0.00
448	0.43	-0.05	0.00	+	+	+	+	NA	+	+	NA	NA	13	212.05	8.23	0.00
256	0.47	-0.07	0.00	+	+	+	+	+	+	NA	NA	NA	13	212.19	8.37	0.00
304	0.39	-0.03	0.00	+	+	NA	+	NA	NA	+	NA	NA	11	212.21	8.39	0.00
336	0.35	-0.02	0.00	+	+	NA	NA	+	NA	+	NA	NA	11	212.53	8.70	0.00
112	0.38	-0.03	0.00	+	+	NA	+	+	NA	NA	NA	NA	11	212.61	8.79	0.00
207	0.30	NA	0.00	+	+	NA	NA	+	+	NA	NA	NA	10	212.70	8.88	0.00
64	0.38	-0.03	0.00	+	+	+	+	NA	NA	NA	NA	NA	11	212.72	8.90	0.00
399	0.31	NA	0.00	+	+	NA	NA	NA	+	+	NA	NA	10	212.73	8.91	0.00
288	0.35	-0.02	0.00	+	+	+	NA	NA	NA	+	NA	NA	11	212.74	8.92	0.00
400	0.37	-0.03	0.00	+	+	NA	NA	NA	+	+	NA	NA	11	212.87	9.05	0.00
96	0.38	-0.03	0.00	+	+	+	NA	+	NA	NA	NA	NA	11	212.99	9.17	0.00
512	0.47	-0.07	0.01	+	+	+	+	+	+	+	NA	NA	14	213.13	9.31	0.00
208	0.37	-0.02	0.00	+	+	NA	NA	+	+	NA	NA	NA	11	213.26	9.44	0.00
160	0.37	-0.02	0.00	+	+	+	NA	NA	+	NA	NA	NA	11	213.40	9.57	0.00
318	0.32	0.00	NA	+	+	+	+	NA	NA	+	NA	NA	11	213.46	9.64	0.00
1520	0.43	-0.06	0.00	+	+	NA	+	+	+	+	NA	+	14	213.81	9.99	0.00
368	0.38	-0.03	0.00	+	+	NA	+	+	NA	+	NA	NA	12	213.92	10.10	0.00
463	0.30	NA	0.00	+	+	NA	NA	+	+	+	NA	NA	11	214.08	10.26	0.00
320	0.38	-0.03	0.00	+	+	+	+	NA	NA	+	NA	NA	12	214.09	10.27	0.00
960	0.43	-0.05	0.00	+	+	+	+	NA	+	+	+	NA	14	214.13	10.31	0.00
352	0.38	-0.03	0.00	+	+	+	NA	+	NA	+	NA	NA	12	214.16	10.34	0.00
128	0.41	-0.04	0.00	+	+	+	+	+	NA	NA	NA	NA	12	214.33	10.51	0.00
464	0.36	-0.02	0.00	+	+	NA	NA	+	+	+	NA	NA	12	214.51	10.69	0.00
416	0.36	-0.02	0.00	+	+	+	NA	NA	+	+	NA	NA	12	214.71	10.89	0.00
224	0.39	-0.03	0.00	+	+	+	NA	+	+	NA	NA	NA	12	214.95	11.13	0.00
1024	0.47	-0.07	0.01	+	+	+	+	+	+	+	+	NA	15	215.20	11.38	0.00
1536	0.47	-0.07	0.01	+	+	+	+	+	+	+	NA	+	15	215.22	11.39	0.00
830	0.31	0.00	NA	+	+	+	+	NA	NA	+	+	NA	12	215.38	11.56	0.00
384	0.40	-0.04	0.00	+	+	+	+	+	NA	+	NA	NA	13	215.62	11.80	0.00
832	0.37	-0.03	0.00	+	+	+	+	NA	NA	+	+	NA	13	215.90	12.08	0.00
1487	0.29	NA	0.00	+	+	NA	NA	+	+	+	NA	+	12	215.98	12.16	0.00
480	0.38	-0.03	0.00	+	+	+	NA	+	+	+	NA	NA	13	216.18	12.36	0.00
1488	0.36	-0.03	0.00	+	+	NA	NA	+	+	+	NA	+	13	216.21	12.39	0.00
2048	0.48	-0.07	0.01	+	+	+	+	+	+	+	+	+	16	217.26	13.44	0.00
896	0.39	-0.04	0.00	+	+	+	+	+	NA	+	+	NA	14	217.60	13.78	0.00
1504	0.38	-0.03	0.00	+	+	+	NA	+	+	+	NA	+	14	218.06	14.24	0.00

BASOPHIL COUNT

	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
1	**0.07**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**5**	**-1011.98**	**0.00**	**0.22**
5	0.08	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	-1010.87	1.10	0.13
2	0.07	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1010.63	1.35	0.11
3	0.07	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1010.52	1.46	0.11
12	0.08	0.00	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	-1010.02	1.96	0.08
7	0.08	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1009.35	2.63	0.06
6	0.07	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1009.32	2.66	0.06
4	0.06	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	-1009.17	2.80	0.05
16	0.08	0.00	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	-1008.36	3.61	0.04
8	0.07	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	-1007.80	4.17	0.03
39	0.07	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	-1007.71	4.26	0.03
22	0.07	0.00	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	-1007.67	4.30	0.03
32	0.08	0.00	+	+	+	+	NA	NA	NA	NA	NA	NA	10	-1006.51	5.47	0.01
48	0.08	0.00	+	+	+	NA	+	NA	NA	NA	NA	NA	10	-1006.40	5.58	0.01
40	0.07	0.00	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	-1006.07	5.91	0.01
24	0.07	0.00	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	-1006.06	5.92	0.01
64	0.08	0.00	+	+	+	+	+	NA	NA	NA	NA	NA	11	-1004.56	7.41	0.01
56	0.06	0.00	+	+	NA	+	+	NA	NA	NA	NA	NA	10	-1004.36	7.62	0.00
128	0.08	0.00	+	+	+	+	+	+	NA	NA	NA	NA	12	-1004.07	7.91	0.00
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
1	0.07	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	-1011.98	0.00	0.41
5	0.08	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	-1010.87	1.10	0.24
3	0.07	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1010.52	1.46	0.20
7	0.08	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1009.35	2.63	0.11
39	0.07	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	-1007.71	4.26	0.05
2	0.07	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	-997.47	14.50	0.00
4	0.06	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	-996.04	15.94	0.00
6	0.07	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	-996.02	15.96	0.00
8	0.07	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	-994.51	17.46	0.00
40	0.06	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	-992.87	19.11	0.00
12	0.07	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	-989.23	22.75	0.00
16	0.07	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	-987.39	24.59	0.00
48	0.07	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	-985.33	26.64	0.00
22	0.08	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	-984.57	27.40	0.00
24	0.08	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	-983.09	28.88	0.00
56	0.07	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	-981.50	30.48	0.00
32	0.08	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	-972.69	39.29	0.00
64	0.08	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	-970.60	41.37	0.00
128	0.08	+	+	+	+	+	+	+	NA	NA	NA	NA	46	-961.08	50.90	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
1	0.07	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	-1011.98	0.00	0.19
2	0.03	0.01	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1011.20	0.77	0.13
5	0.08	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	-1010.87	1.10	0.11
3	0.07	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1010.52	1.46	0.09
6	0.04	0.01	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1009.85	2.13	0.07
4	0.02	0.01	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	-1009.84	2.13	0.07
12	0.07	0.00	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	-1009.70	2.28	0.06
7	0.08	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1009.35	2.63	0.05
22	0.01	0.01	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	-1009.23	2.75	0.05
8	0.03	0.01	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	-1008.41	3.57	0.03
16	0.07	0.00	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	-1008.13	3.84	0.03
39	0.07	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	-1007.71	4.26	0.02
24	0.00	0.01	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	-1007.71	4.27	0.02
32	0.05	0.00	+	+	+	+	NA	NA	NA	NA	NA	NA	10	-1006.82	5.15	0.01
40	0.03	0.01	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	-1006.73	5.25	0.01
48	0.07	0.00	+	+	+	NA	+	NA	NA	NA	NA	NA	10	-1006.26	5.72	0.01
56	0.00	0.01	+	+	NA	+	+	NA	NA	NA	NA	NA	10	-1006.21	5.77	0.01
64	0.04	0.00	+	+	+	+	+	NA	NA	NA	NA	NA	11	-1005.08	6.90	0.01
128	0.05	0.00	+	+	+	+	+	+	NA	NA	NA	NA	12	-1003.64	8.33	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
1	0.07	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	-1011.98	0.00	0.11
9	0.08	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	-1010.87	1.10	0.06
3	0.07	NA	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1010.86	1.12	0.06
2	0.07	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1010.63	1.35	0.05
5	0.07	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	-1010.52	1.46	0.05
71	0.07	NA	0.00	+	NA	NA	NA	+	NA	NA	NA	NA	8	-1010.34	1.63	0.05
22	0.08	0.00	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	-1010.02	1.96	0.04
11	0.07	NA	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	7	-1009.51	2.47	0.03
7	0.07	NA	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1009.42	2.56	0.03
13	0.08	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	-1009.35	2.63	0.03
10	0.07	0.00	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	-1009.32	2.66	0.03
6	0.06	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1009.17	2.80	0.03
4	0.08	0.00	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	-1008.99	2.99	0.02
72	0.08	0.00	0.00	+	NA	NA	NA	+	NA	NA	NA	NA	9	-1008.69	3.29	0.02
24	0.09	-0.01	0.00	+	NA	+	NA	NA	NA	NA	NA	NA	9	-1008.68	3.30	0.02
79	0.08	NA	0.00	+	+	NA	NA	+	NA	NA	NA	NA	9	-1008.67	3.31	0.02

139	0.07	NA	0.00	NA	+	NA	NA	NA	+	NA	NA	NA	8	-1008.61	3.36	0.02
30	0.08	0.00	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	-1008.36	3.61	0.02
172	0.10	-0.01	0.00	NA	+	NA	+	NA	+	NA	NA	NA	10	-1008.07	3.91	0.02
15	0.07	NA	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	8	-1008.01	3.97	0.01
14	0.07	0.00	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	-1007.80	4.17	0.01
269	0.07	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	-1007.71	4.26	0.01
42	0.07	0.00	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	-1007.67	4.30	0.01
12	0.08	0.00	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	8	-1007.63	4.35	0.01
8	0.07	0.00	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	8	-1007.57	4.40	0.01
207	0.07	NA	0.00	+	+	NA	NA	+	+	NA	NA	NA	10	-1007.31	4.67	0.01
80	0.09	0.00	0.00	+	+	NA	NA	+	NA	NA	NA	NA	10	-1006.99	4.99	0.01
143	0.07	NA	0.00	+	+	NA	NA	NA	+	NA	NA	NA	9	-1006.97	5.00	0.01
32	0.09	-0.01	0.00	+	+	+	NA	NA	NA	NA	NA	NA	10	-1006.95	5.02	0.01
192	0.11	-0.01	0.00	+	+	+	+	NA	+	NA	NA	NA	12	-1006.80	5.18	0.01
335	0.08	NA	0.00	+	+	NA	NA	+	NA	+	NA	NA	10	-1006.69	5.29	0.01
176	0.10	-0.01	0.00	+	+	NA	+	NA	+	NA	NA	NA	11	-1006.69	5.29	0.01
88	0.09	-0.01	0.00	+	NA	+	NA	+	NA	NA	NA	NA	10	-1006.66	5.32	0.01
240	0.10	-0.01	0.00	+	+	NA	+	+	+	NA	NA	NA	12	-1006.64	5.33	0.01
140	0.07	0.00	0.00	NA	+	NA	NA	NA	+	NA	NA	NA	9	-1006.59	5.38	0.01
62	0.08	0.00	NA	+	+	+	+	NA	NA	NA	NA	NA	10	-1006.51	5.47	0.01
286	0.08	0.00	NA	+	+	+	NA	NA	NA	+	NA	NA	10	-1006.40	5.58	0.01
271	0.07	NA	0.00	+	+	NA	NA	NA	NA	+	NA	NA	9	-1006.27	5.71	0.01
16	0.08	0.00	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	9	-1006.14	5.83	0.01
270	0.07	0.00	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	-1006.07	5.91	0.01
46	0.07	0.00	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	-1006.06	5.92	0.01
44	0.08	0.00	0.00	NA	+	NA	+	NA	NA	NA	NA	NA	9	-1005.84	6.14	0.00
960	0.12	-0.02	0.00	+	+	+	+	NA	+	+	+	NA	14	-1005.56	6.42	0.00
160	0.09	-0.01	0.00	+	+	+	NA	NA	+	NA	NA	NA	11	-1005.46	6.52	0.00
208	0.08	0.00	0.00	+	+	NA	NA	+	+	NA	NA	NA	11	-1005.43	6.54	0.00
1520	0.11	-0.01	0.00	+	+	NA	+	+	+	+	NA	+	14	-1005.39	6.58	0.00
463	0.07	NA	0.00	+	+	NA	NA	+	+	+	NA	NA	11	-1005.39	6.59	0.00
399	0.07	NA	0.00	+	+	NA	NA	NA	+	+	NA	NA	10	-1005.34	6.64	0.00
432	0.10	-0.01	0.00	+	+	NA	+	NA	+	+	NA	NA	12	-1005.24	6.74	0.00
336	0.09	0.00	0.00	+	+	NA	NA	+	NA	+	NA	NA	11	-1005.04	6.94	0.00
448	0.11	-0.01	0.00	+	+	+	+	NA	+	+	NA	NA	13	-1005.02	6.95	0.00
288	0.09	-0.01	0.00	+	+	+	NA	NA	NA	+	NA	NA	11	-1005.01	6.97	0.00
112	0.08	0.00	0.00	+	+	NA	+	+	NA	NA	NA	NA	11	-1004.98	7.00	0.00
144	0.07	0.00	0.00	+	+	NA	NA	NA	+	NA	NA	NA	10	-1004.96	7.01	0.00
64	0.09	-0.01	0.00	+	+	+	+	NA	NA	NA	NA	NA	11	-1004.96	7.02	0.00
96	0.09	-0.01	0.00	+	+	+	NA	+	NA	NA	NA	NA	11	-1004.94	7.04	0.00
496	0.10	-0.01	0.00	+	+	NA	+	+	+	+	NA	NA	13	-1004.86	7.11	0.00
1487	0.07	NA	0.00	+	+	NA	NA	+	+	+	NA	+	12	-1004.78	7.19	0.00
256	0.11	-0.01	0.00	+	+	+	+	+	+	NA	NA	NA	13	-1004.71	7.27	0.00
318	0.08	0.00	NA	+	+	+	+	NA	NA	+	NA	NA	11	-1004.56	7.41	0.00
272	0.08	0.00	0.00	+	+	NA	NA	NA	NA	+	NA	NA	10	-1004.45	7.52	0.00
302	0.06	0.00	NA	+	+	NA	+	NA	NA	+	NA	NA	10	-1004.36	7.62	0.00
48	0.07	0.00	0.00	+	+	NA	+	NA	NA	NA	NA	NA	10	-1004.27	7.71	0.00
1536	0.12	-0.02	0.00	+	+	+	+	+	+	+	NA	+	15	-1004.14	7.83	0.00
830	0.08	0.00	NA	+	+	+	+	NA	NA	+	+	NA	12	-1004.07	7.91	0.00
1024	0.13	-0.02	0.00	+	+	+	+	+	+	+	+	NA	15	-1003.80	8.17	0.00
416	0.09	-0.01	0.00	+	+	+	NA	NA	+	+	NA	NA	12	-1003.56	8.41	0.00
464	0.08	0.00	0.00	+	+	NA	NA	+	+	+	NA	NA	12	-1003.53	8.45	0.00
224	0.08	0.00	0.00	+	+	+	NA	+	+	NA	NA	NA	12	-1003.40	8.58	0.00
400	0.07	0.00	0.00	+	+	NA	NA	NA	+	+	NA	NA	11	-1003.35	8.63	0.00
368	0.08	0.00	0.00	+	+	NA	+	+	NA	+	NA	NA	12	-1003.03	8.94	0.00
320	0.09	-0.01	0.00	+	+	+	+	NA	NA	+	NA	NA	12	-1003.02	8.95	0.00
352	0.09	-0.01	0.00	+	+	+	NA	+	NA	+	NA	NA	12	-1002.98	9.00	0.00
512	0.11	-0.02	0.00	+	+	+	+	+	+	+	NA	NA	14	-1002.93	9.04	0.00
128	0.09	0.00	0.00	+	+	+	+	+	NA	NA	NA	NA	12	-1002.92	9.05	0.00
1488	0.08	0.00	0.00	+	+	NA	NA	+	+	+	NA	+	13	-1002.78	9.19	0.00
304	0.07	0.00	0.00	+	+	NA	+	NA	NA	+	NA	NA	11	-1002.60	9.38	0.00
832	0.09	-0.01	0.00	+	+	+	+	NA	NA	+	+	NA	13	-1002.35	9.63	0.00
2048	0.12	-0.02	0.00	+	+	+	+	+	+	+	+	+	16	-1002.06	9.91	0.00
480	0.08	0.00	0.00	+	+	+	NA	+	+	+	NA	NA	13	-1001.49	10.49	0.00
1504	0.09	-0.01	0.00	+	+	+	NA	+	+	+	NA	+	14	-1000.99	10.99	0.00
384	0.09	0.00	0.00	+	+	+	+	+	NA	+	NA	NA	13	-1000.97	11.00	0.00
896	0.09	-0.01	0.00	+	+	+	+	+	NA	+	+	NA	14	-1000.26	11.71	0.00

EOSINOPHIL COUNT

	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
6	0.14	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-689.27	0.00	0.27
8	0.14	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	-687.61	1.67	0.12
5	**0.12**	**NA**	**NA**	**+**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**6**	**-687.43**	**1.85**	**0.11**
22	0.14	0.00	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	-687.25	2.03	0.10
40	0.14	0.00	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	-686.83	2.44	0.08
16	0.13	0.00	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	-686.44	2.83	0.07
7	0.11	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-685.79	3.48	0.05
24	0.14	0.00	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	-685.55	3.72	0.04
39	0.12	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	-685.55	3.72	0.04
48	0.13	0.00	+	+	+	NA	+	NA	NA	NA	NA	NA	10	-685.36	3.91	0.04
56	0.14	0.00	+	+	NA	+	+	NA	NA	NA	NA	NA	10	-684.76	4.51	0.03
32	0.13	0.00	+	+	+	+	NA	NA	NA	NA	NA	NA	10	-684.40	4.87	0.02
64	0.13	0.00	+	+	+	+	+	NA	NA	NA	NA	NA	11	-683.30	5.98	0.01
128	0.14	0.00	+	+	+	+	+	+	NA	NA	NA	NA	12	-681.92	7.36	0.01
2	0.12	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-681.57	7.70	0.01
1	0.10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	-681.22	8.06	0.00
4	0.11	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	-680.09	9.18	0.00
3	0.10	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-679.76	9.51	0.00
12	0.11	0.00	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	-678.41	10.86	0.00
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
5	0.12	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	-687.43	0.00	0.53
7	0.11	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-685.79	1.64	0.23
39	0.12	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	-685.55	1.88	0.21
1	0.10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	-681.22	6.21	0.02
3	0.10	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-679.76	7.66	0.01
6	0.14	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	-672.79	14.63	0.00
8	0.13	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	-671.10	16.32	0.00
40	0.14	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	-670.23	17.20	0.00
16	0.11	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	-665.43	22.00	0.00
2	0.12	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	-665.10	22.32	0.00
48	0.12	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	-664.68	22.75	0.00
4	0.11	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	-663.62	23.80	0.00
22	0.14	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	-662.69	24.73	0.00
24	0.14	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	-660.77	26.66	0.00
56	0.15	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	-659.94	27.49	0.00
12	0.10	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	-657.28	30.14	0.00
32	0.12	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	-654.97	32.45	0.00
64	0.13	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	-654.32	33.11	0.00
128	0.12	+	+	+	+	+	+	+	NA	NA	NA	NA	46	-639.00	48.42	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
5	0.12	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	-687.43	0.00	0.29
6	0.19	-0.01	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-686.03	1.39	0.14
7	0.11	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-685.79	1.64	0.13
39	0.12	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	-685.55	1.88	0.11
8	0.18	-0.01	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	-684.32	3.11	0.06
22	0.18	-0.01	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	-684.06	3.36	0.05
40	0.19	-0.01	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	-684.05	3.38	0.05
48	0.24	-0.01	+	+	+	NA	+	NA	NA	NA	NA	NA	10	-682.82	4.61	0.03
16	0.22	-0.01	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	-682.77	4.66	0.03
24	0.17	-0.01	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	-682.33	5.10	0.02
56	0.18	-0.01	+	+	NA	+	+	NA	NA	NA	NA	NA	10	-681.99	5.43	0.02
1	0.10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	-681.22	6.21	0.01
64	0.25	-0.01	+	+	+	+	+	NA	NA	NA	NA	NA	11	-680.77	6.65	0.01
32	0.22	-0.01	+	+	+	+	NA	NA	NA	NA	NA	NA	10	-680.70	6.72	0.01
3	0.10	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-679.76	7.66	0.01
128	0.27	-0.02	+	+	+	+	+	+	NA	NA	NA	NA	12	-679.48	7.95	0.01
2	0.14	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-679.37	8.06	0.01
4	0.13	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	-677.86	9.56	0.00
12	0.18	-0.01	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	-676.53	10.90	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
10	0.14	0.00	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	-689.27	0.00	0.12
11	0.13	NA	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	7	-688.36	0.91	0.08
12	0.16	-0.01	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	8	-687.87	1.41	0.06
14	0.14	0.00	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	-687.61	1.67	0.05
9	0.12	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	-687.43	1.85	0.05
42	0.14	0.00	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	-687.25	2.03	0.04
270	0.14	0.00	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	-686.83	2.44	0.04
15	0.12	NA	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	8	-686.67	2.60	0.03
30	0.13	0.00	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	-686.44	2.83	0.03
139	0.13	NA	0.00	NA	+	NA	NA	NA	+	NA	NA	NA	8	-686.42	2.85	0.03
16	0.15	-0.01	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	9	-686.25	3.03	0.03
271	0.13	NA	0.00	+	+	NA	NA	NA	NA	+	NA	NA	9	-686.04	3.24	0.02
140	0.15	-0.01	0.00	NA	+	NA	NA	NA	+	NA	NA	NA	9	-685.81	3.46	0.02
44	0.16	-0.01	0.00	NA	+	NA	+	NA	NA	NA	NA	NA	9	-685.81	3.47	0.02
13	0.11	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	-685.79	3.48	0.02
46	0.14	0.00	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	-685.55	3.72	0.02
269	0.12	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	-685.55	3.72	0.02
286	0.13	0.00	NA	+	+	+	NA	NA	NA	+	NA	NA	10	-685.36	3.91	0.02
272	0.15	-0.01	0.00	+	+	NA	NA	NA	NA	+	NA	NA	10	-685.34	3.94	0.02
79	0.12	NA	0.00	+	+	NA	NA	+	NA	NA	NA	NA	9	-685.01	4.26	0.01
96	0.12	0.00	0.00	+	+	+	NA	+	NA	NA	NA	NA	11	-685.01	4.27	0.01
32	0.14	-0.01	0.00	+	+	+	NA	NA	NA	NA	NA	NA	10	-684.92	4.35	0.01
302	0.14	0.00	NA	+	+	NA	+	NA	NA	+	NA	NA	10	-684.76	4.51	0.01
143	0.12	NA	0.00	+	+	NA	NA	NA	+	NA	NA	NA	9	-684.69	4.59	0.01
80	0.15	-0.01	0.00	+	+	NA	NA	+	NA	NA	NA	NA	10	-684.42	4.85	0.01
62	0.13	0.00	NA	+	+	+	+	NA	NA	NA	NA	NA	10	-684.40	4.87	0.01
48	0.15	-0.01	0.00	+	+	NA	+	NA	NA	NA	NA	NA	10	-684.18	5.09	0.01
144	0.15	-0.01	0.00	+	+	NA	NA	NA	+	NA	NA	NA	10	-684.18	5.10	0.01
335	0.13	NA	0.00	+	+	NA	NA	+	NA	+	NA	NA	10	-684.16	5.12	0.01
352	0.12	0.00	0.00	+	+	+	NA	+	NA	+	NA	NA	12	-684.02	5.26	0.01
399	0.13	NA	0.00	+	+	NA	NA	NA	+	+	NA	NA	10	-684.00	5.27	0.01
172	0.16	-0.01	0.00	NA	+	NA	+	NA	+	NA	NA	NA	10	-683.88	5.39	0.01
288	0.15	-0.01	0.00	+	+	+	NA	NA	NA	+	NA	NA	11	-683.76	5.52	0.01
336	0.15	-0.01	0.00	+	+	NA	NA	+	NA	+	NA	NA	11	-683.37	5.91	0.01
318	0.13	0.00	NA	+	+	+	+	NA	NA	+	NA	NA	11	-683.30	5.98	0.01
304	0.16	-0.01	0.00	+	+	NA	+	NA	NA	+	NA	NA	11	-683.28	5.99	0.01
400	0.16	-0.01	0.00	+	+	NA	NA	NA	+	+	NA	NA	11	-683.26	6.01	0.01
207	0.12	NA	0.00	+	+	NA	NA	+	+	NA	NA	NA	10	-683.07	6.20	0.01
128	0.12	0.00	0.00	+	+	+	+	+	NA	NA	NA	NA	12	-682.94	6.33	0.01
224	0.12	0.00	0.00	+	+	+	NA	+	+	NA	NA	NA	12	-682.93	6.35	0.01
160	0.14	-0.01	0.00	+	+	+	NA	NA	+	NA	NA	NA	11	-682.86	6.42	0.00
64	0.14	-0.01	0.00	+	+	+	+	NA	NA	NA	NA	NA	11	-682.84	6.43	0.00
208	0.15	-0.01	0.00	+	+	NA	NA	+	+	NA	NA	NA	11	-682.36	6.92	0.00
112	0.15	-0.01	0.00	+	+	NA	+	+	NA	NA	NA	NA	11	-682.35	6.92	0.00
176	0.16	-0.01	0.00	+	+	NA	+	NA	+	NA	NA	NA	11	-682.29	6.99	0.00
463	0.13	NA	0.00	+	+	NA	NA	+	+	+	NA	NA	11	-682.14	7.13	0.00
384	0.12	0.00	0.00	+	+	+	+	+	NA	+	NA	NA	13	-681.97	7.30	0.00
480	0.12	0.00	0.00	+	+	+	NA	+	+	+	NA	NA	13	-681.95	7.32	0.00
830	0.14	0.00	NA	+	+	+	+	NA	NA	+	+	NA	12	-681.92	7.36	0.00
320	0.15	-0.01	0.00	+	+	+	+	NA	NA	+	NA	NA	12	-681.68	7.60	0.00
416	0.15	-0.01	0.00	+	+	+	NA	NA	+	+	NA	NA	12	-681.68	7.60	0.00
1487	0.13	NA	0.00	+	+	NA	NA	+	+	+	NA	+	12	-681.58	7.70	0.00
2	0.12	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-681.57	7.70	0.00
432	0.16	-0.01	0.00	+	+	NA	+	NA	+	+	NA	NA	12	-681.32	7.95	0.00
368	0.15	-0.01	0.00	+	+	NA	+	+	NA	+	NA	NA	12	-681.29	7.98	0.00
464	0.15	-0.01	0.00	+	+	NA	NA	+	+	+	NA	NA	12	-681.28	7.99	0.00
1	0.10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	-681.22	8.06	0.00
192	0.15	-0.01	0.00	+	+	+	+	NA	+	NA	NA	NA	12	-681.01	8.26	0.00
256	0.12	0.00	0.00	+	+	+	+	+	+	NA	NA	NA	13	-680.95	8.33	0.00
3	0.11	NA	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-680.81	8.46	0.00
240	0.16	-0.01	0.00	+	+	NA	+	+	+	NA	NA	NA	12	-680.50	8.77	0.00
4	0.14	-0.01	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	-680.42	8.85	0.00
1488	0.15	-0.01	0.00	+	+	NA	NA	+	+	+	NA	+	13	-680.37	8.91	0.00
1504	0.13	0.00	0.00	+	+	+	NA	+	+	+	NA	+	14	-680.31	8.96	0.00
832	0.15	-0.01	0.00	+	+	+	+	NA	NA	+	+	NA	13	-680.19	9.09	0.00
6	0.11	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-680.09	9.18	0.00
896	0.13	0.00	0.00	+	+	+	+	+	NA	+	+	NA	14	-680.03	9.24	0.00
512	0.13	0.00	0.00	+	+	+	+	+	+	+	NA	NA	14	-679.92	9.35	0.00
448	0.15	-0.01	0.00	+	+	+	+	NA	+	+	NA	NA	13	-679.77	9.50	0.00
5	0.10	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	-679.76	9.51	0.00
496	0.16	-0.01	0.00	+	+	NA	+	+	+	+	NA	NA	13	-679.36	9.91	0.00
7	0.11	NA	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-679.32	9.96	0.00
8	0.13	-0.01	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	8	-679.01	10.26	0.00
1520	0.16	-0.01	0.00	+	+	NA	+	+	+	+	NA	+	14	-678.73	10.55	0.00
960	0.16	-0.01	0.00	+	+	+	+	NA	+	+	+	NA	14	-678.44	10.83	0.00
22	0.11	0.00	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	-678.41	10.86	0.00
1536	0.13	0.00	0.00	+	+	+	+	+	+	+	NA	+	15	-678.41	10.86	0.00
1024	0.13	0.00	0.00	+	+	+	+	+	+	+	+	NA	15	-678.03	11.24	0.00
2048	0.12	0.01	0.00	+	+	+	+	+	+	+	+	+	16	-677.80	11.48	0.00
71	0.10	NA	0.00	+	NA	NA	NA	+	NA	NA	NA	NA	8	-677.37	11.90	0.00
24	0.13	-0.01	0.00	+	NA	+	NA	NA	NA	NA	NA	NA	9	-677.22	12.06	0.00
72	0.13	-0.01	0.00	+	NA	NA	NA	+	NA	NA	NA	NA	9	-676.99	12.28	0.00
88	0.11	0.00	0.00	+	NA	+	NA	+	NA	NA	NA	NA	10	-676.89	12.38	0.00

LYMPHOCYTE COUNT

	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
4	2.56	-0.04	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1489.61	0.00	0.17
12	2.48	-0.02	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	1489.81	0.20	0.15
16	2.53	-0.02	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	1489.91	0.30	0.14
8	2.62	-0.04	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	1490.14	0.53	0.13
40	2.64	-0.04	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	1490.87	1.26	0.09
48	2.56	-0.02	+	+	+	NA	+	NA	NA	NA	NA	NA	10	1491.09	1.48	0.08
32	2.49	-0.01	+	+	+	+	NA	NA	NA	NA	NA	NA	10	1491.13	1.52	0.08
24	2.58	-0.03	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	1491.53	1.93	0.06
56	2.61	-0.03	+	+	NA	+	+	NA	NA	NA	NA	NA	10	1492.38	2.77	0.04
64	2.52	-0.01	+	+	+	+	+	NA	NA	NA	NA	NA	11	1492.44	2.83	0.04
128	2.50	-0.01	+	+	+	+	+	+	NA	NA	NA	NA	12	1494.41	4.80	0.01
3	2.36	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1496.03	6.43	0.01
39	2.44	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	1497.32	7.72	0.00
7	2.39	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1497.42	7.81	0.00
2	2.25	-0.04	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1549.96	60.35	0.00
6	2.29	-0.04	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1551.21	61.60	0.00
22	2.30	-0.04	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	1553.26	63.65	0.00
1	2.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1554.21	64.61	0.00
5	2.09	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1555.77	66.17	0.00
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
4	2.64	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	1488.82	0.00	0.45
8	2.69	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	1489.48	0.66	0.32
40	2.72	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	1490.48	1.66	0.19
3	2.36	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1496.03	7.22	0.01
12	2.57	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	1496.98	8.16	0.01
39	2.44	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	1497.32	8.51	0.01
7	2.39	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1497.42	8.60	0.01
16	2.61	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	1497.66	8.85	0.01
48	2.64	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	1499.00	10.18	0.00
24	2.69	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	1504.90	16.08	0.00
56	2.72	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	1505.93	17.12	0.00
32	2.61	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	1516.08	27.26	0.00
64	2.64	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	1517.47	28.66	0.00
128	2.72	+	+	+	+	+	+	+	NA	NA	NA	NA	46	1520.39	31.57	0.00
2	2.35	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	1548.42	59.60	0.00
6	2.38	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	1549.77	60.95	0.00
1	2.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1554.21	65.40	0.00
5	2.09	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1555.77	66.96	0.00
22	2.41	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	1567.05	78.23	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
3	2.36	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1496.03	0.00	0.28
39	2.44	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	1497.32	1.29	0.15
7	2.39	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1497.42	1.38	0.14
4	1.42	0.09	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1497.92	1.89	0.11
12	0.02	0.21	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	1499.03	2.99	0.06
40	1.70	0.07	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	1499.29	3.25	0.06
8	1.59	0.07	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	1499.36	3.32	0.05
16	0.18	0.20	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	1500.43	4.39	0.03
48	0.42	0.18	+	+	+	NA	+	NA	NA	NA	NA	NA	10	1500.56	4.53	0.03
56	2.54	-0.01	+	+	NA	+	+	NA	NA	NA	NA	NA	10	1500.83	4.80	0.03
24	2.28	0.01	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	1501.07	5.03	0.02
32	0.70	0.15	+	+	+	+	NA	NA	NA	NA	NA	NA	10	1502.40	6.37	0.01
64	1.22	0.11	+	+	+	+	+	NA	NA	NA	NA	NA	11	1502.41	6.38	0.01
128	1.22	0.11	+	+	+	+	+	+	NA	NA	NA	NA	11	1502.41	6.38	0.01
1	2.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1554.21	58.18	0.00
2	-0.41	0.22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1555.30	59.27	0.00
5	2.09	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1555.77	59.74	0.00
6	-0.32	0.22	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1556.92	60.89	0.00
22	0.51	0.14	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	1558.54	62.50	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
8	**2.82**	**-0.15**	**0.01**	**+**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**8**	**1487.20**	**0.00**	**0.09**
24	2.73	-0.13	0.01	+	NA	+	NA	NA	NA	NA	NA	NA	9	1487.87	0.67	0.07
16	2.86	-0.15	0.01	+	+	NA	NA	NA	NA	NA	NA	NA	9	1487.96	0.76	0.06
72	2.77	-0.14	0.01	+	NA	NA	NA	+	NA	NA	NA	NA	9	1488.01	0.81	0.06
32	2.77	-0.13	0.01	+	+	+	NA	NA	NA	NA	NA	NA	10	1488.27	1.07	0.05
80	2.81	-0.14	0.01	+	+	NA	NA	+	NA	NA	NA	NA	10	1488.47	1.27	0.05
272	2.88	-0.14	0.01	+	+	NA	NA	NA	NA	+	NA	NA	10	1488.99	1.79	0.04
6	2.56	-0.04	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1489.61	2.41	0.03
288	2.79	-0.13	0.01	+	+	+	NA	NA	NA	+	NA	NA	11	1489.64	2.44	0.03
22	2.48	-0.02	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	1489.81	2.61	0.02
336	2.83	-0.14	0.01	+	+	NA	NA	+	NA	+	NA	NA	11	1489.84	2.64	0.02
48	2.83	-0.14	0.01	+	+	NA	+	NA	NA	NA	NA	NA	10	1489.88	2.69	0.02
30	2.53	-0.02	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	1489.91	2.71	0.02
88	2.72	-0.13	0.01	+	NA	+	NA	+	NA	NA	NA	NA	10	1489.92	2.72	0.02
144	2.84	-0.15	0.01	+	+	NA	NA	NA	+	NA	NA	NA	10	1489.94	2.74	0.02
64	2.73	-0.12	0.01	+	+	+	+	NA	NA	NA	NA	NA	11	1490.10	2.90	0.02
14	2.62	-0.04	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	1490.14	2.94	0.02
160	2.75	-0.12	0.01	+	+	+	NA	NA	+	NA	NA	NA	11	1490.15	2.95	0.02
112	2.77	-0.13	0.01	+	+	NA	+	+	NA	NA	NA	NA	11	1490.28	3.08	0.02
96	2.75	-0.12	0.01	+	+	+	NA	+	NA	NA	NA	NA	11	1490.32	3.12	0.02
208	2.78	-0.14	0.01	+	+	NA	NA	+	+	NA	NA	NA	11	1490.32	3.13	0.02
270	2.64	-0.04	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	1490.87	3.67	0.01
304	2.85	-0.14	0.01	+	+	NA	+	NA	NA	+	NA	NA	11	1490.95	3.76	0.01
400	2.86	-0.14	0.01	+	+	NA	NA	NA	+	+	NA	NA	11	1491.01	3.81	0.01
286	2.56	-0.02	NA	+	+	+	NA	NA	NA	+	NA	NA	10	1491.09	3.89	0.01
62	2.49	-0.01	NA	+	+	+	+	NA	NA	NA	NA	NA	10	1491.13	3.93	0.01
320	2.76	-0.11	0.01	+	+	+	+	NA	NA	+	NA	NA	12	1491.52	4.32	0.01
46	2.58	-0.03	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	1491.53	4.34	0.01
416	2.77	-0.12	0.01	+	+	+	NA	NA	+	+	NA	NA	12	1491.58	4.38	0.01
352	2.77	-0.12	0.01	+	+	+	NA	+	NA	+	NA	NA	12	1491.68	4.48	0.01
368	2.79	-0.13	0.01	+	+	NA	+	+	NA	+	NA	NA	12	1491.71	4.51	0.01
464	2.81	-0.13	0.01	+	+	NA	NA	+	+	+	NA	NA	12	1491.76	4.56	0.01
176	2.80	-0.13	0.01	+	+	NA	+	NA	+	NA	NA	NA	11	1491.88	4.68	0.01
192	2.72	-0.11	0.01	+	+	+	+	NA	+	NA	NA	NA	12	1492.15	4.96	0.01
128	2.72	-0.11	0.01	+	+	+	+	+	NA	NA	NA	NA	12	1492.16	4.97	0.01
224	2.73	-0.12	0.01	+	+	+	NA	+	+	NA	NA	NA	12	1492.22	5.02	0.01
240	2.76	-0.13	0.01	+	+	NA	+	+	+	NA	NA	NA	12	1492.35	5.15	0.01
302	2.61	-0.03	NA	+	+	NA	+	NA	NA	+	NA	NA	10	1492.38	5.18	0.01
318	2.52	-0.01	NA	+	+	+	+	NA	NA	+	NA	NA	11	1492.44	5.24	0.01
7	2.45	NA	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1492.73	5.53	0.01
432	2.82	-0.12	0.01	+	+	NA	+	NA	+	+	NA	NA	12	1492.91	5.71	0.01
71	2.41	NA	0.00	+	NA	NA	NA	+	NA	NA	NA	NA	8	1492.93	5.73	0.01
79	2.46	NA	0.00	+	+	NA	NA	+	NA	NA	NA	NA	9	1493.25	6.05	0.00
832	2.74	-0.11	0.01	+	+	+	+	NA	NA	+	+	NA	13	1493.33	6.13	0.00
1488	2.81	-0.14	0.01	+	+	NA	NA	+	+	+	NA	+	13	1493.39	6.20	0.00
15	2.50	NA	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	8	1493.42	6.22	0.00
448	2.74	-0.11	0.01	+	+	+	+	NA	+	+	NA	NA	13	1493.56	6.36	0.00
384	2.74	-0.11	0.01	+	+	+	+	+	NA	+	NA	NA	13	1493.58	6.38	0.00
480	2.75	-0.11	0.01	+	+	+	NA	+	+	+	NA	NA	13	1493.64	6.44	0.00
496	2.78	-0.12	0.01	+	+	NA	+	+	+	+	NA	NA	13	1493.76	6.56	0.00
271	2.53	NA	0.00	+	+	NA	NA	NA	NA	+	NA	NA	9	1493.89	6.69	0.00
256	2.70	-0.10	0.01	+	+	+	+	+	+	NA	NA	NA	13	1494.22	7.03	0.00
335	2.49	NA	0.00	+	+	NA	NA	+	NA	+	NA	NA	10	1494.24	7.04	0.00
207	2.43	NA	0.00	+	+	NA	NA	+	+	NA	NA	NA	10	1494.25	7.06	0.00
830	2.50	-0.01	NA	+	+	+	+	NA	NA	+	+	NA	12	1494.41	7.21	0.00
143	2.48	NA	0.00	+	+	NA	NA	NA	+	NA	NA	NA	9	1494.75	7.56	0.00
1504	2.73	-0.11	0.01	+	+	+	NA	+	+	+	NA	+	14	1495.06	7.87	0.00
1520	2.76	-0.12	0.01	+	+	NA	+	+	+	+	NA	+	14	1495.28	8.09	0.00
960	2.71	-0.10	0.01	+	+	+	+	NA	+	+	+	NA	14	1495.32	8.12	0.00
896	2.71	-0.10	0.01	+	+	+	+	+	NA	+	+	NA	14	1495.32	8.13	0.00
399	2.51	NA	0.00	+	+	NA	NA	NA	+	+	NA	NA	10	1495.39	8.19	0.00
463	2.46	NA	0.00	+	+	NA	NA	+	+	+	NA	NA	11	1495.45	8.25	0.00
512	2.71	-0.10	0.01	+	+	+	+	+	+	+	NA	NA	14	1495.61	8.42	0.00
5	2.36	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1496.03	8.84	0.00
1536	2.66	-0.08	0.01	+	+	+	+	+	+	+	NA	+	15	1496.83	9.64	0.00
1024	2.66	-0.08	0.01	+	+	+	+	+	+	+	+	NA	15	1497.27	10.07	0.00
269	2.44	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	1497.32	10.13	0.00
13	2.39	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	1497.42	10.22	0.00
1487	2.46	NA	0.00	+	+	NA	NA	+	+	+	NA	+	12	1497.44	10.24	0.00
2048	2.75	-0.12	0.01	+	+	+	+	+	+	+	+	+	16	1497.95	10.75	0.00
4	2.55	-0.17	0.01	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1546.48	59.28	0.00
12	2.58	-0.17	0.01	NA	+	NA	NA	NA	NA	NA	NA	NA	8	1547.91	60.71	0.00
44	2.63	-0.18	0.01	NA	+	NA	+	NA	NA	NA	NA	NA	9	1549.63	62.43	0.00
140	2.62	-0.17	0.01	NA	+	NA	NA	NA	+	NA	NA	NA	9	1549.64	62.45	0.00
2	2.25	-0.04	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1549.96	62.77	0.00
10	2.29	-0.04	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	1551.21	64.02	0.00
172	2.63	-0.18	0.01	NA	+	NA	+	NA	+	NA	NA	NA	10	1551.70	64.50	0.00
3	2.14	NA	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1552.97	65.77	0.00
42	2.30	-0.04	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	1553.26	66.06	0.00
1	2.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1554.21	67.02	0.00
11	2.17	NA	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	7	1554.34	67.15	0.00
9	2.09	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	1555.77	68.58	0.00
139	2.17	NA	0.00	NA	+	NA	NA	NA	+	NA	NA	NA	8	1556.40	69.20	0.00

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	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
40	3.13	0.02	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	1095.77	0.00	0.20
56	3.09	0.03	+	+	NA	+	+	NA	NA	NA	NA	NA	10	1095.92	0.14	0.19
24	3.06	0.03	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	1096.28	0.51	0.16
8	3.11	0.02	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	1096.50	0.73	0.14
48	3.14	0.02	+	+	+	NA	+	NA	NA	NA	NA	NA	10	1097.76	1.99	0.07
64	3.09	0.03	+	+	+	+	+	NA	NA	NA	NA	NA	11	1097.96	2.18	0.07
32	3.06	0.03	+	+	+	+	NA	NA	NA	NA	NA	NA	10	1098.34	2.56	0.06
16	3.11	0.02	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	1098.56	2.79	0.05
128	3.11	0.03	+	+	+	+	+	+	NA	NA	NA	NA	12	1099.72	3.94	0.03
7	3.24	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1101.18	5.41	0.01
39	3.26	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	1101.38	5.60	0.01
6	3.04	0.02	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1102.32	6.54	0.01
22	3.00	0.03	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	1102.94	7.16	0.01
4	3.20	0.02	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1106.83	11.06	0.00
5	3.17	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1107.15	11.38	0.00
12	3.19	0.02	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	1108.74	12.96	0.00
3	3.30	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1109.37	13.60	0.00
2	3.13	0.02	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1112.90	17.13	0.00
1	3.24	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1115.62	19.84	0.00
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
40	3.26	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	1086.32	0.00	0.48
8	3.23	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	1086.45	0.13	0.45
6	3.18	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	1090.91	4.58	0.05
24	3.26	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	1094.54	8.22	0.01
56	3.28	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	1094.76	8.44	0.01
22	3.21	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	1098.38	12.06	0.00
4	3.32	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	1098.50	12.18	0.00
16	3.23	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	1099.01	12.69	0.00
48	3.25	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	1099.05	12.72	0.00
7	3.24	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1101.18	14.86	0.00
39	3.26	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	1101.38	15.05	0.00
2	3.26	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	1103.44	17.12	0.00
32	3.26	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	1106.15	19.82	0.00
64	3.28	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	1106.83	20.51	0.00
5	3.17	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1107.15	20.83	0.00
3	3.30	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1109.37	23.05	0.00
12	3.31	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	1112.25	25.93	0.00
1	3.24	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1115.62	29.29	0.00
128	3.26	+	+	+	+	+	+	+	NA	NA	NA	NA	46	1122.58	36.26	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
40	1.92	0.14	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	1088.48	0.00	0.21
8	1.90	0.14	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	1088.53	0.06	0.20
24	1.74	0.16	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	1089.57	1.10	0.12
56	1.78	0.16	+	+	NA	+	+	NA	NA	NA	NA	NA	10	1089.80	1.33	0.11
16	1.73	0.16	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	1090.12	1.65	0.09
48	1.79	0.16	+	+	+	NA	+	NA	NA	NA	NA	NA	10	1090.30	1.83	0.08
32	1.39	0.20	+	+	+	+	NA	NA	NA	NA	NA	NA	10	1090.49	2.02	0.08
64	1.50	0.19	+	+	+	+	+	NA	NA	NA	NA	NA	11	1091.19	2.71	0.05
128	1.53	0.19	+	+	+	+	+	+	NA	NA	NA	NA	12	1093.02	4.55	0.02
6	1.79	0.15	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1093.51	5.03	0.02
22	1.64	0.16	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	1094.78	6.30	0.01
4	2.15	0.12	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1100.12	11.65	0.00
7	3.24	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1101.18	12.71	0.00
12	1.91	0.15	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	1101.34	12.86	0.00
39	3.26	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	1101.38	12.90	0.00
2	2.03	0.13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1105.60	17.12	0.00
5	3.17	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1107.15	18.68	0.00
3	3.30	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1109.37	20.90	0.00
1	3.24	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1115.62	27.14	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
272	3.36	-0.08	0.01	+	+	NA	NA	NA	NA	+	NA	NA	10	1087.78	0.00	0.09
16	**3.35**	**-0.09**	**0.01**	**+**	**+**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**9**	**1087.86**	**0.07**	**0.08**
144	3.31	-0.08	0.01	+	+	NA	NA	NA	+	NA	NA	NA	10	1088.70	0.92	0.06
400	3.33	-0.07	0.01	+	+	NA	NA	NA	+	+	NA	NA	11	1088.85	1.07	0.05
48	3.31	-0.08	0.01	+	+	NA	+	NA	NA	NA	NA	NA	10	1089.28	1.49	0.04
304	3.33	-0.07	0.01	+	+	NA	+	NA	NA	+	NA	NA	11	1089.35	1.56	0.04
176	3.38	-0.11	0.01	+	+	NA	+	NA	+	NA	NA	NA	11	1089.56	1.78	0.04
288	3.38	-0.09	0.01	+	+	+	NA	NA	NA	+	NA	NA	11	1089.66	1.88	0.03
432	3.40	-0.10	0.01	+	+	NA	+	NA	+	+	NA	NA	12	1089.81	2.02	0.03
336	3.37	-0.08	0.01	+	+	NA	NA	+	NA	+	NA	NA	11	1089.82	2.03	0.03
32	3.36	-0.09	0.01	+	+	+	NA	NA	NA	NA	NA	NA	10	1089.87	2.09	0.03
80	3.35	-0.09	0.01	+	+	NA	NA	+	NA	NA	NA	NA	10	1089.92	2.13	0.03
96	3.44	-0.12	0.01	+	+	+	NA	+	NA	NA	NA	NA	11	1090.66	2.88	0.02
352	3.45	-0.12	0.01	+	+	+	NA	+	NA	+	NA	NA	12	1090.68	2.89	0.02
208	3.31	-0.08	0.01	+	+	NA	NA	+	+	NA	NA	NA	11	1090.73	2.94	0.02
160	3.32	-0.08	0.01	+	+	+	NA	NA	+	NA	NA	NA	11	1090.76	2.98	0.02
416	3.34	-0.08	0.01	+	+	+	NA	NA	+	+	NA	NA	12	1090.83	3.04	0.02
464	3.33	-0.07	0.01	+	+	NA	NA	+	+	+	NA	NA	12	1090.93	3.14	0.02
224	3.40	-0.12	0.01	+	+	+	NA	+	+	NA	NA	NA	12	1091.14	3.35	0.02
399	3.14	NA	0.00	+	+	NA	NA	NA	+	+	NA	NA	10	1091.28	3.50	0.02
320	3.35	-0.08	0.01	+	+	+	+	NA	NA	+	NA	NA	12	1091.28	3.50	0.02
64	3.32	-0.08	0.01	+	+	+	+	NA	NA	NA	NA	NA	11	1091.33	3.54	0.01
112	3.31	-0.07	0.01	+	+	NA	+	+	NA	NA	NA	NA	11	1091.33	3.54	0.01
271	3.17	NA	0.00	+	+	NA	NA	NA	NA	+	NA	NA	9	1091.40	3.61	0.01
368	3.33	-0.07	0.01	+	+	NA	+	+	NA	+	NA	NA	12	1091.41	3.63	0.01
480	3.42	-0.11	0.01	+	+	+	NA	+	+	+	NA	NA	13	1091.50	3.71	0.01
240	3.38	-0.11	0.01	+	+	NA	+	+	+	NA	NA	NA	12	1091.51	3.73	0.01
256	3.49	-0.15	0.01	+	+	+	+	+	+	NA	NA	NA	13	1091.59	3.80	0.01
143	3.12	NA	0.00	+	+	NA	NA	NA	+	NA	NA	NA	9	1091.61	3.83	0.01
192	3.38	-0.11	0.01	+	+	+	+	NA	+	NA	NA	NA	12	1091.64	3.85	0.01
448	3.41	-0.10	0.01	+	+	+	+	NA	+	+	NA	NA	13	1091.85	4.06	0.01
496	3.39	-0.10	0.01	+	+	NA	+	+	+	+	NA	NA	13	1091.88	4.09	0.01
128	3.40	-0.11	0.01	+	+	+	+	+	NA	NA	NA	NA	12	1091.90	4.11	0.01
384	3.42	-0.11	0.01	+	+	+	+	+	NA	+	NA	NA	13	1092.12	4.34	0.01
512	3.50	-0.15	0.01	+	+	+	+	+	+	+	NA	NA	14	1092.15	4.36	0.01
15	3.14	NA	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	8	1092.20	4.42	0.01
1488	3.33	-0.07	0.01	+	+	NA	NA	+	+	+	NA	+	13	1092.92	5.13	0.01
1504	3.43	-0.11	0.01	+	+	+	NA	+	+	+	NA	+	14	1093.17	5.38	0.01
1536	3.54	-0.16	0.01	+	+	+	+	+	+	+	NA	+	15	1093.25	5.46	0.01
12	3.29	-0.09	0.01	NA	+	NA	NA	NA	NA	NA	NA	NA	8	1093.32	5.54	0.01
832	3.35	-0.08	0.01	+	+	+	+	NA	NA	+	+	NA	13	1093.34	5.55	0.01
463	3.14	NA	0.00	+	+	NA	NA	+	+	+	NA	NA	11	1093.34	5.55	0.01
335	3.17	NA	0.00	+	+	NA	NA	+	NA	+	NA	NA	10	1093.46	5.67	0.01
207	3.11	NA	0.00	+	+	NA	NA	+	+	NA	NA	NA	10	1093.52	5.73	0.01
1024	3.54	-0.16	0.01	+	+	+	+	+	+	+	+	NA	15	1093.52	5.73	0.00
1520	3.40	-0.10	0.01	+	+	NA	+	+	+	+	NA	+	14	1093.66	5.88	0.00
960	3.42	-0.11	0.01	+	+	+	+	NA	+	+	+	NA	14	1093.76	5.98	0.00
896	3.43	-0.11	0.01	+	+	+	+	+	NA	+	+	NA	14	1093.99	6.20	0.00
79	3.14	NA	0.00	+	+	NA	NA	+	NA	NA	NA	NA	9	1094.22	6.43	0.00
140	3.26	-0.08	0.01	NA	+	NA	NA	NA	+	NA	NA	NA	9	1094.69	6.91	0.00
172	3.35	-0.12	0.01	NA	+	NA	+	NA	+	NA	NA	NA	10	1094.95	7.17	0.00
1487	3.15	NA	0.00	+	+	NA	NA	+	+	+	NA	+	12	1094.97	7.19	0.00
2048	3.51	-0.15	0.01	+	+	+	+	+	+	+	+	+	16	1095.10	7.31	0.00
44	3.27	-0.08	0.01	NA	+	NA	+	NA	NA	NA	NA	NA	9	1095.16	7.37	0.00
270	3.13	0.02	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	1095.77	7.99	0.00
302	3.09	0.03	NA	+	+	NA	+	NA	NA	+	NA	NA	10	1095.92	8.13	0.00
46	3.06	0.03	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	1096.28	8.49	0.00
14	3.11	0.02	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	1096.50	8.72	0.00
286	3.14	0.02	NA	+	+	+	NA	NA	NA	+	NA	NA	10	1097.76	9.97	0.00
11	3.07	NA	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	7	1097.85	10.06	0.00
318	3.09	0.03	NA	+	+	+	+	NA	NA	+	NA	NA	11	1097.96	10.17	0.00
139	3.05	NA	0.00	NA	+	NA	NA	NA	+	NA	NA	NA	8	1098.00	10.22	0.00
62	3.06	0.03	NA	+	+	+	+	NA	NA	NA	NA	NA	10	1098.34	10.55	0.00
30	3.11	0.02	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	1098.56	10.77	0.00
830	3.11	0.03	NA	+	+	+	+	NA	NA	+	+	NA	12	1099.72	11.93	0.00
8	3.43	-0.08	0.01	+	NA	NA	NA	NA	NA	NA	NA	NA	8	1099.85	12.06	0.00
13	3.24	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	1101.18	13.40	0.00
269	3.26	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	1101.38	13.59	0.00
72	3.42	-0.08	0.01	+	NA	NA	NA	+	NA	NA	NA	NA	9	1101.68	13.89	0.00
24	3.42	-0.08	0.01	+	NA	+	NA	NA	NA	NA	NA	NA	9	1101.84	14.06	0.00
10	3.04	0.02	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	1102.32	14.53	0.00
88	3.49	-0.11	0.01	+	NA	+	NA	+	NA	NA	NA	NA	10	1102.90	15.11	0.00
42	3.00	0.03	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	1102.94	15.15	0.00
7	3.23	NA	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1103.54	15.76	0.00
71	3.22	NA	0.00	+	NA	NA	NA	+	NA	NA	NA	NA	8	1105.25	17.46	0.00
4	3.37	-0.09	0.01	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1105.73	17.95	0.00
6	3.20	0.02	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1106.83	19.05	0.00
9	3.17	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	1107.15	19.37	0.00
22	3.19	0.02	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	1108.74	20.95	0.00
5	3.30	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1109.37	21.59	0.00
3	3.16	NA	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1109.42	21.63	0.00
2	3.13	0.02	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1112.90	25.12	0.00
1	3.24	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1115.62	27.83	0.00

ALPHA2-GLOBULIN

	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	logLik	AIC	delta	weight
5	5.88	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	-1129.41	2270.94	0.00	0.21
6	6.02	-0.03	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1128.62	2271.42	0.48	0.16
22	5.95	-0.01	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	-1128.11	2272.45	1.50	0.10
1	**5.79**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**5**	**-1131.22**	**2272.53**	**1.59**	**0.09**
7	5.85	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1129.28	2272.73	1.78	0.09
8	6.00	-0.03	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	-1128.50	2273.23	2.29	0.07
2	5.89	-0.02	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1130.77	2273.67	2.72	0.05
3	5.76	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1131.05	2274.23	3.29	0.04
24	5.93	-0.01	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	-1128.04	2274.36	3.42	0.04
39	5.84	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	-1129.26	2274.74	3.80	0.03
40	5.99	-0.03	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	-1128.45	2275.17	4.23	0.03
16	5.99	-0.02	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	-1128.50	2275.28	4.34	0.02
4	5.86	-0.02	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	-1130.60	2275.38	4.43	0.02
56	5.91	-0.01	+	+	NA	+	+	NA	NA	NA	NA	NA	10	-1127.95	2276.24	5.30	0.01
32	5.92	-0.01	+	+	+	+	NA	NA	NA	NA	NA	NA	10	-1128.03	2276.41	5.46	0.01
48	5.97	-0.02	+	+	+	NA	+	NA	NA	NA	NA	NA	10	-1128.44	2277.22	6.28	0.01
12	5.88	-0.02	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	-1130.58	2277.38	6.44	0.01
64	5.89	-0.01	+	+	+	+	+	NA	NA	NA	NA	NA	11	-1127.93	2278.27	7.33	0.01
128	5.89	-0.01	+	+	+	+	+	+	NA	NA	NA	NA	12	-1127.92	2280.32	9.38	0.00
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**logLik**	**AIC**	**delta**	**weight**
5	5.88	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	-1129.41	2270.94	0.00	0.45
1	5.79	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	-1131.22	2272.53	1.59	0.20
7	5.85	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1129.28	2272.73	1.78	0.19
3	5.76	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1131.05	2274.23	3.29	0.09
39	5.84	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	-1129.26	2274.74	3.80	0.07
6	6.03	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	-1125.17	2283.21	12.26	0.00
8	6.00	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	-1125.02	2285.00	14.06	0.00
2	5.92	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	-1127.35	2285.45	14.51	0.00
40	5.99	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	-1124.94	2286.95	16.01	0.00
4	5.89	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	-1127.14	2287.15	16.20	0.00
22	6.11	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	-1119.70	2293.66	22.72	0.00
24	6.09	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	-1119.59	2295.61	24.67	0.00
56	6.06	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	-1119.38	2297.37	26.43	0.00
16	6.01	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	-1120.94	2298.32	27.37	0.00
48	6.00	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	-1120.87	2300.35	29.41	0.00
12	5.91	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	-1123.07	2300.40	29.45	0.00
32	6.08	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	-1116.03	2310.66	39.71	0.00
64	6.05	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	-1115.81	2312.45	41.51	0.00
128	6.08	+	+	+	+	+	+	+	NA	NA	NA	NA	46	-1113.44	2326.05	55.10	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**logLik**	**AIC**	**delta**	**weight**
5	5.88	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	-1129.41	2270.94	0.00	0.25
6	9.22	-0.30	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1128.93	2272.04	1.09	0.15
1	5.79	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	-1131.22	2272.53	1.59	0.11
7	5.85	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1129.28	2272.73	1.78	0.10
8	9.12	-0.30	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	-1128.82	2273.87	2.93	0.06
2	8.46	-0.24	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1130.91	2273.95	3.01	0.06
22	8.89	-0.27	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	-1128.91	2274.03	3.09	0.05
3	5.76	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1131.05	2274.23	3.29	0.05
39	5.84	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	-1129.26	2274.74	3.80	0.04
4	8.36	-0.24	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	-1130.76	2275.70	4.75	0.02
16	9.81	-0.36	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	-1128.78	2275.85	4.91	0.02
24	8.81	-0.27	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	-1128.80	2275.88	4.94	0.02
40	9.10	-0.29	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	-1128.81	2275.90	4.96	0.02
12	9.18	-0.31	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	-1130.70	2277.63	6.69	0.01
32	9.49	-0.33	+	+	+	+	NA	NA	NA	NA	NA	NA	10	-1128.77	2277.89	6.95	0.01
48	9.76	-0.35	+	+	+	NA	+	NA	NA	NA	NA	NA	10	-1128.77	2277.89	6.95	0.01
56	8.77	-0.26	+	+	NA	+	+	NA	NA	NA	NA	NA	10	-1128.78	2277.91	6.96	0.01
64	9.40	-0.32	+	+	+	+	+	NA	NA	NA	NA	NA	11	-1128.76	2279.93	8.99	0.00
128	9.40	-0.32	+	+	+	+	+	+	NA	NA	NA	NA	11	-1128.76	2279.93	8.99	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**logLik**	**AIC**	**delta**	**weight**
9	5.88	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	-1129.41	2270.94	0.00	0.10
10	6.02	-0.03	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	-1128.62	2271.42	0.48	0.08
11	5.94	NA	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	7	-1128.91	2271.99	1.05	0.06
139	5.89	NA	0.00	NA	+	NA	NA	NA	+	NA	NA	NA	8	-1128.01	2272.24	1.30	0.05
42	5.95	-0.01	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	-1128.11	2272.45	1.50	0.05
1	5.79	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	-1131.22	2272.53	1.59	0.05
12	6.20	-0.10	0.01	NA	+	NA	NA	NA	NA	NA	NA	NA	8	-1128.20	2272.62	1.67	0.04
13	5.85	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	-1129.28	2272.73	1.78	0.04
14	6.00	-0.03	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	-1128.50	2273.23	2.29	0.03
140	6.11	-0.08	0.01	NA	+	NA	NA	NA	+	NA	NA	NA	9	-1127.55	2273.39	2.45	0.03
2	5.89	-0.02	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1130.77	2273.67	2.72	0.03
15	5.92	NA	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	8	-1128.79	2273.80	2.86	0.02
44	6.10	-0.08	0.01	NA	+	NA	+	NA	NA	NA	NA	NA	9	-1127.84	2273.96	3.02	0.02
3	5.83	NA	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	-1130.98	2274.10	3.16	0.02
143	5.88	NA	0.00	+	+	NA	NA	NA	+	NA	NA	NA	9	-1127.94	2274.17	3.23	0.02
5	5.76	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	-1131.05	2274.23	3.29	0.02

172	6.29	-0.15	0.01	NA	+	NA	+	NA	+	NA	NA	NA	10	-1126.96	2274.26	3.32	0.02
46	5.93	-0.01	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	-1128.04	2274.36	3.42	0.02
16	6.18	-0.10	0.01	+	+	NA	NA	NA	NA	NA	NA	NA	9	-1128.06	2274.40	3.45	0.02
4	6.10	-0.11	0.01	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	-1130.23	2274.63	3.68	0.02
269	5.84	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	-1129.26	2274.74	3.80	0.02
270	5.99	-0.03	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	-1128.45	2275.17	4.23	0.01
30	5.99	-0.02	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	-1128.50	2275.28	4.34	0.01
144	6.10	-0.09	0.01	+	+	NA	NA	NA	+	NA	NA	NA	10	-1127.47	2275.29	4.34	0.01
6	5.86	-0.02	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1130.60	2275.38	4.43	0.01
271	5.91	NA	0.00	+	+	NA	NA	NA	NA	+	NA	NA	9	-1128.75	2275.78	4.83	0.01
7	5.80	NA	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	7	-1130.82	2275.81	4.87	0.01
48	6.09	-0.08	0.01	+	+	NA	+	NA	NA	NA	NA	NA	10	-1127.75	2275.85	4.90	0.01
79	5.92	NA	0.00	+	+	NA	NA	+	NA	NA	NA	NA	9	-1128.79	2275.86	4.91	0.01
399	5.86	NA	0.00	+	+	NA	NA	NA	+	+	NA	NA	10	-1127.86	2276.06	5.12	0.01
176	6.28	-0.16	0.01	+	+	NA	+	NA	+	NA	NA	NA	11	-1126.83	2276.07	5.13	0.01
207	5.87	NA	0.00	+	+	NA	NA	+	+	NA	NA	NA	10	-1127.93	2276.20	5.26	0.01
302	5.91	-0.01	NA	+	+	NA	+	NA	NA	+	NA	NA	10	-1127.95	2276.24	5.30	0.01
272	6.17	-0.11	0.01	+	+	NA	NA	NA	NA	+	NA	NA	10	-1127.97	2276.29	5.35	0.01
8	6.07	-0.11	0.01	+	NA	NA	NA	NA	NA	NA	NA	NA	8	-1130.04	2276.30	5.36	0.01
62	5.92	-0.01	NA	+	+	+	+	NA	NA	NA	NA	NA	10	-1128.03	2276.41	5.46	0.01
80	6.18	-0.10	0.01	+	+	NA	NA	+	NA	NA	NA	NA	10	-1128.06	2276.45	5.51	0.01
32	6.18	-0.10	0.01	+	+	+	NA	NA	NA	NA	NA	NA	10	-1128.06	2276.46	5.51	0.01
400	6.09	-0.09	0.01	+	+	NA	NA	NA	+	+	NA	NA	11	-1127.34	2277.10	6.16	0.00
286	5.97	-0.02	NA	+	+	+	NA	NA	NA	+	NA	NA	10	-1128.44	2277.22	6.28	0.00
160	6.09	-0.08	0.01	+	+	+	NA	NA	+	NA	NA	NA	11	-1127.47	2277.35	6.40	0.00
208	6.09	-0.09	0.01	+	+	NA	NA	+	+	NA	NA	NA	11	-1127.47	2277.35	6.41	0.00
22	5.88	-0.02	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	-1130.58	2277.38	6.44	0.00
304	6.08	-0.08	0.01	+	+	NA	+	NA	NA	+	NA	NA	11	-1127.64	2277.69	6.75	0.00
71	5.81	NA	0.00	+	NA	NA	NA	+	NA	NA	NA	NA	8	-1130.80	2277.82	6.88	0.00
335	5.90	NA	0.00	+	+	NA	NA	+	NA	+	NA	NA	10	-1128.75	2277.83	6.89	0.00
432	6.27	-0.16	0.01	+	+	NA	+	NA	+	+	NA	NA	12	-1126.68	2277.86	6.92	0.00
64	6.09	-0.08	0.01	+	+	+	+	NA	NA	NA	NA	NA	11	-1127.75	2277.91	6.97	0.00
112	6.09	-0.08	0.01	+	+	NA	+	+	NA	NA	NA	NA	11	-1127.75	2277.92	6.97	0.00
463	5.85	NA	0.00	+	+	NA	NA	+	+	+	NA	NA	11	-1127.82	2278.05	7.11	0.00
240	6.27	-0.16	0.01	+	+	NA	+	+	+	NA	NA	NA	12	-1126.81	2278.10	7.16	0.00
192	6.26	-0.15	0.01	+	+	+	+	NA	+	NA	NA	NA	12	-1126.81	2278.10	7.16	0.00
72	6.09	-0.11	0.01	+	NA	NA	NA	+	NA	NA	NA	NA	9	-1129.99	2278.27	7.32	0.00
318	5.89	-0.01	NA	+	+	+	+	NA	NA	+	NA	NA	11	-1127.93	2278.27	7.33	0.00
24	6.10	-0.11	0.01	+	NA	+	NA	NA	NA	NA	NA	NA	9	-1130.00	2278.28	7.34	0.00
288	6.17	-0.11	0.01	+	+	+	NA	NA	NA	+	NA	NA	11	-1127.97	2278.35	7.41	0.00
336	6.17	-0.11	0.01	+	+	NA	NA	+	NA	+	NA	NA	11	-1127.97	2278.36	7.41	0.00
96	6.14	-0.09	0.01	+	+	+	NA	+	NA	NA	NA	NA	11	-1128.03	2278.48	7.54	0.00
416	6.06	-0.09	0.01	+	+	+	NA	NA	+	+	NA	NA	12	-1127.32	2279.14	8.19	0.00
464	6.07	-0.09	0.01	+	+	NA	NA	+	+	+	NA	NA	12	-1127.33	2279.14	8.20	0.00
224	6.07	-0.08	0.01	+	+	+	NA	+	+	NA	NA	NA	12	-1127.46	2279.42	8.47	0.00
320	6.06	-0.08	0.01	+	+	+	+	NA	NA	+	NA	NA	12	-1127.63	2279.75	8.81	0.00
368	6.07	-0.08	0.01	+	+	NA	+	+	NA	+	NA	NA	12	-1127.63	2279.76	8.81	0.00
496	6.25	-0.16	0.01	+	+	NA	+	+	+	+	NA	NA	13	-1126.63	2279.84	8.89	0.00
448	6.24	-0.16	0.01	+	+	+	+	NA	+	+	NA	NA	13	-1126.64	2279.85	8.90	0.00
128	6.06	-0.07	0.00	+	+	+	+	+	NA	NA	NA	NA	12	-1127.74	2279.97	9.03	0.00
1487	5.85	NA	0.00	+	+	NA	NA	+	+	+	NA	+	12	-1127.82	2280.12	9.18	0.00
256	6.27	-0.16	0.01	+	+	+	+	+	+	NA	NA	NA	13	-1126.81	2280.18	9.24	0.00
830	5.89	-0.01	NA	+	+	+	+	NA	NA	+	+	NA	12	-1127.92	2280.32	9.38	0.00
88	6.08	-0.10	0.01	+	NA	+	NA	+	NA	NA	NA	NA	10	-1129.99	2280.33	9.38	0.00
352	6.13	-0.09	0.01	+	+	+	NA	+	NA	+	NA	NA	12	-1127.95	2280.40	9.45	0.00
1488	6.07	-0.09	0.01	+	+	NA	NA	+	+	+	NA	+	13	-1127.32	2281.21	10.27	0.00
480	6.06	-0.08	0.01	+	+	+	NA	+	+	+	NA	NA	13	-1127.32	2281.22	10.28	0.00
832	6.06	-0.08	0.01	+	+	+	+	NA	NA	+	+	NA	13	-1127.60	2281.77	10.82	0.00
384	6.05	-0.07	0.00	+	+	+	+	+	NA	+	NA	NA	13	-1127.63	2281.82	10.88	0.00
1520	6.26	-0.16	0.01	+	+	NA	+	+	+	+	NA	+	14	-1126.62	2281.89	10.95	0.00
512	6.26	-0.17	0.01	+	+	+	+	+	+	+	NA	NA	14	-1126.63	2281.92	10.98	0.00
960	6.24	-0.16	0.01	+	+	+	+	NA	+	+	+	NA	14	-1126.64	2281.93	10.99	0.00
1504	6.06	-0.08	0.01	+	+	+	NA	+	+	+	NA	+	14	-1127.32	2283.29	12.35	0.00
896	6.03	-0.07	0.00	+	+	+	+	+	NA	+	+	NA	14	-1127.58	2283.83	12.89	0.00
1536	6.28	-0.17	0.01	+	+	+	+	+	+	+	NA	+	15	-1126.61	2283.98	13.03	0.00
1024	6.27	-0.17	0.01	+	+	+	+	+	+	+	+	NA	15	-1126.63	2284.01	13.07	0.00
2048	6.20	-0.14	0.01	+	+	+	+	+	+	+	+	+	16	-1126.45	2285.76	14.82	0.00

BETA-GLOBULIN

	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
8	5.29	0.24	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	2648.81	0.00	0.23
40	5.36	0.24	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	2648.91	0.10	0.22
16	5.16	0.26	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	2649.70	0.89	0.15
48	5.25	0.26	+	+	+	NA	+	NA	NA	NA	NA	NA	10	2650.20	1.38	0.12
24	5.30	0.23	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	2650.84	2.03	0.08
56	5.38	0.23	+	+	NA	+	+	NA	NA	NA	NA	NA	10	2650.90	2.08	0.08
32	5.17	0.26	+	+	+	+	NA	NA	NA	NA	NA	NA	10	2651.76	2.95	0.05
64	5.27	0.26	+	+	+	+	+	NA	NA	NA	NA	NA	11	2652.22	3.41	0.04
128	5.32	0.25	+	+	+	+	+	+	NA	NA	NA	NA	12	2654.02	5.21	0.02
12	5.44	0.26	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	2660.20	11.38	0.00
4	5.63	0.22	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	2660.42	11.60	0.00
6	5.58	0.24	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2660.50	11.68	0.00
22	5.55	0.24	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	2662.46	13.64	0.00
2	5.90	0.22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2670.60	21.79	0.00
7	6.99	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2675.44	26.63	0.00
39	7.07	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	2675.99	27.18	0.00
3	7.21	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2684.56	35.75	0.00
5	7.28	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2688.70	39.89	0.00
1	7.49	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2697.02	48.21	0.00
	I	AT	P	S	AT:P	AT:S	P:S	AT:P:S	NA	NA	NA	NA	df	AIC	delta	weight
24	5.88	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	2656.37	0.00	0.29
56	5.95	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	2656.57	0.20	0.27
8	5.72	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	2657.10	0.73	0.20
40	5.79	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	2657.62	1.25	0.16
16	5.85	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	2660.50	4.13	0.04
48	5.90	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	2661.74	5.38	0.02
32	5.98	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	2662.87	6.50	0.01
64	6.05	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	2663.95	7.58	0.01
4	6.02	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	2668.87	12.50	0.00
6	6.02	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	2669.39	13.02	0.00
22	6.15	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	2670.18	13.81	0.00
12	6.10	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	2671.91	15.54	0.00
128	5.99	+	+	+	+	+	+	+	NA	NA	NA	NA	46	2673.92	17.55	0.00
7	6.99	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2675.44	19.07	0.00
39	7.07	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	2675.99	19.62	0.00
2	6.29	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	2679.52	23.16	0.00
3	7.21	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2684.56	28.19	0.00
5	7.28	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2688.70	32.33	0.00
1	7.49	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2697.02	40.66	0.00
	I	AT	P	S	AT:P	AT:S	P:S	AT:P:S	NA	NA	NA	NA	df	AIC	delta	weight
8	4.85	0.29	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	2651.52	0.00	0.21
16	4.62	0.33	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	2651.53	0.01	0.21
40	4.91	0.29	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	2652.06	0.54	0.16
48	4.69	0.33	+	+	+	NA	+	NA	NA	NA	NA	NA	10	2652.54	1.03	0.13
24	4.88	0.28	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	2653.53	2.01	0.08
32	4.63	0.33	+	+	+	+	NA	NA	NA	NA	NA	NA	10	2653.59	2.07	0.08
56	4.95	0.28	+	+	NA	+	+	NA	NA	NA	NA	NA	10	2654.01	2.49	0.06
64	4.72	0.32	+	+	+	+	+	NA	NA	NA	NA	NA	11	2654.59	3.07	0.05
128	4.84	0.30	+	+	+	+	+	+	NA	NA	NA	NA	12	2655.67	4.15	0.03
12	4.91	0.32	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	2662.07	10.55	0.00
6	5.14	0.29	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2663.21	11.69	0.00
4	5.22	0.27	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	2663.33	11.81	0.00
22	5.11	0.29	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	2665.21	13.69	0.00
2	5.50	0.27	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2673.52	22.01	0.00
7	6.99	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2675.44	23.92	0.00
39	7.07	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	2675.99	24.47	0.00
3	7.21	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2684.56	33.04	0.00
5	7.28	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2688.70	37.18	0.00
1	7.49	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2697.02	45.50	0.00
	I	AL	I(AL^2)	P	S	AL:P	AL:S	I(AL^2):P	I(AL^2):S	P:S	AL:P:S	I(AL^2):P:S	df	AIC	delta	weight
96	6.00	-0.10	0.03	+	+	+	NA	+	NA	NA	NA	NA	11	2647.08	0.00	0.09
79	5.76	NA	0.02	+	+	NA	NA	+	NA	NA	NA	NA	9	2647.86	0.79	0.06
15	**5.84**	**NA**	**0.02**	**+**	**+**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**8**	**2647.90**	**0.82**	**0.06**
352	6.04	-0.09	0.03	+	+	+	NA	+	NA	+	NA	NA	12	2648.09	1.01	0.05
271	5.91	NA	0.02	+	+	NA	NA	NA	NA	+	NA	NA	9	2648.49	1.42	0.04
14	5.29	0.24	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	2648.81	1.74	0.04
270	5.36	0.24	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	2648.91	1.84	0.03
335	5.82	NA	0.02	+	+	NA	NA	+	NA	+	NA	NA	10	2648.95	1.87	0.03
80	5.49	0.11	0.01	+	+	NA	NA	+	NA	NA	NA	NA	10	2649.04	1.97	0.03
128	6.02	-0.10	0.03	+	+	+	+	+	NA	NA	NA	NA	12	2649.14	2.06	0.03
224	6.00	-0.10	0.03	+	+	+	NA	+	+	NA	NA	NA	12	2649.15	2.08	0.03
16	5.60	0.10	0.01	+	+	NA	NA	NA	NA	NA	NA	NA	9	2649.24	2.17	0.03
143	5.87	NA	0.02	+	+	NA	NA	NA	+	NA	NA	NA	9	2649.57	2.49	0.02
272	5.64	0.11	0.01	+	+	NA	NA	NA	NA	+	NA	NA	10	2649.61	2.53	0.02
30	5.16	0.26	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	2649.70	2.63	0.02
207	5.78	NA	0.02	+	+	NA	NA	+	+	NA	NA	NA	10	2649.74	2.66	0.02
336	5.54	0.12	0.01	+	+	NA	NA	+	NA	+	NA	NA	11	2649.94	2.86	0.02
399	5.95	NA	0.02	+	+	NA	NA	NA	+	+	NA	NA	10	2650.00	2.92	0.02
384	6.07	-0.09	0.03	+	+	+	+	+	NA	+	NA	NA	13	2650.13	3.05	0.02
480	6.05	-0.09	0.03	+	+	+	NA	+	+	+	NA	NA	13	2650.16	3.08	0.02
286	5.25	0.26	NA	+	+	+	NA	NA	NA	+	NA	NA	10	2650.20	3.12	0.02
32	5.47	0.13	0.01	+	+	+	NA	NA	NA	NA	NA	NA	10	2650.32	3.24	0.02
1504	6.12	-0.10	0.03	+	+	+	NA	+	+	+	NA	+	14	2650.62	3.55	0.01
463	5.86	NA	0.02	+	+	NA	NA	+	+	+	NA	NA	11	2650.70	3.62	0.01
46	5.30	0.23	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	2650.84	3.77	0.01
302	5.38	0.23	NA	+	+	NA	+	NA	NA	+	NA	NA	10	2650.90	3.82	0.01
288	5.53	0.14	0.01	+	+	+	NA	NA	NA	+	NA	NA	11	2651.01	3.94	0.01
112	5.54	0.09	0.01	+	+	NA	+	+	NA	NA	NA	NA	11	2651.03	3.96	0.01
208	5.52	0.10	0.01	+	+	NA	NA	+	+	NA	NA	NA	11	2651.05	3.98	0.01
256	6.10	-0.14	0.03	+	+	+	+	+	+	NA	NA	NA	13	2651.07	3.99	0.01
144	5.65	0.08	0.01	+	+	NA	NA	NA	+	NA	NA	NA	10	2651.09	4.02	0.01
48	5.67	0.08	0.01	+	+	NA	+	NA	NA	NA	NA	NA	10	2651.11	4.03	0.01
896	6.17	-0.12	0.03	+	+	+	+	+	NA	+	+	NA	14	2651.31	4.23	0.01
400	5.70	0.10	0.01	+	+	NA	NA	NA	+	+	NA	NA	11	2651.35	4.28	0.01
304	5.73	0.09	0.01	+	+	NA	+	NA	NA	+	NA	NA	11	2651.39	4.31	0.01
62	5.17	0.26	NA	+	+	+	+	NA	NA	NA	NA	NA	10	2651.76	4.68	0.01
368	5.60	0.10	0.01	+	+	NA	+	+	NA	+	NA	NA	12	2651.87	4.79	0.01
464	5.58	0.11	0.01	+	+	NA	NA	+	+	+	NA	NA	12	2651.88	4.81	0.01
512	6.13	-0.12	0.03	+	+	+	+	+	+	+	NA	NA	14	2652.14	5.06	0.01
318	5.27	0.26	NA	+	+	+	+	NA	NA	+	NA	NA	11	2652.22	5.14	0.01
1536	6.29	-0.17	0.03	+	+	+	+	+	+	+	NA	+	15	2652.25	5.17	0.01
64	5.53	0.11	0.01	+	+	+	+	NA	NA	NA	NA	NA	11	2652.26	5.18	0.01
160	5.51	0.12	0.01	+	+	+	NA	NA	+	NA	NA	NA	11	2652.27	5.19	0.01
1487	5.88	NA	0.02	+	+	NA	NA	+	+	+	NA	+	12	2652.48	5.40	0.01
2048	6.06	-0.07	0.03	+	+	+	+	+	+	+	+	+	16	2652.65	5.58	0.01
416	5.59	0.12	0.01	+	+	+	NA	NA	+	+	NA	NA	12	2652.87	5.79	0.00
320	5.61	0.11	0.01	+	+	+	+	NA	NA	+	NA	NA	12	2652.88	5.80	0.00
240	5.56	0.08	0.01	+	+	NA	+	+	+	NA	NA	NA	12	2653.09	6.02	0.00
1024	6.29	-0.17	0.03	+	+	+	+	+	+	+	+	NA	15	2653.12	6.04	0.00
176	5.65	0.08	0.01	+	+	NA	+	NA	+	NA	NA	NA	11	2653.16	6.09	0.00
432	5.69	0.10	0.01	+	+	NA	+	NA	+	+	NA	NA	12	2653.43	6.35	0.00
1488	5.58	0.12	0.01	+	+	NA	NA	+	+	+	NA	+	13	2653.45	6.37	0.00
496	5.60	0.10	0.01	+	+	NA	+	+	+	+	NA	NA	13	2653.95	6.88	0.00
830	5.32	0.25	NA	+	+	+	+	NA	NA	+	+	NA	12	2654.02	6.95	0.00
192	5.53	0.11	0.01	+	+	+	+	NA	+	NA	NA	NA	12	2654.34	7.26	0.00
832	5.63	0.11	0.01	+	+	+	+	NA	NA	+	+	NA	13	2654.82	7.75	0.00
448	5.59	0.12	0.01	+	+	+	+	NA	+	+	NA	NA	13	2654.95	7.88	0.00
1520	5.63	0.10	0.01	+	+	NA	+	+	+	+	NA	+	14	2655.46	8.38	0.00
960	5.62	0.12	0.01	+	+	+	+	NA	+	+	+	NA	14	2656.91	9.83	0.00
71	6.03	NA	0.02	+	NA	NA	NA	+	NA	NA	NA	NA	8	2658.73	11.65	0.00
88	6.20	-0.07	0.03	+	NA	+	NA	+	NA	NA	NA	NA	10	2658.73	11.66	0.00
11	6.14	NA	0.02	NA	+	NA	NA	NA	NA	NA	NA	NA	7	2659.73	12.65	0.00
72	5.74	0.12	0.01	+	NA	NA	NA	+	NA	NA	NA	NA	9	2659.75	12.67	0.00
7	6.16	NA	0.02	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2660.09	13.01	0.00
22	5.44	0.26	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	2660.20	13.12	0.00
6	5.63	0.22	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2660.42	13.34	0.00
10	5.58	0.24	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	2660.50	13.42	0.00
12	5.89	0.10	0.01	NA	+	NA	NA	NA	NA	NA	NA	NA	8	2661.02	13.94	0.00
24	5.69	0.15	0.01	+	NA	+	NA	NA	NA	NA	NA	NA	9	2661.30	14.22	0.00
8	5.90	0.10	0.01	+	NA	NA	NA	NA	NA	NA	NA	NA	8	2661.33	14.25	0.00
139	6.15	NA	0.02	NA	+	NA	NA	NA	+	NA	NA	NA	8	2661.75	14.67	0.00
42	5.55	0.24	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	2662.46	15.38	0.00
44	5.88	0.10	0.01	NA	+	NA	+	NA	NA	NA	NA	NA	9	2663.08	16.00	0.00
140	5.89	0.10	0.01	NA	+	NA	NA	NA	+	NA	NA	NA	9	2663.08	16.00	0.00
172	5.85	0.12	0.01	NA	+	NA	+	NA	+	NA	NA	NA	10	2665.10	18.02	0.00
3	6.44	NA	0.02	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2670.41	23.33	0.00
2	5.90	0.22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2670.60	23.53	0.00
4	6.17	0.11	0.01	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	2671.58	24.50	0.00
13	6.99	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	2675.44	28.36	0.00
269	7.07	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	2675.99	28.91	0.00
5	7.21	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2684.56	37.48	0.00
9	7.28	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	2688.70	41.62	0.00
1	7.49	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2697.02	49.95	0.00

GAMMA-GLOBULIN

	I	**AL**	**P**	**S**	**AL:P**	**AL:S**	**P:S**	**AL:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
4	13.21	0.41	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	3642.15	0.00	0.32
12	12.87	0.48	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	3642.70	0.55	0.24
8	13.19	0.41	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	3644.19	2.04	0.11
16	12.88	0.48	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	3644.75	2.60	0.09
24	12.99	0.45	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	3645.44	3.30	0.06
32	12.64	0.53	+	+	+	+	NA	NA	NA	NA	NA	NA	10	3645.83	3.69	0.05
40	13.24	0.42	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	3646.05	3.90	0.05
48	12.92	0.48	+	+	+	NA	+	NA	NA	NA	NA	NA	10	3646.73	4.59	0.03
56	13.04	0.46	+	+	NA	+	+	NA	NA	NA	NA	NA	10	3647.36	5.21	0.02
64	12.67	0.53	+	+	+	+	+	NA	NA	NA	NA	NA	11	3647.86	5.71	0.02
128	12.74	0.51	+	+	+	+	+	+	NA	NA	NA	NA	12	3649.82	7.67	0.01
3	15.91	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	3669.97	27.83	0.00
7	15.92	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	3672.01	29.87	0.00
39	15.97	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	3673.99	31.84	0.00
2	15.36	0.40	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	3747.84	105.70	0.00
22	14.87	0.49	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	3748.65	106.50	0.00
6	15.36	0.40	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	3749.88	107.74	0.00
1	17.72	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	3767.53	125.38	0.00
5	17.73	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	3769.56	127.42	0.00
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
4	14.23	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	3654.50	0.00	0.67
8	14.21	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	3656.59	2.09	0.24
40	14.25	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	3658.61	4.12	0.09
24	14.32	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	3666.03	11.53	0.00
12	14.20	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	3666.07	11.57	0.00
56	14.36	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	3668.12	13.62	0.00
16	14.20	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	3668.24	13.75	0.00
3	15.91	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	3669.97	15.48	0.00
48	14.21	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	3670.43	15.93	0.00
7	15.92	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	3672.01	17.52	0.00
39	15.97	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	3673.99	19.49	0.00
32	14.27	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	3679.84	25.35	0.00
64	14.26	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	3682.10	27.60	0.00
128	14.37	+	+	+	+	+	+	+	NA	NA	NA	NA	46	3697.22	42.73	0.00
2	16.28	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	3761.97	107.48	0.00
6	16.28	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	3764.08	109.58	0.00
1	17.72	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	3767.53	113.03	0.00
5	17.73	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	3769.56	115.07	0.00
22	16.19	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	3772.10	117.60	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
4	**12.32**	**0.52**	**+**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**7**	**3640.45**	**0.00**	**0.31**
12	11.81	0.61	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	3640.73	0.28	0.27
8	12.28	0.52	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	3642.47	2.02	0.11
16	11.81	0.61	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	3642.78	2.34	0.10
24	12.10	0.55	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	3644.16	3.71	0.05
32	11.53	0.66	+	+	+	+	NA	NA	NA	NA	NA	NA	10	3644.24	3.79	0.05
40	12.32	0.52	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	3644.40	3.95	0.04
48	11.83	0.61	+	+	+	NA	+	NA	NA	NA	NA	NA	10	3644.82	4.37	0.03
56	12.14	0.56	+	+	NA	+	+	NA	NA	NA	NA	NA	10	3646.12	5.67	0.02
64	11.55	0.66	+	+	+	+	+	NA	NA	NA	NA	NA	11	3646.30	5.85	0.02
128	11.64	0.64	+	+	+	+	+	+	NA	NA	NA	NA	12	3648.28	7.83	0.01
3	15.91	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	3669.97	29.52	0.00
7	15.92	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	3672.01	31.56	0.00
39	15.97	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	3673.99	33.54	0.00
2	14.53	0.50	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	3747.01	106.56	0.00
22	14.00	0.58	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	3748.90	108.45	0.00
6	14.51	0.50	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	3749.05	108.60	0.00
1	17.72	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	3767.53	127.08	0.00
5	17.73	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	3769.56	129.11	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
71	13.94	NA	0.04	+	NA	NA	NA	+	NA	NA	NA	NA	8	3641.37	0.00	0.12
7	14.17	NA	0.03	+	NA	NA	NA	NA	NA	NA	NA	NA	7	3641.62	0.25	0.10
6	13.21	0.41	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	3642.15	0.77	0.08
22	12.87	0.48	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	3642.70	1.32	0.06
72	13.50	0.18	0.03	+	NA	NA	NA	+	NA	NA	NA	NA	9	3642.86	1.49	0.06
8	13.74	0.17	0.02	+	NA	NA	NA	NA	NA	NA	NA	NA	8	3643.15	1.78	0.05
79	13.94	NA	0.04	+	+	NA	NA	+	NA	NA	NA	NA	9	3643.43	2.06	0.04
88	14.14	-0.08	0.05	+	NA	+	NA	+	NA	NA	NA	NA	10	3643.57	2.20	0.04
15	14.13	NA	0.03	+	+	NA	NA	NA	NA	NA	NA	NA	8	3643.64	2.27	0.04
24	13.39	0.25	0.02	+	NA	+	NA	NA	NA	NA	NA	NA	9	3643.76	2.39	0.04
14	13.19	0.41	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	3644.19	2.82	0.03
30	12.88	0.48	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	3644.75	3.38	0.02
80	13.50	0.18	0.03	+	+	NA	NA	+	NA	NA	NA	NA	10	3644.92	3.55	0.02
207	13.86	NA	0.04	+	+	NA	NA	+	+	NA	NA	NA	10	3645.03	3.66	0.02
16	13.71	0.17	0.02	+	+	NA	NA	NA	NA	NA	NA	NA	9	3645.18	3.81	0.02
46	12.99	0.45	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	3645.44	4.07	0.02
335	13.96	NA	0.04	+	+	NA	NA	+	NA	+	NA	NA	10	3645.48	4.11	0.01
143	14.08	NA	0.03	+	+	NA	NA	NA	+	NA	NA	NA	9	3645.49	4.12	0.01
271	14.18	NA	0.03	+	+	NA	NA	NA	NA	+	NA	NA	9	3645.58	4.21	0.01
96	14.13	-0.08	0.05	+	+	+	NA	+	NA	NA	NA	NA	11	3645.64	4.27	0.01
32	13.39	0.25	0.02	+	+	+	NA	NA	NA	NA	NA	NA	10	3645.83	4.46	0.01
62	12.64	0.53	NA	+	+	+	+	NA	NA	NA	NA	NA	10	3645.83	4.46	0.01
270	13.24	0.42	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	3646.05	4.68	0.01
112	13.19	0.27	0.02	+	+	NA	+	+	NA	NA	NA	NA	11	3646.24	4.87	0.01
208	13.28	0.23	0.03	+	+	NA	NA	+	+	NA	NA	NA	11	3646.26	4.89	0.01
224	13.94	-0.05	0.05	+	+	+	NA	+	+	NA	NA	NA	12	3646.70	5.32	0.01
286	12.92	0.48	NA	+	+	+	NA	NA	NA	+	NA	NA	10	3646.73	5.36	0.01
48	13.48	0.24	0.02	+	+	NA	+	NA	NA	NA	NA	NA	10	3646.77	5.40	0.01
128	13.83	0.00	0.04	+	+	+	+	+	NA	NA	NA	NA	12	3646.77	5.40	0.01
144	13.57	0.20	0.02	+	+	NA	NA	NA	+	NA	NA	NA	10	3646.87	5.50	0.01
336	13.52	0.19	0.03	+	+	NA	NA	+	NA	+	NA	NA	11	3646.96	5.59	0.01
272	13.75	0.18	0.02	+	+	NA	NA	NA	NA	+	NA	NA	10	3647.10	5.72	0.01
463	13.86	NA	0.04	+	+	NA	NA	+	+	+	NA	NA	11	3647.10	5.73	0.01
64	13.10	0.33	0.02	+	+	+	+	NA	NA	NA	NA	NA	11	3647.26	5.89	0.01
160	13.19	0.29	0.02	+	+	+	NA	NA	+	NA	NA	NA	11	3647.32	5.95	0.01
302	13.04	0.46	NA	+	+	NA	+	NA	NA	+	NA	NA	10	3647.36	5.99	0.01
399	14.13	NA	0.03	+	+	NA	NA	NA	+	+	NA	NA	10	3647.46	6.09	0.01
352	14.15	-0.07	0.05	+	+	+	NA	+	NA	+	NA	NA	12	3647.70	6.33	0.00
288	13.42	0.25	0.02	+	+	+	NA	NA	NA	+	NA	NA	11	3647.84	6.47	0.00
318	12.67	0.53	NA	+	+	+	+	NA	NA	+	NA	NA	11	3647.86	6.49	0.00
368	13.21	0.27	0.02	+	+	NA	+	+	NA	+	NA	NA	12	3648.31	6.94	0.00
240	13.22	0.25	0.02	+	+	NA	+	+	+	NA	NA	NA	12	3648.31	6.94	0.00
464	13.29	0.23	0.03	+	+	NA	NA	+	+	+	NA	NA	12	3648.32	6.95	0.00
304	13.52	0.24	0.02	+	+	NA	+	NA	NA	+	NA	NA	11	3648.72	7.34	0.00
480	13.95	-0.05	0.05	+	+	+	NA	+	+	+	NA	NA	13	3648.78	7.41	0.00
256	13.95	-0.06	0.05	+	+	+	+	+	+	NA	NA	NA	13	3648.78	7.41	0.00
176	13.40	0.27	0.01	+	+	NA	+	NA	+	NA	NA	NA	11	3648.80	7.43	0.00
400	13.61	0.21	0.02	+	+	NA	NA	NA	+	+	NA	NA	11	3648.82	7.44	0.00
384	13.84	0.00	0.04	+	+	+	+	+	NA	+	NA	NA	13	3648.85	7.48	0.00
1487	13.88	NA	0.04	+	+	NA	NA	+	+	+	NA	+	12	3649.15	7.78	0.00
320	13.13	0.33	0.02	+	+	+	+	NA	NA	+	NA	NA	12	3649.31	7.94	0.00
192	13.09	0.33	0.02	+	+	+	+	NA	+	NA	NA	NA	12	3649.34	7.97	0.00
416	13.22	0.29	0.02	+	+	+	NA	NA	+	+	NA	NA	12	3649.37	8.00	0.00
830	12.74	0.51	NA	+	+	+	+	NA	NA	+	+	NA	12	3649.82	8.45	0.00
1488	13.29	0.24	0.02	+	+	NA	NA	+	+	+	NA	+	13	3650.30	8.93	0.00
496	13.23	0.26	0.02	+	+	NA	+	+	+	+	NA	NA	13	3650.39	9.01	0.00
1504	14.03	-0.06	0.05	+	+	+	NA	+	+	+	NA	+	14	3650.40	9.03	0.00
896	13.99	-0.04	0.04	+	+	+	+	+	NA	+	+	NA	14	3650.53	9.16	0.00
432	13.44	0.28	0.01	+	+	NA	+	NA	+	+	NA	NA	12	3650.74	9.37	0.00
512	13.95	-0.06	0.05	+	+	+	+	+	+	+	NA	NA	14	3650.87	9.49	0.00
832	13.16	0.33	0.01	+	+	+	+	NA	NA	+	+	NA	13	3651.34	9.96	0.00
448	13.12	0.33	0.01	+	+	+	+	NA	+	+	NA	NA	13	3651.39	10.02	0.00
1520	13.25	0.26	0.02	+	+	NA	+	+	+	+	NA	+	14	3652.38	11.01	0.00
1024	14.19	-0.13	0.05	+	+	+	+	+	+	+	+	NA	15	3652.45	11.08	0.00
1536	14.12	-0.10	0.05	+	+	+	+	+	+	+	NA	+	15	3652.46	11.09	0.00
960	13.16	0.33	0.01	+	+	+	+	NA	+	+	+	NA	14	3653.42	12.05	0.00
2048	14.17	-0.12	0.05	+	+	+	+	+	+	+	+	+	16	3654.55	13.18	0.00
5	15.91	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	3669.97	28.60	0.00
13	15.92	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	3672.01	30.64	0.00
269	15.97	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	3673.99	32.62	0.00
2	15.36	0.40	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	3747.84	106.47	0.00
3	16.31	NA	0.03	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	3748.52	107.14	0.00
42	14.87	0.49	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	3748.65	107.27	0.00
4	15.68	0.26	0.01	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	3749.58	108.21	0.00
10	15.36	0.40	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	3749.88	108.51	0.00
11	16.29	NA	0.03	NA	+	NA	NA	NA	NA	NA	NA	NA	7	3750.55	109.18	0.00
44	14.98	0.44	0.00	NA	+	NA	+	NA	NA	NA	NA	NA	9	3750.67	109.30	0.00
139	16.12	NA	0.04	NA	+	NA	NA	NA	+	NA	NA	NA	8	3750.87	109.50	0.00
140	15.24	0.34	0.01	NA	+	NA	NA	NA	+	NA	NA	NA	9	3751.25	109.87	0.00
12	15.66	0.26	0.01	NA	+	NA	NA	NA	NA	NA	NA	NA	8	3751.62	110.25	0.00
172	14.81	0.51	0.00	NA	+	NA	+	NA	+	NA	NA	NA	10	3752.55	111.18	0.00
1	17.72	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	3767.53	126.16	0.00
9	17.73	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	3769.56	128.19	0.00

HAPTOGLOBIN

	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
40	-0.04	0.06	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	1852.38	0.00	0.32
56	-0.07	0.07	+	+	NA	+	+	NA	NA	NA	NA	NA	10	1854.08	1.70	0.14
48	-0.09	0.07	+	+	+	NA	+	NA	NA	NA	NA	NA	10	1854.09	1.71	0.14
8	-0.09	0.06	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	1854.65	2.27	0.10
16	-0.15	0.07	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	1855.75	3.37	0.06
64	-0.12	0.08	+	+	+	+	+	NA	NA	NA	NA	NA	11	1855.78	3.40	0.06
24	-0.13	0.06	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	1856.23	3.85	0.05
6	-0.01	0.06	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1856.69	4.31	0.04
32	-0.19	0.08	+	+	+	+	NA	NA	NA	NA	NA	NA	10	1857.23	4.85	0.03
128	-0.13	0.08	+	+	+	+	+	+	NA	NA	NA	NA	12	1857.78	5.40	0.02
22	-0.07	0.07	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	1857.83	5.45	0.02
39	0.40	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	1858.67	6.29	0.01
7	0.33	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1860.38	8.00	0.01
4	0.05	0.05	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1861.40	9.02	0.00
12	-0.04	0.07	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	1861.79	9.41	0.00
2	0.12	0.05	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1862.90	10.52	0.00
5	0.42	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1863.43	11.06	0.00
3	0.43	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1865.99	13.61	0.00
1	0.50	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1868.50	16.12	0.00
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
56	0.21	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	1850.99	0.00	0.35
24	0.17	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	1851.54	0.55	0.26
40	0.10	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	1852.32	1.33	0.18
8	0.05	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	1853.45	2.47	0.10
22	0.24	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	1854.50	3.52	0.06
6	0.13	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	1856.34	5.35	0.02
39	0.40	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	1858.67	7.68	0.01
7	0.33	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1860.38	9.39	0.00
32	0.20	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	1860.61	9.62	0.00
4	0.17	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	1861.19	10.20	0.00
64	0.24	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	1861.23	10.25	0.00
48	0.14	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	1862.23	11.24	0.00
16	0.10	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	1862.56	11.58	0.00
2	0.25	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	1863.40	12.41	0.00
5	0.42	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1863.43	12.45	0.00
3	0.43	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1865.99	15.01	0.00
128	0.20	+	+	+	+	+	+	+	NA	NA	NA	NA	46	1867.13	16.15	0.00
1	0.50	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1868.50	17.51	0.00
12	0.21	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	1870.49	19.51	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
16	**-4.24**	**0.48**	**+**	**+**	**+**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**9**	**1835.31**	**0.00**	**0.30**
48	-4.11	0.47	+	+	+	NA	+	NA	NA	NA	NA	NA	10	1835.74	0.43	0.24
32	-4.55	0.52	+	+	+	+	NA	NA	NA	NA	NA	NA	10	1836.87	1.57	0.14
64	-4.36	0.50	+	+	+	+	+	NA	NA	NA	NA	NA	11	1837.52	2.22	0.10
128	-4.23	0.49	+	+	+	+	+	+	NA	NA	NA	NA	12	1838.48	3.18	0.06
40	-3.10	0.36	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	1838.73	3.43	0.05
8	-3.14	0.36	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	1839.35	4.05	0.04
56	-3.03	0.36	+	+	NA	+	+	NA	NA	NA	NA	NA	10	1840.73	5.42	0.02
24	-3.11	0.36	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	1841.40	6.10	0.01
12	-4.00	0.47	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	1842.42	7.12	0.01
6	-3.00	0.36	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1842.59	7.28	0.01
22	-3.02	0.36	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	1844.64	9.33	0.00
4	-2.79	0.34	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1847.40	12.10	0.00
2	-2.67	0.33	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1849.85	14.54	0.00
39	0.40	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	1858.67	23.36	0.00
7	0.33	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1860.38	25.07	0.00
5	0.42	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1863.43	28.13	0.00
3	0.43	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1865.99	30.69	0.00
1	0.50	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1868.50	33.19	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
352	0.61	-0.24	0.02	+	+	+	NA	+	NA	+	NA	NA	12	1843.60	0.00	0.12
96	0.59	-0.25	0.03	+	+	+	NA	+	NA	NA	NA	NA	11	1843.94	0.34	0.10
272	0.37	-0.12	0.01	+	+	NA	NA	NA	NA	+	NA	NA	10	1845.24	1.65	0.05
480	0.59	-0.23	0.02	+	+	+	NA	+	+	+	NA	NA	13	1845.40	1.80	0.05
224	0.56	-0.24	0.03	+	+	+	NA	+	+	NA	NA	NA	12	1845.55	1.95	0.05
384	0.60	-0.23	0.02	+	+	+	+	+	NA	+	NA	NA	13	1845.63	2.03	0.04
128	0.56	-0.24	0.03	+	+	+	+	+	NA	NA	NA	NA	12	1845.89	2.30	0.04
256	0.72	-0.31	0.03	+	+	+	+	+	+	NA	NA	NA	13	1845.91	2.32	0.04
512	0.74	-0.30	0.03	+	+	+	+	+	+	+	NA	NA	14	1846.01	2.41	0.04
336	0.32	-0.12	0.02	+	+	NA	NA	+	NA	+	NA	NA	11	1846.39	2.80	0.03
16	0.34	-0.13	0.02	+	+	NA	NA	NA	NA	NA	NA	NA	9	1846.45	2.85	0.03
80	0.29	-0.13	0.02	+	+	NA	NA	+	NA	NA	NA	NA	10	1846.91	3.31	0.02
271	0.07	NA	0.01	+	+	NA	NA	NA	NA	+	NA	NA	9	1846.99	3.40	0.02
288	0.33	-0.11	0.01	+	+	+	NA	NA	NA	+	NA	NA	11	1847.13	3.53	0.02
400	0.36	-0.12	0.01	+	+	NA	NA	NA	+	+	NA	NA	11	1847.30	3.70	0.02
304	0.37	-0.12	0.01	+	+	NA	+	NA	NA	+	NA	NA	11	1847.30	3.71	0.02
1504	0.59	-0.23	0.02	+	+	+	NA	+	+	+	NA	+	14	1847.46	3.86	0.02
896	0.60	-0.23	0.02	+	+	+	+	+	NA	+	+	NA	14	1847.72	4.12	0.02
1536	0.77	-0.30	0.03	+	+	+	+	+	+	+	NA	+	15	1847.85	4.25	0.01
335	0.04	NA	0.01	+	+	NA	NA	+	NA	+	NA	NA	10	1847.91	4.31	0.01
32	0.29	-0.12	0.02	+	+	+	NA	NA	NA	NA	NA	NA	10	1847.99	4.39	0.01
1024	0.77	-0.30	0.03	+	+	+	+	+	+	+	+	NA	15	1848.01	4.41	0.01
464	0.30	-0.11	0.02	+	+	NA	NA	+	+	+	NA	NA	12	1848.39	4.80	0.01
144	0.33	-0.13	0.02	+	+	NA	NA	NA	+	NA	NA	NA	10	1848.46	4.86	0.01
368	0.32	-0.12	0.02	+	+	NA	+	+	NA	+	NA	NA	12	1848.47	4.87	0.01
48	0.34	-0.13	0.02	+	+	NA	+	NA	NA	NA	NA	NA	10	1848.51	4.91	0.01
432	0.46	-0.16	0.02	+	+	NA	+	NA	+	+	NA	NA	12	1848.60	5.01	0.01
399	0.06	NA	0.01	+	+	NA	NA	NA	+	+	NA	NA	10	1848.79	5.19	0.01
208	0.27	-0.12	0.02	+	+	NA	NA	+	+	NA	NA	NA	11	1848.80	5.20	0.01
12	0.42	-0.13	0.02	NA	+	NA	NA	NA	NA	NA	NA	NA	8	1848.87	5.27	0.01
112	0.28	-0.12	0.02	+	+	NA	+	+	NA	NA	NA	NA	11	1848.95	5.36	0.01
79	-0.03	NA	0.01	+	+	NA	NA	+	NA	NA	NA	NA	9	1849.10	5.50	0.01
15	0.02	NA	0.01	+	+	NA	NA	NA	NA	NA	NA	NA	8	1849.12	5.52	0.01
416	0.32	-0.11	0.01	+	+	+	NA	NA	+	+	NA	NA	12	1849.17	5.58	0.01
320	0.34	-0.12	0.01	+	+	+	+	NA	NA	+	NA	NA	12	1849.20	5.61	0.01
2048	0.69	-0.27	0.03	+	+	+	+	+	+	+	+	+	16	1849.31	5.72	0.01
496	0.42	-0.16	0.02	+	+	NA	+	+	+	+	NA	NA	13	1849.48	5.88	0.01
463	0.02	NA	0.01	+	+	NA	NA	+	+	+	NA	NA	11	1849.55	5.95	0.01
176	0.44	-0.17	0.02	+	+	NA	+	NA	+	NA	NA	NA	11	1849.70	6.10	0.01
240	0.39	-0.17	0.02	+	+	NA	+	+	+	NA	NA	NA	12	1849.72	6.12	0.01
160	0.27	-0.12	0.02	+	+	+	NA	NA	+	NA	NA	NA	11	1849.96	6.36	0.01
64	0.29	-0.12	0.02	+	+	+	+	NA	NA	NA	NA	NA	11	1850.05	6.45	0.00
1488	0.31	-0.12	0.02	+	+	NA	NA	+	+	+	NA	+	13	1850.28	6.69	0.00
448	0.42	-0.15	0.02	+	+	+	+	NA	+	+	NA	NA	13	1850.41	6.81	0.00
207	-0.05	NA	0.01	+	+	NA	NA	+	+	NA	NA	NA	10	1850.44	6.84	0.00
140	0.39	-0.12	0.02	NA	+	NA	NA	NA	+	NA	NA	NA	9	1850.69	7.09	0.00
143	0.00	NA	0.01	+	+	NA	NA	NA	+	NA	NA	NA	9	1850.75	7.15	0.00
44	0.39	-0.12	0.02	NA	+	NA	+	NA	NA	NA	NA	NA	9	1850.84	7.24	0.00
832	0.32	-0.11	0.02	+	+	+	+	NA	NA	+	+	NA	13	1850.87	7.27	0.00
192	0.38	-0.16	0.02	+	+	+	+	NA	+	NA	NA	NA	12	1851.06	7.46	0.00
11	0.10	NA	0.01	NA	+	NA	NA	NA	NA	NA	NA	NA	7	1851.29	7.69	0.00
1520	0.41	-0.16	0.02	+	+	NA	+	+	+	+	NA	+	14	1851.52	7.92	0.00
1487	0.01	NA	0.01	+	+	NA	NA	+	+	+	NA	+	12	1851.61	8.01	0.00
88	0.66	-0.23	0.02	+	NA	+	NA	+	NA	NA	NA	NA	10	1852.19	8.59	0.00
172	0.48	-0.16	0.02	NA	+	NA	+	NA	+	NA	NA	NA	10	1852.19	8.60	0.00
960	0.40	-0.15	0.02	+	+	+	+	NA	+	+	+	NA	14	1852.27	8.67	0.00
270	-0.04	0.06	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	1852.38	8.78	0.00
139	0.07	NA	0.01	NA	+	NA	NA	NA	+	NA	NA	NA	8	1852.53	8.94	0.00
72	0.39	-0.12	0.02	+	NA	NA	NA	+	NA	NA	NA	NA	9	1854.05	10.46	0.00
302	-0.07	0.07	NA	+	+	NA	+	NA	NA	+	NA	NA	10	1854.08	10.48	0.00
286	-0.09	0.07	NA	+	+	+	NA	NA	NA	+	NA	NA	10	1854.09	10.49	0.00
14	-0.09	0.06	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	1854.65	11.05	0.00
8	0.47	-0.13	0.01	+	NA	NA	NA	NA	NA	NA	NA	NA	8	1854.69	11.10	0.00
24	0.38	-0.11	0.01	+	NA	+	NA	NA	NA	NA	NA	NA	9	1855.43	11.84	0.00
30	-0.15	0.07	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	1855.75	12.16	0.00
318	-0.12	0.08	NA	+	+	+	+	NA	NA	+	NA	NA	11	1855.78	12.18	0.00
71	0.09	NA	0.01	+	NA	NA	NA	+	NA	NA	NA	NA	8	1855.78	12.18	0.00
46	-0.13	0.06	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	1856.23	12.64	0.00
4	0.54	-0.13	0.01	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1856.44	12.85	0.00
10	-0.01	0.06	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	1856.69	13.09	0.00
7	0.15	NA	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1856.86	13.27	0.00
62	-0.19	0.08	NA	+	+	+	+	NA	NA	NA	NA	NA	10	1857.23	13.64	0.00
830	-0.13	0.08	NA	+	+	+	+	NA	NA	+	+	NA	12	1857.78	14.18	0.00
42	-0.07	0.07	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	1857.83	14.23	0.00
3	0.22	NA	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1858.44	14.84	0.00
269	0.40	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	1858.67	15.07	0.00
13	0.33	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	1860.38	16.78	0.00
6	0.05	0.05	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1861.40	17.80	0.00
22	-0.04	0.07	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	1861.79	18.20	0.00
2	0.12	0.05	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1862.90	19.30	0.00
9	0.42	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	1863.43	19.84	0.00
5	0.43	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1865.99	22.40	0.00
1	0.50	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1868.50	24.90	0.00

HEMAGGLUTINATION SCORE

	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
12	3.78	0.05	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	2042.86	0.00	0.20
1	4.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2043.22	0.36	0.17
16	3.75	0.05	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	2044.41	1.55	0.09
5	4.02	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2044.46	1.61	0.09
2	3.99	0.01	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2044.67	1.81	0.08
3	4.05	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2045.13	2.28	0.06
6	3.93	0.02	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2045.71	2.86	0.05
48	3.78	0.05	+	+	+	NA	+	NA	NA	NA	NA	NA	10	2045.79	2.94	0.05
7	4.01	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2046.37	3.52	0.03
32	3.76	0.05	+	+	+	+	NA	NA	NA	NA	NA	NA	10	2046.44	3.59	0.03
4	3.97	0.01	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	2046.59	3.73	0.03
39	4.05	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	2047.33	4.47	0.02
8	3.92	0.02	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	2047.62	4.76	0.02
22	3.95	0.01	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	2047.68	4.83	0.02
64	3.80	0.05	+	+	+	+	+	NA	NA	NA	NA	NA	11	2047.80	4.95	0.02
40	3.95	0.02	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	2048.32	5.47	0.01
128	3.84	0.04	+	+	+	+	+	+	NA	NA	NA	NA	12	2049.26	6.40	0.01
24	3.94	0.01	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	2049.57	6.71	0.01
56	3.98	0.01	+	+	NA	+	+	NA	NA	NA	NA	NA	10	2050.21	7.35	0.01
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
1	4.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2043.22	0.00	0.44
5	4.02	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2044.46	1.24	0.24
3	4.05	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2045.13	1.91	0.17
7	4.01	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2046.37	3.15	0.09
39	4.05	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	2047.33	4.11	0.06
2	4.07	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	2055.79	12.57	0.00
6	4.02	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	2056.81	13.59	0.00
12	3.89	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	2057.06	13.84	0.00
4	4.04	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	2057.68	14.46	0.00
8	4.00	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	2058.68	15.46	0.00
16	3.86	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	2058.75	15.54	0.00
40	4.04	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	2059.08	15.86	0.00
48	3.89	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	2059.90	16.68	0.00
22	3.97	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	2072.64	29.42	0.00
24	3.95	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	2074.57	31.35	0.00
56	3.99	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	2074.75	31.53	0.00
32	3.82	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	2077.67	34.45	0.00
64	3.86	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	2079.01	35.79	0.00
128	3.95	+	+	+	+	+	+	+	NA	NA	NA	NA	46	2091.70	48.48	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
12	**2.43**	**0.19**	**+**	**NA**	**+**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**8**	**2038.76**	**0.00**	**0.37**
16	2.36	0.20	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	2040.07	1.31	0.19
32	2.60	0.17	+	+	+	+	NA	NA	NA	NA	NA	NA	10	2041.61	2.86	0.09
48	2.41	0.19	+	+	+	NA	+	NA	NA	NA	NA	NA	10	2041.64	2.89	0.09
64	2.70	0.16	+	+	+	+	+	NA	NA	NA	NA	NA	11	2043.00	4.25	0.04
1	4.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2043.22	4.46	0.04
2	3.53	0.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2043.84	5.08	0.03
5	4.02	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2044.46	5.71	0.02
22	3.76	0.03	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	2044.65	5.89	0.02
6	3.42	0.07	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2044.76	6.01	0.02
128	2.69	0.16	+	+	+	+	+	+	NA	NA	NA	NA	12	2045.06	6.31	0.02
3	4.05	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2045.13	6.38	0.02
4	3.51	0.06	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	2045.71	6.95	0.01
7	4.01	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2046.37	7.62	0.01
24	3.73	0.03	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	2046.43	7.67	0.01
8	3.39	0.07	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	2046.62	7.86	0.01
56	3.79	0.03	+	+	NA	+	+	NA	NA	NA	NA	NA	10	2046.85	8.09	0.01
39	4.05	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	2047.33	8.57	0.01
40	3.42	0.07	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	2047.46	8.70	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
71	3.90	NA	0.00	+	NA	NA	NA	+	NA	NA	NA	NA	8	2041.95	0.00	0.12
22	3.78	0.05	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	2042.86	0.91	0.08
1	4.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2043.22	1.27	0.06
79	3.86	NA	0.00	+	+	NA	NA	+	NA	NA	NA	NA	9	2043.45	1.50	0.06
72	3.94	-0.02	0.01	+	NA	NA	NA	+	NA	NA	NA	NA	9	2043.95	2.00	0.04
30	3.75	0.05	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	2044.41	2.46	0.03
9	4.02	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	2044.46	2.52	0.03
3	4.02	NA	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2044.49	2.54	0.03
2	3.99	0.01	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2044.67	2.72	0.03
24	3.86	0.02	0.00	+	NA	+	NA	NA	NA	NA	NA	NA	9	2044.71	2.76	0.03
335	3.89	NA	0.00	+	+	NA	NA	+	NA	+	NA	NA	10	2044.93	2.98	0.03
5	4.05	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2045.13	3.19	0.02
207	3.88	NA	0.00	+	+	NA	NA	+	+	NA	NA	NA	10	2045.31	3.36	0.02
80	3.91	-0.02	0.01	+	+	NA	NA	+	NA	NA	NA	NA	10	2045.44	3.50	0.02
11	3.96	NA	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	7	2045.47	3.53	0.02
10	3.93	0.02	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	2045.71	3.77	0.02
286	3.78	0.05	NA	+	+	+	NA	NA	NA	+	NA	NA	10	2045.79	3.84	0.02
88	4.00	-0.04	0.01	+	NA	+	NA	+	NA	NA	NA	NA	10	2045.87	3.92	0.02
32	3.83	0.01	0.00	+	+	+	NA	NA	NA	NA	NA	NA	10	2046.21	4.26	0.01
13	4.01	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	2046.37	4.43	0.01
7	4.00	NA	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2046.40	4.45	0.01
4	4.08	-0.02	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	2046.41	4.47	0.01
62	3.76	0.05	NA	+	+	+	+	NA	NA	NA	NA	NA	10	2046.44	4.50	0.01
6	3.97	0.01	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2046.59	4.64	0.01
463	3.91	NA	0.00	+	+	NA	NA	+	+	+	NA	NA	11	2046.69	4.75	0.01
336	3.93	-0.02	0.01	+	+	NA	NA	+	NA	+	NA	NA	11	2046.96	5.01	0.01
139	3.99	NA	0.00	NA	+	NA	NA	NA	+	NA	NA	NA	8	2046.98	5.04	0.01
208	3.95	-0.03	0.01	+	+	NA	NA	+	+	NA	NA	NA	11	2047.23	5.29	0.01
269	4.05	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	2047.33	5.38	0.01
96	3.98	-0.05	0.01	+	+	+	NA	+	NA	NA	NA	NA	11	2047.34	5.39	0.01
15	3.95	NA	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	8	2047.37	5.42	0.01
12	4.03	-0.03	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	8	2047.38	5.44	0.01
112	3.93	-0.03	0.01	+	+	NA	+	+	NA	NA	NA	NA	11	2047.47	5.52	0.01
14	3.92	0.02	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	2047.62	5.67	0.01
288	3.86	0.02	0.00	+	+	+	NA	NA	NA	+	NA	NA	11	2047.64	5.70	0.01
42	3.95	0.01	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	2047.68	5.74	0.01
240	3.79	0.04	0.00	+	+	NA	+	+	+	NA	NA	NA	12	2047.80	5.85	0.01
318	3.80	0.05	NA	+	+	+	+	NA	NA	+	NA	NA	11	2047.80	5.86	0.01
160	3.88	0.00	0.00	+	+	+	NA	NA	+	NA	NA	NA	11	2047.90	5.95	0.01
271	3.99	NA	0.00	+	+	NA	NA	NA	NA	+	NA	NA	9	2048.09	6.14	0.01
64	3.86	0.01	0.00	+	+	+	+	NA	NA	NA	NA	NA	11	2048.20	6.25	0.01
192	3.72	0.07	0.00	+	+	+	+	NA	+	NA	NA	NA	12	2048.29	6.34	0.01
8	4.06	-0.03	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	8	2048.32	6.37	0.00
270	3.95	0.02	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	2048.32	6.38	0.00
172	3.88	0.04	0.00	NA	+	NA	+	NA	+	NA	NA	NA	10	2048.44	6.49	0.00
1487	3.92	NA	0.00	+	+	NA	NA	+	+	+	NA	+	12	2048.58	6.64	0.00
464	3.97	-0.02	0.01	+	+	NA	NA	+	+	+	NA	NA	12	2048.66	6.72	0.00
140	4.09	-0.04	0.00	NA	+	NA	NA	NA	+	NA	NA	NA	9	2048.76	6.81	0.00
143	3.97	NA	0.00	+	+	NA	NA	NA	+	NA	NA	NA	9	2048.83	6.88	0.00
352	3.99	-0.04	0.01	+	+	+	NA	+	NA	+	NA	NA	12	2048.88	6.93	0.00
368	3.96	-0.02	0.01	+	+	NA	+	+	NA	+	NA	NA	12	2048.96	7.01	0.00
496	3.81	0.04	0.00	+	+	NA	+	+	+	+	NA	NA	13	2049.09	7.15	0.00
224	4.00	-0.05	0.01	+	+	+	NA	+	+	NA	NA	NA	12	2049.18	7.24	0.00
416	3.91	0.00	0.00	+	+	+	NA	NA	+	+	NA	NA	12	2049.22	7.28	0.00
830	3.84	0.04	NA	+	+	+	+	NA	NA	+	+	NA	12	2049.26	7.31	0.00
44	4.07	-0.04	0.00	NA	+	NA	+	NA	NA	NA	NA	NA	9	2049.26	7.31	0.00
16	4.02	-0.03	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	9	2049.27	7.32	0.00
399	4.01	NA	0.00	+	+	NA	NA	NA	+	+	NA	NA	10	2049.36	7.41	0.00
128	3.99	-0.05	0.01	+	+	+	+	+	NA	NA	NA	NA	12	2049.38	7.44	0.00
448	3.75	0.08	0.00	+	+	+	+	NA	+	+	NA	NA	13	2049.48	7.53	0.00
46	3.94	0.01	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	2049.57	7.62	0.00
320	3.90	0.01	0.00	+	+	+	+	NA	NA	+	NA	NA	12	2049.60	7.65	0.00
256	3.83	0.02	0.00	+	+	+	+	+	+	NA	NA	NA	13	2049.83	7.88	0.00
272	4.04	-0.02	0.00	+	+	NA	NA	NA	NA	+	NA	NA	10	2050.07	8.12	0.00
302	3.98	0.01	NA	+	+	NA	+	NA	NA	+	NA	NA	10	2050.21	8.26	0.00
176	3.87	0.04	0.00	+	+	NA	+	NA	+	NA	NA	NA	11	2050.37	8.42	0.00
144	4.07	-0.04	0.00	+	+	NA	NA	NA	+	NA	NA	NA	10	2050.58	8.63	0.00
1488	3.97	-0.02	0.00	+	+	NA	NA	+	+	+	NA	+	13	2050.60	8.65	0.00
480	4.02	-0.04	0.01	+	+	+	NA	+	+	+	NA	NA	13	2050.64	8.70	0.00
2048	4.10	-0.07	0.01	+	+	+	+	+	+	+	+	+	16	2050.64	8.70	0.00
432	3.89	0.05	0.00	+	+	NA	+	NA	+	+	NA	NA	12	2050.84	8.90	0.00
384	4.01	-0.05	0.01	+	+	+	+	+	NA	+	NA	NA	13	2050.91	8.96	0.00
48	4.06	-0.04	0.00	+	+	NA	+	NA	NA	NA	NA	NA	10	2051.11	9.16	0.00
512	3.85	0.03	0.00	+	+	+	+	+	+	+	NA	NA	14	2051.15	9.20	0.00
832	3.92	0.01	0.00	+	+	+	+	NA	NA	+	+	NA	13	2051.16	9.21	0.00
1520	3.82	0.04	0.00	+	+	NA	+	+	+	+	NA	+	14	2051.17	9.23	0.00
400	4.10	-0.04	0.00	+	+	NA	NA	NA	+	+	NA	NA	11	2051.21	9.26	0.00
960	3.77	0.07	0.00	+	+	+	+	NA	+	+	+	NA	14	2051.36	9.42	0.00
304	4.09	-0.04	0.00	+	+	NA	+	NA	NA	+	NA	NA	11	2051.83	9.89	0.00
896	4.08	-0.06	0.01	+	+	+	+	+	NA	+	+	NA	14	2052.03	10.09	0.00
1504	4.04	-0.05	0.01	+	+	+	NA	+	+	+	NA	+	14	2052.50	10.55	0.00
1024	3.91	0.01	0.00	+	+	+	+	+	+	+	+	NA	15	2052.79	10.84	0.00
1536	3.86	0.03	0.00	+	+	+	+	+	+	+	NA	+	15	2053.21	11.27	0.00

HEMOLYSIS SCORE

	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
4	2.20	0.02	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1767.08	0.00	0.19
3	2.30	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1767.34	0.26	0.16
40	2.23	0.02	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	1768.04	0.96	0.11
12	2.17	0.03	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	1768.97	1.89	0.07
39	2.36	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	1768.99	1.91	0.07
8	2.19	0.02	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	1769.13	2.04	0.07
7	2.31	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1769.36	2.28	0.06
56	2.26	0.02	+	+	NA	+	+	NA	NA	NA	NA	NA	10	1769.85	2.77	0.05
48	2.23	0.03	+	+	+	NA	+	NA	NA	NA	NA	NA	10	1770.09	3.01	0.04
2	2.11	0.02	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1770.43	3.35	0.03
1	2.22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1770.71	3.63	0.03
16	2.17	0.03	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	1771.02	3.94	0.03
24	2.21	0.02	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	1771.05	3.97	0.03
64	2.25	0.02	+	+	+	+	+	NA	NA	NA	NA	NA	11	1771.91	4.83	0.02
6	2.10	0.02	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1772.44	5.36	0.01
5	2.22	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1772.75	5.67	0.01
32	2.19	0.02	+	+	+	+	NA	NA	NA	NA	NA	NA	10	1772.98	5.89	0.01
128	2.22	0.03	+	+	+	+	+	+	NA	NA	NA	NA	12	1773.42	6.33	0.01
22	2.14	0.01	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	1774.08	7.00	0.01
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
3	2.30	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1767.34	0.00	0.48
39	2.36	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	1768.99	1.65	0.21
7	2.31	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1769.36	2.02	0.17
1	2.22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1770.71	3.37	0.09
5	2.22	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1772.75	5.41	0.03
4	2.28	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	1776.94	9.60	0.00
40	2.33	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	1777.80	10.45	0.00
8	2.28	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	1779.05	11.70	0.00
2	2.21	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	1779.20	11.86	0.00
12	2.21	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	1779.95	12.61	0.00
48	2.27	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	28	1780.63	13.28	0.00
6	2.20	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	1781.29	13.95	0.00
16	2.21	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	1782.12	14.78	0.00
64	2.32	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	1785.35	18.01	0.00
32	2.27	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	1785.98	18.64	0.00
56	2.39	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	1787.25	19.91	0.00
24	2.34	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	1788.32	20.98	0.00
22	2.28	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	1790.37	23.02	0.00
128	2.34	+	+	+	+	+	+	+	NA	NA	NA	NA	46	1796.50	29.16	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
12	**-1.52**	**0.38**	**+**	**NA**	**+**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**8**	**1761.33**	**0.00**	**0.41**
16	-1.52	0.38	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	1763.38	2.05	0.15
48	-1.38	0.37	+	+	+	NA	+	NA	NA	NA	NA	NA	10	1763.71	2.38	0.12
32	-1.31	0.36	+	+	+	+	NA	NA	NA	NA	NA	NA	10	1765.37	4.04	0.05
64	-0.99	0.33	+	+	+	+	+	NA	NA	NA	NA	NA	11	1765.55	4.22	0.05
128	-0.99	0.33	+	+	+	+	+	+	NA	NA	NA	NA	11	1765.55	4.22	0.05
4	0.42	0.19	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1765.83	4.50	0.04
3	2.30	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1767.34	6.01	0.02
40	0.44	0.19	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	1767.40	6.07	0.02
56	1.05	0.13	+	+	NA	+	+	NA	NA	NA	NA	NA	10	1767.78	6.45	0.02
8	0.41	0.19	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	1767.87	6.54	0.02
24	0.93	0.14	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	1768.68	7.35	0.01
2	0.21	0.20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1768.70	7.37	0.01
39	2.36	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	1768.99	7.66	0.01
7	2.31	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1769.36	8.03	0.01
1	2.22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1770.71	9.38	0.00
6	0.19	0.20	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1770.72	9.39	0.00
22	0.76	0.14	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	1771.28	9.95	0.00
5	2.22	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1772.75	11.42	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
7	2.24	NA	0.00	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1766.29	0.00	0.10
6	2.20	0.02	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	1767.08	0.79	0.06
271	2.28	NA	0.00	+	+	NA	NA	NA	NA	+	NA	NA	9	1767.27	0.97	0.06
5	2.30	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	1767.34	1.05	0.06
8	2.35	-0.05	0.01	+	NA	NA	NA	NA	NA	NA	NA	NA	8	1767.67	1.38	0.05
71	2.22	NA	0.00	+	NA	NA	NA	+	NA	NA	NA	NA	8	1768.03	1.74	0.04
270	2.23	0.02	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	1768.04	1.75	0.04
15	2.23	NA	0.00	+	+	NA	NA	NA	NA	NA	NA	NA	8	1768.32	2.03	0.03
399	2.30	NA	0.00	+	+	NA	NA	NA	+	+	NA	NA	10	1768.89	2.60	0.03
272	2.37	-0.04	0.00	+	+	NA	NA	NA	NA	+	NA	NA	10	1768.91	2.62	0.03
22	2.17	0.03	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	1768.97	2.68	0.03
269	2.36	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	1768.99	2.70	0.02
14	2.19	0.02	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	1769.13	2.83	0.02
335	2.27	NA	0.00	+	+	NA	NA	+	NA	+	NA	NA	10	1769.27	2.98	0.02
13	2.31	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	1769.36	3.07	0.02
72	2.33	-0.05	0.01	+	NA	NA	NA	+	NA	NA	NA	NA	9	1769.47	3.18	0.02
3	2.15	NA	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1769.57	3.28	0.02
24	2.33	-0.04	0.01	+	NA	+	NA	NA	NA	NA	NA	NA	9	1769.62	3.32	0.02
16	2.35	-0.05	0.01	+	+	NA	NA	NA	NA	NA	NA	NA	9	1769.71	3.41	0.02
302	2.26	0.02	NA	+	+	NA	+	NA	NA	+	NA	NA	10	1769.85	3.56	0.02
79	2.21	NA	0.00	+	+	NA	NA	+	NA	NA	NA	NA	9	1770.09	3.80	0.01
286	2.23	0.03	NA	+	+	+	NA	NA	NA	+	NA	NA	10	1770.09	3.80	0.01
143	2.24	NA	0.00	+	+	NA	NA	NA	+	NA	NA	NA	9	1770.14	3.85	0.01
400	2.41	-0.05	0.01	+	+	NA	NA	NA	+	+	NA	NA	11	1770.33	4.04	0.01
304	2.43	-0.05	0.01	+	+	NA	+	NA	NA	+	NA	NA	11	1770.41	4.12	0.01
2	2.11	0.02	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	1770.43	4.14	0.01
1	2.22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	1770.71	4.42	0.01
4	2.28	-0.05	0.01	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	1770.81	4.52	0.01
336	2.36	-0.04	0.01	+	+	NA	NA	+	NA	+	NA	NA	11	1770.93	4.64	0.01
463	2.29	NA	0.00	+	+	NA	NA	+	+	+	NA	NA	11	1770.94	4.65	0.01
288	2.37	-0.04	0.00	+	+	+	NA	NA	NA	+	NA	NA	11	1770.98	4.68	0.01
30	2.17	0.03	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	1771.02	4.73	0.01
46	2.21	0.02	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	1771.05	4.76	0.01
88	2.42	-0.08	0.01	+	NA	+	NA	+	NA	NA	NA	NA	10	1771.08	4.79	0.01
144	2.38	-0.06	0.01	+	+	NA	NA	NA	+	NA	NA	NA	10	1771.31	5.02	0.01
48	2.40	-0.06	0.01	+	+	NA	+	NA	NA	NA	NA	NA	10	1771.36	5.07	0.01
80	2.33	-0.05	0.01	+	+	NA	NA	+	NA	NA	NA	NA	10	1771.53	5.24	0.01
11	2.14	NA	0.00	NA	+	NA	NA	NA	NA	NA	NA	NA	7	1771.56	5.27	0.01
32	2.32	-0.04	0.01	+	+	+	NA	NA	NA	NA	NA	NA	10	1771.67	5.38	0.01
1487	2.27	NA	0.00	+	+	NA	NA	+	+	+	NA	+	12	1771.75	5.46	0.01
318	2.25	0.02	NA	+	+	+	+	NA	NA	+	NA	NA	11	1771.91	5.62	0.01
207	2.23	NA	0.00	+	+	NA	NA	+	+	NA	NA	NA	10	1771.97	5.68	0.01
432	2.40	-0.04	0.00	+	+	NA	+	NA	+	+	NA	NA	12	1772.39	6.10	0.00
464	2.41	-0.05	0.01	+	+	NA	NA	+	+	+	NA	NA	12	1772.40	6.11	0.00
416	2.42	-0.05	0.01	+	+	+	NA	NA	+	+	NA	NA	12	1772.40	6.11	0.00
10	2.10	0.02	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	1772.44	6.15	0.00
368	2.42	-0.05	0.01	+	+	NA	+	+	NA	+	NA	NA	12	1772.48	6.18	0.00
320	2.43	-0.05	0.01	+	+	+	+	NA	NA	+	NA	NA	12	1772.49	6.20	0.00
352	2.44	-0.07	0.01	+	+	+	NA	+	NA	+	NA	NA	12	1772.61	6.32	0.00
9	2.22	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	1772.75	6.46	0.00
12	2.27	-0.05	0.01	NA	+	NA	NA	NA	NA	NA	NA	NA	8	1772.80	6.51	0.00
1488	2.41	-0.06	0.01	+	+	NA	NA	+	+	+	NA	+	13	1772.86	6.57	0.00
62	2.19	0.02	NA	+	+	+	+	NA	NA	NA	NA	NA	10	1772.98	6.69	0.00
139	2.16	NA	0.00	NA	+	NA	NA	NA	+	NA	NA	NA	8	1773.08	6.79	0.00
96	2.42	-0.08	0.01	+	+	+	NA	+	NA	NA	NA	NA	11	1773.13	6.84	0.00
208	2.36	-0.05	0.01	+	+	NA	NA	+	+	NA	NA	NA	11	1773.23	6.93	0.00
112	2.38	-0.06	0.01	+	+	NA	+	+	NA	NA	NA	NA	11	1773.25	6.96	0.00
160	2.36	-0.05	0.01	+	+	+	NA	NA	+	NA	NA	NA	11	1773.32	7.03	0.00
64	2.38	-0.06	0.01	+	+	+	+	NA	NA	NA	NA	NA	11	1773.36	7.07	0.00
176	2.37	-0.05	0.01	+	+	NA	+	NA	+	NA	NA	NA	11	1773.37	7.08	0.00
830	2.22	0.03	NA	+	+	+	+	NA	NA	+	+	NA	12	1773.42	7.12	0.00
832	2.41	-0.05	0.01	+	+	+	+	NA	NA	+	+	NA	13	1773.73	7.44	0.00
44	2.35	-0.07	0.01	NA	+	NA	+	NA	NA	NA	NA	NA	9	1773.98	7.69	0.00
140	2.32	-0.06	0.01	NA	+	NA	NA	NA	+	NA	NA	NA	9	1773.98	7.69	0.00
42	2.14	0.01	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	1774.08	7.79	0.00
480	2.48	-0.07	0.01	+	+	+	NA	+	+	+	NA	NA	13	1774.18	7.89	0.00
384	2.49	-0.08	0.01	+	+	+	+	+	NA	+	NA	NA	13	1774.23	7.94	0.00
448	2.40	-0.04	0.00	+	+	+	+	NA	+	+	NA	NA	13	1774.46	8.17	0.00
496	2.40	-0.04	0.00	+	+	NA	+	+	+	+	NA	NA	13	1774.47	8.17	0.00
1520	2.37	-0.04	0.01	+	+	NA	+	+	+	+	NA	+	14	1774.78	8.49	0.00
1504	2.44	-0.07	0.01	+	+	+	NA	+	+	+	NA	+	14	1774.90	8.61	0.00
224	2.44	-0.08	0.01	+	+	+	NA	+	+	NA	NA	NA	12	1774.92	8.63	0.00
128	2.45	-0.09	0.01	+	+	+	+	+	NA	NA	NA	NA	12	1774.92	8.63	0.00
240	2.36	-0.05	0.01	+	+	NA	+	+	+	NA	NA	NA	12	1775.30	9.01	0.00
192	2.36	-0.05	0.01	+	+	+	+	NA	+	NA	NA	NA	12	1775.40	9.11	0.00
960	2.36	-0.03	0.00	+	+	+	+	NA	+	+	+	NA	14	1775.56	9.27	0.00
896	2.45	-0.07	0.01	+	+	+	+	+	NA	+	+	NA	14	1775.69	9.40	0.00
172	2.34	-0.07	0.01	NA	+	NA	+	NA	+	NA	NA	NA	10	1776.03	9.74	0.00
512	2.47	-0.07	0.01	+	+	+	+	+	+	+	NA	NA	14	1776.26	9.97	0.00
1536	2.39	-0.05	0.01	+	+	+	+	+	+	+	NA	+	15	1776.86	10.57	0.00
256	2.44	-0.09	0.01	+	+	+	+	+	+	NA	NA	NA	13	1777.00	10.71	0.00
2048	2.52	-0.10	0.01	+	+	+	+	+	+	+	+	+	16	1777.21	10.92	0.00
1024	2.40	-0.05	0.01	+	+	+	+	+	+	+	+	NA	15	1777.59	11.30	0.00

Table S6. Set of models fitted to assess senescence patterns of 4 parasitic traits. We tested the effect of population ("P"), sex ("S), 4 age functions (linear "AL", factor "AF", threshold "AT" and quadratic "I(AL^2)") with all two and three-way interactions between them. "I" is the Intercept, "df" is the number of parameters, "delta" is the difference of AIC between the candidate model and the model having the lowest AIC, and "weight" the AIC weight of each model. We selected the model with the lowest AIC, and when some models had very similar explanatory power (delta<2), we selected the one which included fewer terms (shown in bold).

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	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	D	weight
3	**1811.06**	**NA**	**+**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**6**	**10681.60**	**0.00**	**0.17**
1	2560.37	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	10681.95	0.36	0.14
7	2371.13	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	10682.31	0.71	0.12
5	3123.42	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	10682.62	1.02	0.10
12	2343.35	-90.07	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	10683.45	1.85	0.07
4	1969.59	-24.94	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	10683.55	1.95	0.06
2	2705.98	-22.52	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	10683.92	2.32	0.05
39	2112.87	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	10684.10	2.50	0.05
8	2535.34	-25.66	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	10684.27	2.67	0.05
16	2877.10	-89.39	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	10684.28	2.68	0.04
6	3275.03	-23.28	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	10684.58	2.98	0.04
40	2278.91	-26.48	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	10686.06	4.46	0.02
48	2652.07	-89.15	+	+	+	NA	+	NA	NA	NA	NA	NA	10	10686.15	4.55	0.02
24	2459.91	-10.33	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	10686.23	4.63	0.02
32	2850.06	-83.97	+	+	+	+	NA	NA	NA	NA	NA	NA	10	10686.34	4.74	0.02
22	3183.74	-5.93	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	10686.50	4.91	0.01
56	2200.22	-10.86	+	+	NA	+	+	NA	NA	NA	NA	NA	10	10688.02	6.42	0.01
64	2621.37	-83.21	+	+	+	+	+	NA	NA	NA	NA	NA	11	10688.22	6.62	0.01
128	2700.64	-99.13	+	+	+	+	+	+	NA	NA	NA	NA	12	10690.16	8.57	0.00
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
3	1811.06	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	10681.60	0.00	0.29
1	2560.37	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	10681.95	0.36	0.24
7	2371.13	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	10682.31	0.71	0.20
5	3123.42	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	10682.62	1.02	0.18
39	2112.87	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	10684.10	2.50	0.08
4	2184.63	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	10696.10	14.50	0.00
2	2932.06	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	10696.46	14.86	0.00
8	2729.48	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	10696.97	15.37	0.00
6	3480.50	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	10697.28	15.68	0.00
40	2479.55	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	10698.85	17.25	0.00
12	2635.16	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	10708.46	26.86	0.00
16	3160.29	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	10709.46	27.86	0.00
48	2948.43	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	10711.50	29.90	0.00
24	3081.15	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	10711.79	30.19	0.00
22	3820.39	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	10712.01	30.41	0.00
56	2814.96	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	10713.72	32.12	0.00
32	3517.49	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	10726.48	44.88	0.00
64	3293.00	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	10728.59	46.99	0.00
128	3608.61	+	+	+	+	+	+	+	NA	NA	NA	NA	46	10742.07	60.47	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT: P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
3	1811.06	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	10681.60	0.00	0.19
1	2560.37	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	10681.95	0.36	0.16
7	2371.13	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	10682.31	0.71	0.13
5	3123.42	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	10682.62	1.02	0.11
4	1573.95	24.86	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	10683.64	2.04	0.07
12	4545.80	-290.02	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	10683.91	2.31	0.06
2	2341.49	22.99	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	10683.99	2.39	0.06
39	2112.87	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	10684.10	2.50	0.05
16	5156.73	-296.13	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	10684.66	3.06	0.04
6	2973.73	15.64	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	10684.66	3.07	0.04
24	1134.95	135.11	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	10685.70	4.10	0.02
22	1904.69	133.05	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	10685.99	4.40	0.02
48	4890.73	-292.75	+	+	+	NA	+	NA	NA	NA	NA	NA	10	10686.51	4.91	0.02
32	4576.05	-233.27	+	+	+	+	NA	NA	NA	NA	NA	NA	10	10686.69	5.09	0.01
56	859.46	136.15	+	+	NA	+	+	NA	NA	NA	NA	NA	10	10687.49	5.89	0.01
64	4244.86	-223.39	+	+	+	+	+	NA	NA	NA	NA	NA	11	10688.54	6.94	0.01
128	4173.13	-215.71	+	+	+	+	+	+	NA	NA	NA	NA	12	10690.63	9.03	0.00
8	-806.68	166.04	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	10708.52	26.92	0.00
40	-1264.69	164.54	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	10709.85	28.25	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
5	1811.06	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	10681.60	0.00	0.10
1	2560.37	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	10681.95	0.36	0.08
13	2371.13	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	10682.31	0.71	0.07
9	3123.42	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	10682.62	1.02	0.06
71	2112.37	NA	-6.73	+	NA	NA	NA	+	NA	NA	NA	NA	8	10683.27	1.67	0.04
22	2343.35	-90.07	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	10683.45	1.85	0.04
6	1969.59	-24.94	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	10683.55	1.95	0.04
7	1841.60	NA	-0.58	+	NA	NA	NA	NA	NA	NA	NA	NA	7	10683.64	2.05	0.04
2	2705.98	-22.52	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	10683.92	2.32	0.03
3	2582.86	NA	-0.42	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	10683.99	2.40	0.03
79	2651.70	NA	-6.77	+	+	NA	NA	+	NA	NA	NA	NA	9	10684.09	2.49	0.03
269	2112.87	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	10684.10	2.50	0.03
14	2535.34	-25.66	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	10684.27	2.67	0.03
30	2877.10	-89.39	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	10684.28	2.68	0.03
15	2410.33	NA	-0.72	+	+	NA	NA	NA	NA	NA	NA	NA	8	10684.36	2.76	0.03
10	3275.03	-23.28	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	10684.58	2.98	0.02
11	3154.79	NA	-0.57	NA	+	NA	NA	NA	NA	NA	NA	NA	7	10684.66	3.06	0.02
72	2295.09	-72.19	-1.48	+	NA	NA	NA	+	NA	NA	NA	NA	9	10685.20	3.60	0.02
8	2110.13	-107.47	7.26	+	NA	NA	NA	NA	NA	NA	NA	NA	8	10685.39	3.79	0.02
24	2440.96	-150.00	5.37	+	NA	+	NA	NA	NA	NA	NA	NA	9	10685.40	3.80	0.01
4	2844.48	-102.11	7.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	10685.76	4.16	0.01
143	2345.85	NA	1.51	+	+	NA	NA	NA	+	NA	NA	NA	9	10685.96	4.37	0.01
335	2434.43	NA	-6.73	+	+	NA	NA	+	NA	+	NA	NA	10	10685.97	4.37	0.01
80	2820.02	-67.89	-1.82	+	+	NA	NA	+	NA	NA	NA	NA	10	10686.04	4.45	0.01
270	2278.91	-26.48	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	10686.06	4.46	0.01
207	2614.62	NA	-5.59	+	+	NA	NA	+	+	NA	NA	NA	10	10686.09	4.50	0.01
16	2659.09	-102.13	6.73	+	+	NA	NA	NA	NA	NA	NA	NA	9	10686.14	4.54	0.01
286	2652.07	-89.15	NA	+	+	+	NA	NA	NA	+	NA	NA	10	10686.15	4.55	0.01
271	2154.20	NA	-0.78	+	+	NA	NA	NA	NA	+	NA	NA	9	10686.16	4.56	0.01
139	3077.85	NA	1.77	NA	+	NA	NA	NA	+	NA	NA	NA	8	10686.21	4.61	0.01
46	2459.91	-10.33	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	10686.23	4.63	0.01
32	2962.02	-144.11	4.91	+	+	+	NA	NA	NA	NA	NA	NA	10	10686.25	4.65	0.01
62	2850.06	-83.97	NA	+	+	+	+	NA	NA	NA	NA	NA	10	10686.34	4.74	0.01
12	3396.82	-96.79	6.47	NA	+	NA	NA	NA	NA	NA	NA	NA	8	10686.46	4.87	0.01
42	3183.74	-5.93	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	10686.50	4.91	0.01
88	2317.61	-82.36	-0.66	+	NA	+	NA	+	NA	NA	NA	NA	10	10687.27	5.67	0.01
399	2083.93	NA	1.48	+	+	NA	NA	NA	+	+	NA	NA	10	10687.76	6.16	0.00
144	2556.01	-83.16	7.33	+	+	NA	NA	NA	+	NA	NA	NA	10	10687.86	6.26	0.00
336	2603.01	-69.62	-1.64	+	+	NA	NA	+	NA	+	NA	NA	11	10687.93	6.33	0.00
272	2402.36	-103.28	6.76	+	+	NA	NA	NA	NA	+	NA	NA	10	10687.94	6.34	0.00
302	2200.22	-10.86	NA	+	+	NA	+	NA	NA	+	NA	NA	10	10688.02	6.42	0.00
208	2774.24	-62.06	-1.25	+	+	NA	NA	+	+	NA	NA	NA	11	10688.08	6.48	0.00
140	3277.24	-76.59	7.11	NA	+	NA	NA	NA	+	NA	NA	NA	9	10688.12	6.53	0.00
288	2736.59	-144.51	4.96	+	+	+	NA	NA	NA	+	NA	NA	11	10688.13	6.53	0.00
96	2837.40	-75.86	-1.18	+	+	+	NA	+	NA	NA	NA	NA	11	10688.13	6.53	0.00
112	2829.22	-70.16	-1.77	+	+	NA	+	+	NA	NA	NA	NA	11	10688.13	6.53	0.00
48	2587.03	-81.82	5.99	+	+	NA	+	NA	NA	NA	NA	NA	10	10688.16	6.56	0.00
318	2621.37	-83.21	NA	+	+	+	+	NA	NA	+	NA	NA	11	10688.22	6.62	0.00
64	2947.96	-140.37	4.79	+	+	+	+	NA	NA	NA	NA	NA	11	10688.33	6.73	0.00
44	3305.88	-72.64	5.59	NA	+	NA	+	NA	NA	NA	NA	NA	9	10688.44	6.85	0.00
176	2923.79	-233.40	18.79	+	+	NA	+	NA	+	NA	NA	NA	11	10689.01	7.41	0.00
172	3639.52	-221.07	18.14	NA	+	NA	+	NA	+	NA	NA	NA	10	10689.34	7.74	0.00
192	3179.11	-255.78	15.22	+	+	+	+	NA	+	NA	NA	NA	12	10689.64	8.04	0.00
400	2294.19	-84.08	7.37	+	+	NA	NA	NA	+	+	NA	NA	11	10689.65	8.06	0.00
240	3030.93	-177.72	8.27	+	+	NA	+	+	+	NA	NA	NA	12	10689.66	8.06	0.00
304	2327.23	-82.46	6.00	+	+	NA	+	NA	NA	+	NA	NA	11	10689.96	8.36	0.00
464	2550.93	-63.45	-1.03	+	+	NA	NA	+	+	+	NA	NA	12	10689.97	8.37	0.00
352	2621.00	-77.95	-0.97	+	+	+	NA	+	NA	+	NA	NA	12	10690.02	8.42	0.00
1487	2409.13	NA	-5.98	+	+	NA	NA	+	+	+	NA	+	12	10690.05	8.45	0.00
416	2657.02	-131.29	5.40	+	+	+	NA	NA	+	+	NA	NA	12	10690.10	8.50	0.00
830	2700.64	-99.13	NA	+	+	+	+	NA	NA	+	+	NA	12	10690.16	8.57	0.00
224	2800.86	-75.26	-0.12	+	+	+	NA	+	+	NA	NA	NA	12	10690.17	8.57	0.00
320	2719.14	-139.99	4.82	+	+	+	+	NA	NA	+	NA	NA	12	10690.22	8.62	0.00
128	2844.46	-77.45	-1.16	+	+	+	+	+	NA	NA	NA	NA	12	10690.22	8.62	0.00
432	2660.91	-234.93	18.87	+	+	NA	+	NA	+	+	NA	NA	12	10690.81	9.21	0.00
448	2944.45	-256.66	15.37	+	+	+	+	NA	+	+	NA	NA	13	10691.52	9.92	0.00
496	2804.29	-180.56	8.61	+	+	NA	+	+	+	+	NA	NA	13	10691.54	9.94	0.00
256	3116.41	-219.39	11.82	+	+	+	+	+	+	NA	NA	NA	13	10691.70	10.11	0.00
480	2578.43	-77.34	0.16	+	+	+	NA	+	+	+	NA	NA	13	10692.06	10.46	0.00
1488	2553.53	-60.17	-1.56	+	+	NA	NA	+	+	+	NA	+	13	10692.06	10.47	0.00
384	2625.20	-78.86	-0.96	+	+	+	+	+	NA	+	NA	NA	13	10692.12	10.52	0.00
832	2769.92	-142.87	3.87	+	+	+	+	NA	NA	+	+	NA	13	10692.21	10.61	0.00
960	3023.82	-266.84	14.70	+	+	+	+	NA	+	+	+	NA	14	10693.44	11.84	0.00
512	2890.49	-223.42	12.26	+	+	+	+	+	+	+	NA	NA	14	10693.59	12.00	0.00
1520	2821.56	-177.80	7.77	+	+	NA	+	+	+	+	NA	+	14	10693.61	12.01	0.00
1504	2591.04	-77.35	-0.18	+	+	+	NA	+	+	+	NA	+	14	10694.16	12.56	0.00
896	2674.27	-88.89	-0.90	+	+	+	+	+	NA	+	+	NA	14	10694.18	12.58	0.00
1024	3003.27	-255.65	13.71	+	+	+	+	+	+	+	+	NA	15	10695.55	13.95	0.00
1536	2948.94	-235.59	12.39	+	+	+	+	+	+	+	NA	+	15	10695.63	14.03	0.00
2048	3062.76	-288.10	16.60	+	+	+	+	+	+	+	+	+	16	10697.58	15.98	0.00
463	226.54	NA	1.17	+	+	NA	NA	+	+	+	NA	NA	11	10713.08	31.49	0.00
160	1017.47	-142.82	11.59	+	+	+	NA	NA	+	NA	NA	NA	11	10713.42	31.82	0.00

368	570.09	-119.22	8.43	+	+	NA	+	+	NA	+	NA	NA	12	10714.88	33.28	0.00

PROTOSTRONGYLIDS ABUNDANCE

	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
40	-0.40	0.35	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	2925.97	0.00	0.19
6	-0.51	0.33	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2926.31	0.34	0.16
2	-0.26	0.33	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2926.79	0.82	0.12
56	-0.15	0.31	+	+	NA	+	+	NA	NA	NA	NA	NA	10	2927.21	1.24	0.10
22	-0.31	0.30	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	2927.85	1.88	0.07
48	-0.55	0.37	+	+	+	NA	+	NA	NA	NA	NA	NA	10	2927.92	1.95	0.07
8	-0.55	0.33	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	2928.18	2.21	0.06
4	-0.28	0.33	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	2928.78	2.81	0.05
128	-0.61	0.39	+	+	+	+	+	+	NA	NA	NA	NA	12	2929.20	3.22	0.04
64	-0.24	0.32	+	+	+	+	+	NA	NA	NA	NA	NA	11	2929.27	3.29	0.04
24	-0.34	0.29	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	2929.65	3.68	0.03
16	-0.83	0.37	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	2929.76	3.79	0.03
12	-0.65	0.39	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	2930.09	4.12	0.02
32	-0.60	0.33	+	+	+	+	NA	NA	NA	NA	NA	NA	10	2931.40	5.43	0.01
5	1.77	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2932.73	6.76	0.01
1	2.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2933.19	7.21	0.01
39	1.91	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	2933.19	7.22	0.01
7	1.64	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2934.21	8.24	0.00
3	1.92	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2934.92	8.95	0.00
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
128	0.23	+	+	+	+	+	+	+	NA	NA	NA	NA	46	2926.74	0.00	0.65
40	0.02	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	2930.90	4.16	0.08
6	-0.23	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	2931.27	4.53	0.07
5	1.77	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2932.73	5.99	0.03
2	0.13	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	2933.12	6.38	0.03
1	2.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2933.19	6.44	0.03
39	1.91	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	2933.19	6.45	0.03
8	-0.27	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	2933.37	6.62	0.02
7	1.64	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2934.21	7.47	0.02
3	1.92	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2934.92	8.18	0.01
48	0.03	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	2934.96	8.22	0.01
4	0.13	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	2935.26	8.51	0.01
16	-0.21	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	2935.53	8.79	0.01
12	0.18	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	2937.39	10.65	0.00
56	0.41	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	2940.74	14.00	0.00
22	0.18	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	2940.81	14.07	0.00
64	0.42	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	2941.20	14.46	0.00
32	0.17	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	2941.46	14.72	0.00
24	0.13	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	2942.92	16.17	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
128	**-24.67**	**2.76**	**+**	**+**	**+**	**+**	**+**	**+**	**NA**	**NA**	**NA**	**NA**	**12**	**2892.40**	**0.00**	**1.00**
48	-20.27	2.29	+	+	+	NA	+	NA	NA	NA	NA	NA	10	2920.04	27.64	0.00
16	-21.00	2.34	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	2920.75	28.35	0.00
64	-22.30	2.50	+	+	+	+	+	NA	NA	NA	NA	NA	11	2921.23	28.83	0.00
32	-23.42	2.60	+	+	+	+	NA	NA	NA	NA	NA	NA	10	2921.43	29.03	0.00
12	-19.98	2.26	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	2921.50	29.10	0.00
40	-14.02	1.61	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	2924.96	32.56	0.00
6	-13.97	1.58	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2925.38	32.98	0.00
2	-12.84	1.49	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2926.30	33.89	0.00
56	-14.06	1.62	+	+	NA	+	+	NA	NA	NA	NA	NA	10	2927.04	34.64	0.00
8	-14.13	1.59	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	2927.28	34.88	0.00
22	-14.30	1.61	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	2927.39	34.99	0.00
4	-12.90	1.49	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	2928.31	35.91	0.00
24	-14.41	1.62	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	2929.31	36.91	0.00
5	1.77	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2932.73	40.33	0.00
1	2.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2933.19	40.78	0.00
39	1.91	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	2933.19	40.79	0.00
7	1.64	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2934.21	41.81	0.00
3	1.92	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2934.92	42.52	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
271	0.17	NA	0.03	+	+	NA	NA	NA	NA	+	NA	NA	9	2924.60	0.00	0.06
11	0.02	NA	0.03	NA	+	NA	NA	NA	NA	NA	NA	NA	7	2924.99	0.39	0.05
352	1.64	-0.84	0.10	+	+	+	NA	+	NA	+	NA	NA	12	2925.52	0.92	0.04
1488	0.58	-0.50	0.08	+	+	NA	NA	+	+	+	NA	+	13	2925.69	1.09	0.03
3	0.32	NA	0.03	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2925.90	1.30	0.03
1487	-0.23	NA	0.04	+	+	NA	NA	+	+	+	NA	+	12	2925.92	1.32	0.03
399	0.29	NA	0.03	+	+	NA	NA	NA	+	+	NA	NA	10	2925.97	1.37	0.03
270	-0.40	0.35	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	2925.97	1.37	0.03
335	-0.41	NA	0.04	+	+	NA	NA	+	NA	+	NA	NA	10	2926.05	1.45	0.03
512	2.82	-1.34	0.14	+	+	+	+	+	+	+	NA	NA	14	2926.28	1.68	0.02
10	-0.51	0.33	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	2926.31	1.71	0.02
1504	1.31	-0.79	0.10	+	+	+	NA	+	+	+	NA	+	14	2926.46	1.86	0.02
1520	1.23	-0.76	0.10	+	+	NA	+	+	+	+	NA	+	14	2926.50	1.90	0.02
96	1.46	-0.90	0.10	+	+	+	NA	+	NA	NA	NA	NA	11	2926.57	1.97	0.02
1536	2.33	-1.20	0.13	+	+	+	+	+	+	+	NA	+	15	2926.57	1.97	0.02
139	0.11	NA	0.03	NA	+	NA	NA	NA	+	NA	NA	NA	8	2926.64	2.04	0.02
272	0.31	-0.07	0.04	+	+	NA	NA	NA	NA	+	NA	NA	10	2926.65	2.05	0.02
336	0.49	-0.37	0.06	+	+	NA	NA	+	NA	+	NA	NA	11	2926.65	2.05	0.02
2	-0.26	0.33	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	2926.79	2.19	0.02
15	-0.03	NA	0.03	+	+	NA	NA	NA	NA	NA	NA	NA	8	2926.85	2.25	0.02
256	2.65	-1.40	0.15	+	+	+	+	+	+	NA	NA	NA	13	2926.95	2.35	0.02
12	0.20	-0.08	0.04	NA	+	NA	NA	NA	NA	NA	NA	NA	8	2927.00	2.40	0.02
1024	2.31	-1.18	0.13	+	+	+	+	+	+	+	+	NA	15	2927.20	2.60	0.02
896	1.34	-0.77	0.10	+	+	+	+	+	NA	+	+	NA	14	2927.21	2.61	0.02
302	-0.15	0.31	NA	+	+	NA	+	NA	NA	+	NA	NA	10	2927.21	2.61	0.02
88	1.63	-0.82	0.10	+	NA	+	NA	+	NA	NA	NA	NA	10	2927.47	2.87	0.01
384	1.81	-0.88	0.10	+	+	+	+	+	NA	+	NA	NA	13	2927.47	2.87	0.01
304	0.73	-0.18	0.04	+	+	NA	+	NA	NA	+	NA	NA	11	2927.49	2.89	0.01
480	1.63	-0.84	0.10	+	+	+	NA	+	+	+	NA	NA	13	2927.62	3.02	0.01
79	-0.76	NA	0.04	+	+	NA	NA	+	NA	NA	NA	NA	9	2927.64	3.04	0.01
832	0.38	-0.36	0.07	+	+	+	+	NA	NA	+	+	NA	13	2927.70	3.10	0.01
2048	2.79	-1.38	0.15	+	+	+	+	+	+	+	+	+	16	2927.84	3.24	0.01
42	-0.31	0.30	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	2927.85	3.25	0.01
7	0.30	NA	0.03	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2927.89	3.29	0.01
286	-0.55	0.37	NA	+	+	+	NA	NA	NA	+	NA	NA	10	2927.92	3.32	0.01
4	0.33	0.00	0.03	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	2927.96	3.36	0.01
400	0.52	-0.11	0.04	+	+	NA	NA	NA	+	+	NA	NA	11	2927.98	3.37	0.01
71	-0.11	NA	0.04	+	NA	NA	NA	+	NA	NA	NA	NA	8	2928.11	3.51	0.01
463	-0.44	NA	0.04	+	+	NA	NA	+	+	+	NA	NA	11	2928.12	3.51	0.01
14	-0.55	0.33	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	2928.18	3.58	0.01
72	0.52	-0.38	0.06	+	NA	NA	NA	+	NA	NA	NA	NA	9	2928.23	3.62	0.01
44	0.55	-0.18	0.04	NA	+	NA	+	NA	NA	NA	NA	NA	9	2928.23	3.63	0.01
496	1.44	-0.76	0.09	+	+	NA	+	+	+	+	NA	NA	13	2928.27	3.67	0.01
288	0.35	-0.28	0.05	+	+	+	NA	NA	NA	+	NA	NA	11	2928.40	3.80	0.01
143	0.06	NA	0.03	+	+	NA	NA	NA	+	NA	NA	NA	9	2928.45	3.85	0.01
224	1.38	-0.88	0.11	+	+	+	NA	+	+	NA	NA	NA	12	2928.60	4.00	0.01
140	0.37	-0.11	0.04	NA	+	NA	NA	NA	+	NA	NA	NA	9	2928.62	4.02	0.01
128	1.54	-0.92	0.11	+	+	+	+	+	NA	NA	NA	NA	12	2928.63	4.02	0.01
432	1.22	-0.38	0.06	+	+	NA	+	NA	+	+	NA	NA	12	2928.77	4.17	0.01
6	-0.28	0.33	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	2928.78	4.18	0.01
960	0.86	-0.56	0.08	+	+	+	+	NA	+	+	+	NA	14	2928.83	4.23	0.01
16	0.16	-0.09	0.04	+	+	NA	NA	NA	NA	NA	NA	NA	9	2928.86	4.26	0.01
368	0.56	-0.21	0.05	+	+	NA	+	+	NA	+	NA	NA	12	2929.14	4.54	0.01
830	-0.61	0.39	NA	+	+	+	+	NA	NA	+	+	NA	12	2929.20	4.59	0.01
240	1.20	-0.80	0.10	+	+	NA	+	+	+	NA	NA	NA	12	2929.23	4.63	0.01
318	-0.24	0.32	NA	+	+	+	+	NA	NA	+	NA	NA	11	2929.27	4.66	0.01
80	-0.04	-0.17	0.05	+	+	NA	NA	+	NA	NA	NA	NA	10	2929.38	4.78	0.01
172	1.06	-0.38	0.06	NA	+	NA	+	NA	+	NA	NA	NA	10	2929.44	4.83	0.01
207	-0.82	NA	0.04	+	+	NA	NA	+	+	NA	NA	NA	10	2929.56	4.96	0.00
464	0.36	-0.14	0.04	+	+	NA	NA	+	+	+	NA	NA	12	2929.60	5.00	0.00
46	-0.34	0.29	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	2929.65	5.05	0.00
30	-0.83	0.37	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	2929.76	5.16	0.00
8	0.31	0.00	0.03	+	NA	NA	NA	NA	NA	NA	NA	NA	8	2929.96	5.36	0.00
48	0.52	-0.19	0.04	+	+	NA	+	NA	NA	NA	NA	NA	10	2929.99	5.39	0.00
22	-0.65	0.39	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	2930.09	5.49	0.00
320	0.66	-0.36	0.06	+	+	+	+	NA	NA	+	NA	NA	12	2930.12	5.52	0.00
32	-0.11	-0.08	0.04	+	+	+	NA	NA	NA	NA	NA	NA	10	2930.15	5.55	0.00
448	1.29	-0.64	0.08	+	+	+	+	NA	+	+	NA	NA	13	2930.33	5.72	0.00
144	0.33	-0.12	0.04	+	+	NA	NA	NA	+	NA	NA	NA	10	2930.42	5.82	0.00
416	0.45	-0.30	0.05	+	+	+	NA	NA	+	+	NA	NA	12	2930.43	5.83	0.00
112	0.27	-0.23	0.05	+	+	NA	+	+	NA	NA	NA	NA	11	2930.89	6.29	0.00
24	-0.01	0.00	0.03	+	NA	+	NA	NA	NA	NA	NA	NA	9	2930.94	6.34	0.00
176	1.04	-0.40	0.06	+	+	NA	+	NA	+	NA	NA	NA	11	2931.16	6.56	0.00
208	0.09	-0.18	0.05	+	+	NA	NA	+	+	NA	NA	NA	11	2931.26	6.66	0.00
62	-0.60	0.33	NA	+	+	+	+	NA	NA	NA	NA	NA	10	2931.40	6.80	0.00
64	0.27	-0.36	0.06	+	+	+	+	NA	NA	NA	NA	NA	11	2931.96	7.36	0.00
160	0.09	-0.30	0.06	+	+	+	NA	NA	+	NA	NA	NA	11	2932.14	7.54	0.00
192	0.78	-0.41	0.06	+	+	+	+	NA	+	NA	NA	NA	12	2932.45	7.85	0.00
9	1.77	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	2932.73	8.13	0.00
1	2.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	2933.19	8.59	0.00
269	1.91	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	2933.19	8.59	0.00
13	1.64	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	2934.21	9.61	0.00
5	1.92	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	2934.92	10.32	0.00

TRICHURIS SP. ABUNDANCE

	I	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
128	-9.33	2.87	+	+	+	+	+	+	NA	NA	NA	NA	12	6920.35	0.00	0.65
64	7.41	-0.22	+	+	+	+	+	NA	NA	NA	NA	NA	11	6922.09	1.73	0.27
48	-20.43	4.85	+	+	+	NA	+	NA	NA	NA	NA	NA	10	6926.29	5.94	0.03
32	-13.49	0.74	+	+	+	+	NA	NA	NA	NA	NA	NA	10	6927.50	7.15	0.02
56	-31.77	6.89	+	+	NA	+	+	NA	NA	NA	NA	NA	10	6929.15	8.79	0.01
16	-36.07	5.12	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	6930.19	9.84	0.00
40	-51.84	10.54	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	6931.50	11.15	0.00
24	-42.90	6.63	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	6931.71	11.35	0.00
8	-60.23	9.96	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	6933.26	12.91	0.00
12	-13.21	4.84	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	6938.45	18.09	0.00
4	-35.43	9.02	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	6939.82	19.47	0.00
39	11.96	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	6941.67	21.32	0.00
7	1.89	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	6942.40	22.04	0.00
3	18.50	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	6947.08	26.72	0.00
6	-11.34	9.44	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	6968.65	48.30	0.00
22	8.49	5.99	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	6968.66	48.31	0.00
2	7.34	8.75	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	6971.55	51.19	0.00
5	44.40	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	6973.10	52.74	0.00
1	58.44	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	6975.46	55.10	0.00
	I	**AF**	**P**	**S**	**AF:P**	**AF:S**	**P:S**	**AF:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
128	5.75	+	+	+	+	+	+	+	NA	NA	NA	NA	46	6935.50	0.00	0.65
40	-27.14	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	6939.44	3.94	0.09
48	-1.60	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	28	6940.44	4.93	0.06
8	-36.18	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	6940.78	5.28	0.05
64	19.01	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	6941.06	5.56	0.04
56	-6.78	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	6941.42	5.92	0.03
39	11.96	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	6941.67	6.17	0.03
7	1.89	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	6942.40	6.89	0.02
16	-13.60	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	6943.06	7.56	0.01
24	-17.73	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	6943.56	8.06	0.01
32	3.98	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	6945.39	9.89	0.00
3	18.50	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	6947.08	11.57	0.00
4	-15.45	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	6947.45	11.94	0.00
12	5.13	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	6950.19	14.69	0.00
6	3.88	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	6972.81	37.31	0.00
5	44.40	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	6973.10	37.59	0.00
22	21.55	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	6975.45	39.95	0.00
1	58.44	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	6975.46	39.95	0.00
2	20.42	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	6975.87	40.37	0.00
	I	**AT**	**P**	**S**	**AT:P**	**AT:S**	**P:S**	**AT:P:S**	**NA**	**NA**	**NA**	**NA**	**df**	**AIC**	**delta**	**weight**
128	**-20.85**	**4.19**	**+**	**+**	**+**	**+**	**+**	**+**	**NA**	**NA**	**NA**	**NA**	**12**	**6911.47**	**0.00**	**0.97**
64	18.73	-1.94	+	+	+	+	+	NA	NA	NA	NA	NA	11	6918.79	7.32	0.03
32	-8.36	-0.14	+	+	+	+	NA	NA	NA	NA	NA	NA	10	6924.02	12.54	0.00
48	-41.64	7.32	+	+	+	NA	+	NA	NA	NA	NA	NA	10	6926.69	15.22	0.00
56	-55.63	9.46	+	+	NA	+	+	NA	NA	NA	NA	NA	10	6927.23	15.76	0.00
24	-66.32	9.30	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	6929.22	17.74	0.00
16	-59.39	8.01	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	6929.90	18.42	0.00
40	-92.48	15.11	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	6931.45	19.97	0.00
8	-99.66	14.57	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	6932.64	21.17	0.00
12	-32.43	7.11	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	6938.25	26.78	0.00
4	-69.73	13.02	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	6939.65	28.18	0.00
39	11.96	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	6941.67	30.20	0.00
7	1.89	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	6942.40	30.92	0.00
3	18.50	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	6947.08	35.60	0.00
22	-12.86	8.21	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	6966.46	54.98	0.00
6	-56.26	14.76	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	6968.20	56.73	0.00
2	-31.60	13.33	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	6971.35	59.87	0.00
5	44.40	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	6973.10	61.62	0.00
1	58.44	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	6975.46	63.98	0.00
	I	**AL**	**I(AL^2)**	**P**	**S**	**AL:P**	**AL:S**	**I(AL^2):P**	**I(AL^2):S**	**P:S**	**AL:P:S**	**I(AL^2):P:S**	**df**	**AIC**	**delta**	**weight**
1487	-2.74	NA	0.23	+	+	NA	NA	+	+	+	NA	+	12	6914.86	0.00	0.24
1520	-36.13	13.03	-0.73	+	+	NA	+	+	+	+	NA	+	14	6915.11	0.25	0.21
2048	7.93	-4.14	0.54	+	+	+	+	+	+	+	+	+	16	6915.38	0.51	0.19
1488	4.21	-2.70	0.43	+	+	NA	NA	+	+	+	NA	+	13	6916.90	2.04	0.09
1536	-27.52	9.69	-0.49	+	+	+	+	+	+	+	NA	+	15	6917.13	2.26	0.08
960	-24.44	9.25	-0.50	+	+	+	+	NA	+	+	+	NA	14	6918.00	3.14	0.05
1504	19.07	-8.46	0.86	+	+	+	NA	+	+	+	NA	+	14	6918.58	3.72	0.04
1024	-30.58	11.77	-0.70	+	+	+	+	+	+	+	+	NA	15	6920.06	5.19	0.02
830	-9.33	2.87	NA	+	+	+	+	NA	NA	+	+	NA	12	6920.35	5.49	0.02
463	8.93	NA	-0.07	+	+	NA	NA	+	+	+	NA	NA	11	6920.92	6.06	0.01
832	17.18	-8.05	0.85	+	+	+	+	NA	NA	+	+	NA	13	6921.44	6.58	0.01
416	21.13	-4.65	0.27	+	+	+	NA	NA	+	+	NA	NA	12	6921.51	6.65	0.01
318	7.41	-0.22	NA	+	+	+	+	NA	NA	+	NA	NA	11	6922.09	7.23	0.01
448	-3.05	5.11	-0.48	+	+	+	+	NA	+	+	NA	NA	13	6922.14	7.28	0.01
464	3.41	2.10	-0.22	+	+	NA	NA	+	+	+	NA	NA	12	6922.97	8.11	0.00
480	34.09	-10.38	0.74	+	+	+	NA	+	+	+	NA	NA	13	6923.15	8.29	0.00
496	-22.17	12.52	-1.03	+	+	NA	+	+	+	+	NA	NA	13	6923.46	8.60	0.00
896	22.88	-10.39	1.03	+	+	+	+	+	NA	+	+	NA	14	6923.48	8.61	0.00
320	27.92	-8.49	0.63	+	+	+	+	NA	NA	+	NA	NA	12	6923.62	8.75	0.00
512	8.54	0.03	-0.06	+	+	+	+	+	+	+	NA	NA	14	6923.97	9.10	0.00
384	43.37	-15.25	1.18	+	+	+	+	+	NA	+	NA	NA	13	6925.06	10.20	0.00
368	10.45	-1.97	0.17	+	+	NA	+	+	NA	+	NA	NA	12	6925.17	10.31	0.00
286	-20.43	4.85	NA	+	+	+	NA	NA	NA	+	NA	NA	10	6926.29	11.43	0.00
207	-10.45	NA	0.02	+	+	NA	NA	+	+	NA	NA	NA	10	6926.47	11.61	0.00
160	2.70	-4.87	0.36	+	+	+	NA	NA	+	NA	NA	NA	11	6927.20	12.33	0.00
62	-13.49	0.74	NA	+	+	+	+	NA	NA	NA	NA	NA	10	6927.50	12.64	0.00
399	-13.12	NA	0.48	+	+	NA	NA	NA	+	+	NA	NA	10	6927.89	13.03	0.00
192	-21.54	4.89	-0.38	+	+	+	+	NA	+	NA	NA	NA	12	6927.91	13.05	0.00
288	-16.92	3.35	0.12	+	+	+	NA	NA	NA	+	NA	NA	11	6928.35	13.49	0.00
224	20.39	-12.49	0.99	+	+	+	NA	+	+	NA	NA	NA	12	6928.49	13.63	0.00
208	-12.59	0.80	-0.03	+	+	NA	NA	+	+	NA	NA	NA	11	6928.55	13.69	0.00
335	-10.15	NA	0.41	+	+	NA	NA	+	NA	+	NA	NA	10	6928.61	13.75	0.00
64	8.92	-8.24	0.68	+	+	+	+	NA	NA	NA	NA	NA	11	6928.93	14.06	0.00
302	-31.77	6.89	NA	+	+	NA	+	NA	NA	+	NA	NA	10	6929.15	14.28	0.00
240	-38.12	11.20	-0.84	+	+	NA	+	+	+	NA	NA	NA	12	6929.16	14.29	0.00
352	5.48	-6.13	0.87	+	+	+	NA	+	NA	+	NA	NA	12	6929.30	14.43	0.00
256	-4.54	-2.40	0.21	+	+	+	+	+	+	NA	NA	NA	13	6929.46	14.60	0.00
400	-27.52	5.63	0.07	+	+	NA	NA	NA	+	+	NA	NA	11	6929.70	14.83	0.00
336	-33.05	9.14	-0.28	+	+	NA	NA	+	NA	+	NA	NA	11	6929.93	15.07	0.00
128	28.81	-16.72	1.37	+	+	+	+	+	NA	NA	NA	NA	12	6930.00	15.14	0.00
30	-36.07	5.12	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	6930.19	15.33	0.00
143	-24.98	NA	0.47	+	+	NA	NA	NA	+	NA	NA	NA	9	6930.32	15.46	0.00
112	-6.15	-2.75	0.31	+	+	NA	+	+	NA	NA	NA	NA	11	6930.33	15.47	0.00
432	-47.83	13.62	-0.53	+	+	NA	+	NA	+	+	NA	NA	12	6930.90	16.04	0.00
304	-20.25	2.12	0.37	+	+	NA	+	NA	NA	+	NA	NA	11	6931.04	16.17	0.00
270	-51.84	10.54	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	6931.50	16.64	0.00
46	-42.90	6.63	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	6931.71	16.84	0.00
79	-24.84	NA	0.44	+	+	NA	NA	+	NA	NA	NA	NA	9	6931.98	17.12	0.00
32	-29.48	2.35	0.22	+	+	+	NA	NA	NA	NA	NA	NA	10	6932.20	17.33	0.00
144	-35.27	3.98	0.18	+	+	NA	NA	NA	+	NA	NA	NA	10	6932.26	17.40	0.00
271	-25.82	NA	0.81	+	+	NA	NA	NA	NA	+	NA	NA	9	6932.58	17.72	0.00
96	-3.43	-8.47	1.08	+	+	+	NA	+	NA	NA	NA	NA	11	6932.78	17.92	0.00
14	-60.23	9.96	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	6933.26	18.40	0.00
176	-56.70	12.45	-0.46	+	+	NA	+	NA	+	NA	NA	NA	11	6933.38	18.52	0.00
48	-28.71	0.80	0.45	+	+	NA	+	NA	NA	NA	NA	NA	10	6933.49	18.63	0.00
80	-43.35	7.26	-0.11	+	+	NA	NA	+	NA	NA	NA	NA	10	6933.58	18.71	0.00
272	-52.67	10.90	-0.03	+	+	NA	NA	NA	NA	+	NA	NA	10	6933.58	18.72	0.00
15	-35.42	NA	0.77	+	+	NA	NA	NA	NA	NA	NA	NA	8	6934.00	19.14	0.00
16	-58.08	9.04	0.07	+	+	NA	NA	NA	NA	NA	NA	NA	9	6935.33	20.46	0.00
22	-13.21	4.84	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	6938.45	23.59	0.00
6	-35.43	9.02	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	6939.82	24.96	0.00
71	-2.29	NA	0.41	+	NA	NA	NA	+	NA	NA	NA	NA	8	6940.16	25.29	0.00
24	-13.48	4.95	-0.01	+	NA	+	NA	NA	NA	NA	NA	NA	9	6940.52	25.65	0.00
7	-12.52	NA	0.69	+	NA	NA	NA	NA	NA	NA	NA	NA	7	6940.75	25.88	0.00
88	10.55	-4.98	0.78	+	NA	+	NA	+	NA	NA	NA	NA	10	6941.37	26.51	0.00
72	-25.42	9.00	-0.27	+	NA	NA	NA	+	NA	NA	NA	NA	9	6941.51	26.64	0.00
269	11.96	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	6941.67	26.81	0.00
8	-38.50	10.31	-0.11	+	NA	NA	NA	NA	NA	NA	NA	NA	8	6941.86	27.00	0.00
13	1.89	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	6942.40	27.53	0.00
5	18.50	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	6947.08	32.22	0.00
139	27.00	NA	0.36	NA	+	NA	NA	NA	+	NA	NA	NA	8	6967.84	52.98	0.00
172	-36.70	26.26	-1.67	NA	+	NA	+	NA	+	NA	NA	NA	10	6968.36	53.49	0.00
10	-11.34	9.44	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	6968.65	53.79	0.00
42	8.49	5.99	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	6968.66	53.80	0.00
140	1.34	10.76	-0.47	NA	+	NA	NA	NA	+	NA	NA	NA	9	6969.15	54.29	0.00
11	9.48	NA	0.77	NA	+	NA	NA	NA	NA	NA	NA	NA	7	6969.65	54.79	0.00
12	-18.91	13.31	-0.34	NA	+	NA	NA	NA	NA	NA	NA	NA	8	6970.62	55.75	0.00
44	3.50	8.37	-0.20	NA	+	NA	+	NA	NA	NA	NA	NA	9	6970.69	55.83	0.00
2	7.34	8.75	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	6971.55	56.68	0.00
3	27.55	NA	0.70	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	6972.58	57.72	0.00
9	44.40	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	6973.10	58.23	0.00
4	-3.77	14.35	-0.49	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	6973.40	58.54	0.00
1	58.44	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	6975.46	60.59	0.00

GATTRO-INTESTINAL STRONGYLES ABUNDANCE

	l	AL	P	S	AL:P	AL:S	P:S	AL:P:S	NA	NA	NA	NA	df	AIC	delta	weight
6	2.25	4.62	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	5983.54	0.00	0.19
16	6.01	3.03	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	5984.04	0.50	0.15
8	-1.69	4.70	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	5984.90	1.37	0.09
48	2.10	3.03	+	+	+	NA	+	NA	NA	NA	NA	NA	10	5984.95	1.42	0.09
40	-5.56	4.52	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	5985.29	1.75	0.08
2	11.39	4.26	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	5985.39	1.85	0.07
22	2.99	4.49	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	5985.58	2.04	0.07
32	8.41	2.56	+	+	+	+	NA	NA	NA	NA	NA	NA	10	5985.90	2.37	0.06
12	15.45	2.87	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	5986.83	3.29	0.04
64	4.31	2.61	+	+	+	+	+	NA	NA	NA	NA	NA	11	5986.87	3.33	0.04
4	8.10	4.31	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	5986.90	3.37	0.03
24	-0.69	4.50	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	5986.92	3.39	0.03
56	-4.72	4.36	+	+	NA	+	+	NA	NA	NA	NA	NA	10	5987.34	3.80	0.03
128	7.43	2.02	+	+	+	+	+	+	NA	NA	NA	NA	12	5988.39	4.85	0.02
5	29.13	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	5989.97	6.44	0.01
1	35.32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	5991.03	7.50	0.00
39	20.82	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	5991.31	7.77	0.00
7	26.85	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	5991.73	8.19	0.00
3	33.28	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	5992.83	9.30	0.00
	l	AF	P	S	AF:P	AF:S	P:S	AF:P:S	NA	NA	NA	NA	df	AIC	delta	weight
6	17.95	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	16	5981.87	0.00	0.29
16	23.27	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	27	5983.03	1.15	0.16
8	13.98	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	17	5983.17	1.30	0.15
40	9.40	+	+	+	NA	NA	+	NA	NA	NA	NA	NA	18	5983.49	1.62	0.13
48	18.79	+	+	+	+	NA	+	NA	NA	NA	NA	NA	28	5983.65	1.78	0.12
2	27.02	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	5984.66	2.79	0.07
4	23.67	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	5986.15	4.27	0.03
12	32.48	+	+	NA	+	NA	NA	NA	NA	NA	NA	NA	26	5986.25	4.38	0.03
5	29.13	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	5989.97	8.10	0.01
1	35.32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	5991.03	9.16	0.00
39	20.82	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	5991.31	9.44	0.00
7	26.85	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	5991.73	9.86	0.00
22	19.24	+	NA	+	NA	+	NA	NA	NA	NA	NA	NA	26	5991.90	10.02	0.00
3	33.28	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	5992.83	10.96	0.00
24	14.87	+	+	+	NA	+	NA	NA	NA	NA	NA	NA	27	5992.86	10.98	0.00
56	11.09	+	+	+	NA	+	+	NA	NA	NA	NA	NA	28	5993.79	11.91	0.00
32	24.95	+	+	+	+	+	NA	NA	NA	NA	NA	NA	37	5999.25	17.37	0.00
64	21.30	+	+	+	+	+	+	NA	NA	NA	NA	NA	38	6000.53	18.65	0.00
128	22.64	+	+	+	+	+	+	+	NA	NA	NA	NA	46	6011.27	29.39	0.00
	l	AT	P	S	AT:P	AT:S	P:S	AT:P:S	NA	NA	NA	NA	df	AIC	delta	weight
6	-40.44	9.57	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	5977.06	0.00	0.21
22	-29.66	8.03	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	5977.80	0.73	0.14
8	-44.34	9.64	+	+	NA	NA	NA	NA	NA	NA	NA	NA	8	5978.45	1.38	0.10
40	-48.48	9.47	+	+	NA	NA	+	NA	NA	NA	NA	NA	9	5978.54	1.48	0.10
24	-33.37	8.03	+	+	NA	+	NA	NA	NA	NA	NA	NA	9	5979.05	1.99	0.08
56	-37.81	7.93	+	+	NA	+	+	NA	NA	NA	NA	NA	10	5979.26	2.20	0.07
2	-28.32	8.92	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	5979.37	2.31	0.07
32	-17.81	5.70	+	+	+	+	NA	NA	NA	NA	NA	NA	10	5980.06	3.00	0.05
16	-37.26	8.57	+	+	+	NA	NA	NA	NA	NA	NA	NA	9	5980.14	3.08	0.04
48	-43.36	8.74	+	+	+	NA	+	NA	NA	NA	NA	NA	10	5980.44	3.38	0.04
64	-24.72	6.05	+	+	+	+	+	NA	NA	NA	NA	NA	11	5980.66	3.60	0.03
4	-31.56	8.97	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	5980.90	3.84	0.03
128	-16.74	4.90	+	+	+	+	+	+	NA	NA	NA	NA	12	5981.71	4.65	0.02
12	-26.54	8.23	+	NA	+	NA	NA	NA	NA	NA	NA	NA	8	5982.79	5.73	0.01
5	29.13	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	5989.97	12.91	0.00
1	35.32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	5991.03	13.97	0.00
39	20.82	NA	+	+	NA	NA	+	NA	NA	NA	NA	NA	8	5991.31	14.25	0.00
7	26.85	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	7	5991.73	14.67	0.00
3	33.28	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	5992.83	15.77	0.00
	l	AL	l(AL^2)	P	S	AL:P	AL:S	l(AL^2):P	l(AL^2):S	P:S	AL:P:S	l(AL^2):P:S	df	AIC	delta	weight
96	**45.63**	**-15.30**	**1.55**	**+**	**+**	**+**	**NA**	**+**	**NA**	**NA**	**NA**	**NA**	**11**	**5974.56**	**0.00**	**0.09**
352	41.66	-15.71	1.58	+	+	+	NA	+	NA	+	NA	NA	12	5974.94	0.38	0.08
224	49.41	-15.63	1.48	+	+	+	NA	+	+	NA	NA	NA	12	5975.40	0.84	0.06
480	45.36	-15.98	1.52	+	+	+	NA	+	+	+	NA	NA	13	5976.01	1.45	0.05
128	49.93	-16.33	1.57	+	+	+	+	+	NA	NA	NA	NA	12	5976.07	1.51	0.04
256	39.82	-11.38	1.13	+	+	+	+	+	+	NA	NA	NA	13	5976.25	1.70	0.04
384	45.63	-16.61	1.60	+	+	+	+	+	NA	+	NA	NA	13	5976.58	2.02	0.03
192	26.17	-4.86	0.56	+	+	+	+	NA	+	NA	NA	NA	12	5976.96	2.40	0.03
512	36.40	-11.92	1.18	+	+	+	+	+	+	+	NA	NA	14	5976.98	2.42	0.03
12	25.62	-6.35	0.93	NA	+	NA	NA	NA	NA	NA	NA	NA	8	5977.05	2.49	0.03
160	36.85	-9.49	0.93	+	+	+	NA	NA	+	NA	NA	NA	11	5977.10	2.54	0.03
1504	47.25	-16.01	1.47	+	+	+	NA	+	+	+	NA	+	14	5977.11	2.56	0.03
32	29.53	-8.05	0.94	+	+	+	NA	NA	NA	NA	NA	NA	10	5977.39	2.83	0.02
11	9.96	NA	0.45	NA	+	NA	NA	NA	NA	NA	NA	NA	7	5977.58	3.03	0.02
140	30.28	-7.16	0.92	NA	+	NA	NA	NA	+	NA	NA	NA	9	5977.90	3.34	0.02
448	22.50	-4.99	0.58	+	+	+	+	NA	+	+	NA	NA	13	5978.01	3.45	0.02
416	32.81	-9.54	0.94	+	+	+	NA	NA	+	+	NA	NA	12	5978.06	3.51	0.02
896	48.79	-17.30	1.61	+	+	+	+	+	NA	+	+	NA	14	5978.09	3.54	0.02
288	25.48	-8.17	0.95	+	+	+	NA	NA	NA	+	NA	NA	11	5978.12	3.56	0.02
88	54.56	-14.97	1.51	+	NA	+	NA	+	NA	NA	NA	NA	10	5978.13	3.57	0.02
172	19.85	-2.90	0.59	NA	+	NA	+	NA	+	NA	NA	NA	10	5978.19	3.64	0.02
16	21.63	-6.35	0.94	+	+	NA	NA	NA	NA	NA	NA	NA	9	5978.29	3.73	0.01
64	36.46	-9.92	1.01	+	+	+	+	NA	NA	NA	NA	NA	11	5978.36	3.81	0.01
272	17.67	-6.67	0.95	+	+	NA	NA	NA	NA	+	NA	NA	10	5978.48	3.92	0.01
1536	39.42	-12.62	1.20	+	+	+	+	+	+	+	NA	+	15	5978.49	3.93	0.01
44	29.88	-7.44	0.98	NA	+	NA	+	NA	NA	NA	NA	NA	9	5978.65	4.09	0.01
15	5.98	NA	0.45	+	+	NA	NA	NA	NA	NA	NA	NA	8	5978.82	4.26	0.01
1024	39.34	-12.78	1.23	+	+	+	+	+	+	+	+	NA	15	5978.82	4.27	0.01
144	26.22	-7.23	0.93	+	+	NA	NA	NA	+	NA	NA	NA	10	5978.94	4.38	0.01
240	19.80	-2.65	0.42	+	+	NA	+	+	+	NA	NA	NA	12	5978.97	4.41	0.01
139	11.84	NA	0.40	NA	+	NA	NA	NA	+	NA	NA	NA	8	5979.03	4.47	0.01
208	30.76	-7.52	0.82	+	+	NA	NA	+	+	NA	NA	NA	11	5979.18	4.62	0.01
320	32.20	-9.92	1.01	+	+	+	+	NA	NA	+	NA	NA	12	5979.23	4.68	0.01
400	22.19	-7.50	0.94	+	+	NA	NA	NA	+	+	NA	NA	11	5979.26	4.71	0.01
271	1.58	NA	0.44	+	+	NA	NA	NA	NA	+	NA	NA	9	5979.27	4.71	0.01
80	24.09	-6.37	0.86	+	+	NA	NA	+	NA	NA	NA	NA	10	5979.28	4.73	0.01
176	16.35	-3.10	0.61	+	+	NA	+	NA	+	NA	NA	NA	11	5979.35	4.79	0.01
4	34.53	-6.33	0.90	NA	NA	NA	NA	NA	NA	NA	NA	NA	7	5979.54	4.98	0.01
432	12.51	-3.41	0.63	+	+	NA	+	NA	+	+	NA	NA	12	5979.72	5.16	0.01
48	26.19	-7.61	0.99	+	+	NA	+	NA	NA	NA	NA	NA	10	5979.76	5.20	0.01
79	8.36	NA	0.37	+	+	NA	NA	+	NA	NA	NA	NA	9	5979.82	5.27	0.01
336	20.08	-6.66	0.88	+	+	NA	NA	+	NA	+	NA	NA	11	5979.83	5.27	0.01
2048	33.61	-10.20	1.02	+	+	+	+	+	+	+	+	+	16	5979.90	5.35	0.01
496	16.26	-2.97	0.46	+	+	NA	+	+	+	+	NA	NA	13	5979.91	5.35	0.01
464	26.71	-7.71	0.85	+	+	NA	NA	+	+	+	NA	NA	12	5980.00	5.44	0.01
304	22.02	-7.84	1.00	+	+	NA	+	NA	NA	+	NA	NA	11	5980.03	5.47	0.01
3	18.91	NA	0.42	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	5980.06	5.50	0.01
960	22.56	-5.00	0.58	+	+	+	+	NA	+	+	+	NA	14	5980.12	5.56	0.01
143	7.72	NA	0.40	+	+	NA	NA	NA	+	NA	NA	NA	9	5980.12	5.57	0.01
112	30.38	-8.00	0.91	+	+	NA	+	+	NA	NA	NA	NA	11	5980.39	5.84	0.01
335	4.02	NA	0.38	+	+	NA	NA	+	NA	+	NA	NA	10	5980.60	6.04	0.00
207	11.30	NA	0.28	+	+	NA	NA	+	+	NA	NA	NA	10	5980.60	6.04	0.00
399	3.35	NA	0.39	+	+	NA	NA	NA	+	+	NA	NA	10	5980.68	6.13	0.00
24	38.48	-7.74	0.90	+	NA	+	NA	NA	NA	NA	NA	NA	9	5980.92	6.37	0.00
8	31.18	-6.32	0.90	+	NA	NA	NA	NA	NA	NA	NA	NA	8	5980.97	6.41	0.00
368	26.08	-8.15	0.93	+	+	NA	+	+	NA	+	NA	NA	12	5981.09	6.54	0.00
832	32.83	-9.97	1.00	+	+	+	+	NA	NA	+	+	NA	13	5981.27	6.72	0.00
7	15.56	NA	0.42	+	NA	NA	NA	NA	NA	NA	NA	NA	7	5981.48	6.92	0.00
463	7.08	NA	0.29	+	+	NA	NA	+	+	+	NA	NA	11	5981.59	7.04	0.00
1488	26.69	-7.42	0.81	+	+	NA	NA	+	+	+	NA	+	13	5981.97	7.41	0.00
1520	16.39	-2.94	0.45	+	+	NA	+	+	+	+	NA	+	14	5982.01	7.45	0.00
72	33.37	-6.34	0.84	+	NA	NA	NA	+	NA	NA	NA	NA	9	5982.42	7.87	0.00
71	17.71	NA	0.36	+	NA	NA	NA	+	NA	NA	NA	NA	8	5982.93	8.37	0.00
1487	8.55	NA	0.25	+	+	NA	NA	+	+	+	NA	+	12	5983.14	8.58	0.00
10	2.25	4.62	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	7	5983.54	8.98	0.00
30	6.01	3.03	NA	+	+	+	NA	NA	NA	NA	NA	NA	9	5984.04	9.48	0.00
14	-1.69	4.70	NA	+	+	NA	NA	NA	NA	NA	NA	NA	8	5984.90	10.35	0.00
286	2.10	3.03	NA	+	+	+	NA	NA	NA	+	NA	NA	10	5984.95	10.40	0.00
270	-5.56	4.52	NA	+	+	NA	NA	NA	NA	+	NA	NA	9	5985.29	10.73	0.00
2	11.39	4.26	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	6	5985.39	10.83	0.00
42	2.99	4.49	NA	NA	+	NA	+	NA	NA	NA	NA	NA	8	5985.58	11.02	0.00
62	8.41	2.56	NA	+	+	+	+	NA	NA	NA	NA	NA	10	5985.90	11.35	0.00
22	15.45	2.87	NA	+	NA	+	NA	NA	NA	NA	NA	NA	8	5986.83	12.27	0.00
318	4.31	2.61	NA	+	+	+	+	NA	NA	+	NA	NA	11	5986.87	12.32	0.00
6	8.10	4.31	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	7	5986.90	12.35	0.00
46	-0.69	4.50	NA	+	+	NA	+	NA	NA	NA	NA	NA	9	5986.92	12.37	0.00
302	-4.72	4.36	NA	+	+	NA	+	NA	NA	+	NA	NA	10	5987.34	12.78	0.00
830	7.43	2.02	NA	+	+	+	+	NA	NA	+	+	NA	12	5988.39	13.83	0.00
9	29.13	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	6	5989.97	15.42	0.00
1	35.32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5	5991.03	16.48	0.00
269	20.82	NA	NA	+	+	NA	NA	NA	NA	+	NA	NA	8	5991.31	16.75	0.00
13	26.85	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	7	5991.73	17.18	0.00
5	33.28	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	6	5992.83	18.28	0.00

Table S7. Linear mixed effect models selected for 12 immune parameters and 4 parasitic traits, analysed separately according to sex and roe deer populations (TF: Trois-Fontaines, CH: Chizé). The effect of different age functions (factor, linear, threshold, quadratic) was tested. All models included individual identity, the year of capture and the cohort of individuals as random effects; and were tested with and without body mass (BM) as a covariate. When the age threshold model was selected, "Age of change" represents the age at which the parameter begins to vary, and the "Parameter estimate" of the age function is the slope of the variation with age after the threshold age. Statistical significance of age or body mass function are represented by * for p=0.05, ** for p=0.01 and *** for p=0.001. R2m and R2c are the marginal and conditional variance of the model, respectively. Values are presented ± Standard Error.

					AGE FUNCTION			BODY MASS					
IMMUNE TRAIT	Site	Sex	Best model selected	Age of change	Parameter estimate ±SE	t-value		Parameter estimate ±SE	t-value		r^2m	r^2c	Predicted value at 2 years
INNATE TRAITS													
Neutrophil count	TF	♂	constant	-	-	-	-	-	-	-	0.00	0.51	6.25 ±0.29
		♀	age factor	-	-	-	-	-	-	-	0.10	0.76	6.52 ±0.15
	CH	♂	constant	-	-	-	-	-	-	-	0.00	0.52	5.34 ±0.34
		♀	age threshold	8	0.43 ±0.14	3.00	**	-	-	-	0.05	0.27	5.14 ±0.18
Monocyte count	TF	♂	constant	-	-	-	-	-	-	-	0.00	0.46	0.30 ±0.10
		♀	constant	-	-	-	-	-	-	-	0.00	0.45	0.31 ±0.10
	CH	♂	constant	-	-	-	-	-			0.00	0.35	0.29 ±0.07
		♀	constant	-	-	-	-	-	-	-	0.00	0.59	0.30 ±0.10
Eosinophil count	TF	♂	constant	-	-	-	-	-	-	-	0.00	0.21	0.07 ±0.01
		♀	constant	-	-	-	-	-	-	-	0.00	0.14	0.12 ±0.02
	CH	♂	constant	-	-			-			0.00	0.08	0.10 ±0.02
		♀	constant	-	-	-	-	-	-	-	0.00	0.14	0.11 ±0.01
Basophil count	TF	♂	constant	-	-	-	-	-	-	-	0.00	0.41	0.07 ±0.02
		♀	constant	-	-	-	-	-	-	-	0.00	0.28	0.07 ±0.02
	CH	♂	BM	-	-	-	-	0.01 ±0.003	2.56	*	0.04	0.22	0.07 ±0.02
		♀	age threshold	5	0.01 ±0.004	2.16	*	-	-	-	0.03	0.19	0.08 ±0.01
Hemagglutination	TF	♂	constant	-	-	-	-	-	-	-	0.00	0.31	4.03 ±0.33
		♀	constant	-	-	-	-	-	-	-	0.00	0.36	4.03 ±0.25
	CH	♂	constant	-	-	-	-	-	-	-	0.00	0.54	4.26 ±0.35
		♀	age threshold	10	-0.43 ±0.20	-2.17	*	-	-	-	0.02	0.57	3.98 ±0.36
Hemolysis	TF	♂	constant	-	-	-	-	-	-	-	0.00	0.51	2.24 ±0.41
		♀	age factor	-	-	-	-	-	-	-	0.07	0.62	2.35 ±0.37
	CH	♂	constant	-	-	-	-	-	-	-	0.00	0.76	2.10 ±0.40
		♀	constant	-	-	-	-	-	-	-	0.00	0.62	1.97 ±0.38
INFLAMMATORY MARKERS													
Beta-globulin	TF	♂	age linear	2	0.32 ±0.08	4.17	**	-	-	-	0.09	0.81	6.20 ±0.54
		♀	age linear + BM	2	0.27 ±0.03	7.92	***	-0.14 ±0.04	-2.98	**	0.19	0.41	5.73 ±0.36
	CH	♂	age linear	2	0.20 ±0.10	2.06	*	-	-	-	0.03	0.25	7.12 ±0.51
		♀	age linear	2	0.19 ±0.05	4.00	***	-	-	-	0.08	0.44	6.41 ±0.41

Alpha-1-globulin	TF	♂	BM	-	-	-	-	-0.06 ±0.02	-3.14	**	0.04	0.55	3.35 ±0.20
		♀	age linear + BM	2	0.04 ±0.01	3.39	***	-0.07 ±0.02	-3.62	***	0.06	0.55	3.11 ±0.19
	CH	♂	constant	-	-	-	-	-	-	-	0.00	0.40	3.24 ±0.16
		♀	age linear	2	0.03 ±0.02	1.84	*	-	-	-	0.02	0.43	2.94 ±0.15
Alpha-2-globulin	TF	♂	constant	-	-	-	-	-	-	-	0.00	0.30	5.63 ±0.30
		♀	constant	-	-	-	-	-	-	-	0.00	0.26	5.84 ±0.34
	CH	♂	constant	-	-	-	-	-	-	-	0.00	0.29	5.66 ±0.33
		♀	constant	-	-	-	-	-	-	-	0.00	0.36	5.88 ±0.29
Haptoglobin	TF	♂	age threshold	9	0.43 ±0.12	3.46	***	-	-	-	0.07	0.52	0.34 ±0.10
		♀	age threshold	9	0.47 ±0.07	7.04	***	-	-	-	0.21	0.24	0.15 ±0.06
	CH	♂	constant	-	-	-	-	-	-	-	0.00	0.05	0.69 ±0.14
		♀	age factor	-	-	-	-	-	-	-	0.14	0.16	0.30 ±0.11
ADAPTIVE TRAITS													
Gamma-globulin	TF	♂	age linear + BM	2	0.43 ±0.11	3.88	***	-0.35 ±0.12	-2.98	**	0.10	0.49	13.86 ±0.90
		♀	age linear + BM	2	0.56 ±0.10	5.71	***	-0.29 ±0.12	-2.30	*	0.15	0.55	13.54 ±0.93
	CH	♂	constant	-	-	-	-	-	-	-	0.00	0.74	18.55 ±1.66
		♀	age linear	2	0.42 ±0.12	3.37	***	-	-	-	0.04	0.70	18.43 ±1.57
Lymphocyte count	TF	♂	constant	-	-	-	-	-	-	-	0.00	0.41	2.25 ±0.14
		♀	BM	-	-	-	-	-0.07 ±0.03	-2.20	*	0.03	0.38	2.42 ±0.13
	CH	♂	age linear	2	-0.07 ±0.03	-2.20	*	-	-	-	0.03	0.24	1.90 ±0.17
		♀	age linear	2	-0.06 ±0.02	-2.66	**	-	-	-	0.04	0.37	1.89 ±0.15

					AGE FUNCTION			BODY MASS					
PARASITIC TRAIT	Site	Sex	Best model selected	Age of change	Parameter estimate ±SE	t-value		Parameter estimate ±SE	t-value		r^2m	r^2c	Predicted value at 2 years
Gastro-intestinal strongyles	TF	♂	age threshold + BM	9	43.82 ±10.70	4.10	***	-6.74 ±2.40	-2.81	**	0.15	0.63	34.99 ±10.81
		♀	age threshold	11	42.47 ±13.22	3.21	**	-	-	-	0.08	0.39	15.05 ±4.93
	CH	♂	constant	-	-	-	-	-	-	-	0.00	0.33	34.48 ±10.61
		♀	age linear + BM	2	6.54 ±2.91	2.25	*	-4.70 ±2.35	-2.00	*	0.03	0.95	22.01 ±16.35
***Trichuris* sp.**	TF	♂	age threshold + BM	9	52.44 ±6.65	7.88	***	-2.98 ±1.31	-2.27	*	0.37	0.45	7.97 ±4.75
		♀	age threshold + BM	11	59.99 ±12.21	4.91	***	-3.52 ±1.26	-2.80	**	0.21	0.7	7.35 ±3.40
	CH	♂	age threshold	6	107.34 ±26.74	4.01	***	-	-	-	0.15	0.19	81.42 ±32.81
		♀	constant	-	-	-	-	-	-	-	0.00	0.53	86.40 ±29.43
Protostrongylids	TF	♂	age factor + BM	-	-	-	-	-0.35 ±0.14	-2.55	*	0.17	0.83	0.17 ±0.02
		♀	age threshold	11	10.17 ±1.57	6.47	***	-	-	-	0.23	0.23	0.41 ±0.37
	CH	♂	constant	-	-	-	-	-	-	-	0.00	0.00	1.40 ±0.74
		♀	constant	-	-	-	-	-	-	-	0.00	0.06	0.38 ±0.18
Coccidia	TF	♂	constant	-	-	-	-	-	-	-	0.00	0.99	233.38 ±194.70
		♀	constant	-	-	-	-	-	-	-	0.00	0.00	244.10 ±202.30
	CH	♂	constant	-	-	-	-	-	-	-	0.00	0.25	26.07 ±13.75
		♀	constant	-	-	-	-	-	-	-	0.00	0.96	27.48 ±8.86

Table S8. Linear mixed models describing the relationships between the 12 immune traits and the 4 parasitic traits of the study. Each immune trait was analysed as a function of parasite load, population and the interaction parasite load*population, considering 4 different parasite groups. Models included individual identity as a random effect. The "estimate" for the population or the parasitic trait is the slope of the variation. Statistical significance is represented by . for p=0.1, * for p=0.05, ** for p=0.01 and *** for p=0.001. Values are presented ± 1 SE.

GASTRO-INTESTINAL STRONGYLES	POPULATION			GI STRONGYLES			POPULATION*GI STRONGYLES		
	estimate ±SE	t-value	p	estimate ±SE	t-value	p	estimate ±SE	t-value	p
Neutrophil count	-1.08 ±0.27	-3.89	***	-6.47 E-06 ±0.002	-0.003	-	-0.001 ±0.003	-0.44	-
Monocyte count	-0.03 ±0.03	-0.95	-	9.04 E-05 ±3.22 E-04	0.28	-	-3.82 E-05 ±4.00 E-04	-0.10	-
Basophil count	0.001 ±0.01	0.09	-	3.79 E-05 ±1.12 E-04	0.34	-	6.08 E-05 ±1.39 E-04	0.44	-
Eosinophil count	0.001 ±0.01	0.09	-	-3.14 E-04 ±1.38 E-04	-2.28	*	2.99 E-04 ±1.72 E-04	1.74	-
Lymphocyte count	-0.74 ±0.10	-7.62	***	-7.22 E-04 ±8.78 E-04	-0.82	-	-2.59 E-04 ±0.001	-0.24	-
Alpha1-globulin	-0.04 ±0.06	-0.71	-	0.001 ±0.001	2.35	*	-0.002 ±0.0007	-2.09	*
Alpha2-globulin	0.03 ±0.14	0.8	-	0.001 ±0.001	0.55	-	-0.002 ±0.002	-0.98	-
Beta-globulin	0.89 ±0.21	4.25	***	0.01 ±0.001	2.79	**	-0.004 ±0.002	-1.71	.
Gamma-globulin	4.84 ±0.47	10.26	***	0.01 ±0.004	1.36	-	-0.01 ±0.01	-1.53	-
Haptoglobin	0.16 ±0.1	1.63	-	0.002 ±0.001	2.09	*	-0.001 ±0.001	-0.73	-
Hemagglutination	0.14 ±0.12	1.17	-	2.95 E-04 ±1.19 E-03	0.25	-	-1.62 E-03 ±1.48 E-03	-1.10	-
Hemolysis	-0.11 ±0.10	-1.16	-	-3.49 E-04 ±9.65 E-04	-0.36	-	-8.30 E-05 ±1.19 E-03	-0.07	-

TRICHURIS SP.	POPULATION			*TRICHURIS SP.*			POPULATION**TRICHURIS SP.*		
	estimates ±SE	t-value	p	estimates ±SE	t-value	p	estimates ±SE	t-value	p
Neutrophil count	-0.97 ±0.27	-3.59	***	0.003 ±0.003	1.13	-	-0.005 ±0.003	-1.46	-
Monocyte count	-0.04 ±0.03	-1.15	-	-0.001 ±0.0004	-1.27	-	0.0005 ±0.0005	1.18	-
Basophil count	0.001 ±0.01	0.11	-	2.02 E-05 ±1.56 E-04	0.13	-	5.53 E-06 ±1.60 E-04	0.04	-
Eosinophil count	0.01 ±0.01	0.84	-	3.67 E-05 ±1.94 E-04	0.19	-	-5.18 E-05 ±1.99 E-04	-0.26	-
Lymphocyte count	-0.69 ±0.10	-7.30	***	-3.34 E-05 ±1.22 E-03	-0.03	-	-6.40 E-04 ±1.25 E-03	-0.51	-
Alpha1-globulin	-0.03 ±0.06	-0.53	-	0.002 ±0.0001	2.34	*	-0.002 ±0.0008	-2.67	**
Alpha2-globulin	-0.01 ±0.14	-0.09	-	-0.002 ±0.002	-1.04	-	-0.002 ±0.002	0.86	-
Beta-globulin	0.93 ±0.21	4.45	***	0.01 ±0.003	3.55	***	-0.01 ±0.003	-3.67	***
Gamma-globulin	4.54 ±0.46	9.78	***	0.01 ±0.01	2.28	*	-0.01 ±0.01	-1.86	.
Haptoglobin	0.21 ±0.09	2.24	*	0.01 ±0.001	4.82	***	-0.01 ±0.001	-4.90	***
Hemagglutination	0.14 ±0.12	1.2	-	0.002 ±0.002	1.05	-	-0.002 ±0.002	-1.22	-
Hemolysis	-0.18 ±0.09	-1.97	*	-0.001 ±0.001	-0.75	-	0.002 ±0.001	1.2	-

PROTOSTRONGYLIDS	POPULATION			PROTOSTRONGYLIDS			POPULATION*PROTOSTRONGYLIDS		
	estimates ±SE	t-value	p	estimates ±SE	t-value	p	estimates ±SE	t-value	p
Neutrophil count	-1,10 ±0,26	-4,16	***	0,01 ±0,03	0,26	-	-0,02 ±0,04	-0,40	-
Monocyte count	-0,03 ±0,03	-0,87	-	0,003 ±0,005	0,81	-	-0,01 ±0,01	-0,90	-
Basophil count	0,002 ±0,02	3,83	**	-8,42 E04 ±0,002	-0,52	-	7,19 E04 ±0,002	0,33	-
Eosinophil count	0,008 ±0,014	0,56	-	-0,002 ±0,002	-0,83	-	0,003 ±0,003	1,01	-
Lymphocyte count	-0,76 ±0,10	-8,31	***	-0,02 ±0,01	-1,84	.	0,02 ±0,02	0,93	-
Alpha1-globulin	-0,10 ±0,06	-1,62	-	-0,002 ±0,008	-0,27	-	0,01 ±0,01	1,08	-
Alpha2-globulin	-0,02 ±0,13	-0,14	-	-0,02 ±0,02	-1,19	-	0,003 ±0,03	0,12	-
Beta-globulin	0,76 ±0,20	3,79	***	0,004 ±0,003	0,14	-	0,04 ±0,04	1,02	-
Gamma-globulin	4,55 ±0,45	10,1	***	-0,05 ±0,06	-0,72	-	0,08 ±0,08	0,99	-
Haptoglobin	0,14 ±0,09	1,61	-	0,01 ±0,01	0,41	-	0,002 ±0,02	0,12	-
Hemagglutination	0,11 ±0,11	1,03	-	0,001 ±0,017	0,06	-	-0,03 ±0,02	-1,34	-
Hemolysis	-0,12 ±0,09	-1,34	-	-0,02 ±0,01	-1,14	-	0,01 ±0,02	0,42	-

COCCIDIA	POPULATION			COCCIDIA			POPULATION*COCCIDIA		
	estimates ±SE	t-value	p	estimates ±SE	t-value	p	estimates ±SE	t-value	p
Neutrophil count	-0,92 ±0,27	-3,22	**	0,04 ±0,06	0,66	-	-0,16 ±0,10	-1,63	-
Monocyte count	-0,05 ±0,03	-1,54	-	-0,01 ±0,01	-1,61	-	0,02 ±0,01	1,17	-
Basophil count	-0,01 ±0,01	-0,92	-	-0,001 ±0,003	-0,45	-	0,01 ±0,005	2,44	*
Eosinophil count	-0,002 ±0,02	-0,12	-	-0,01 ±0,004	-2,06	*	0,009 ±0,006	1,43	-
Lymphocyte count	-0,78 ±0,10	-7,59	***	-0,05 ±0,02	-1,92	.	0,02 ±0,04	0,48	-
Alpha1-globulin	-0,11 ±0,07	-1,69	.	0,01 ±0,02	0,66	-	0,02 ±0,02	0,97	-
Alpha2-globulin	0,14 ±0,15	0,93	-	0,09 ±0,0	2,17	*	-0,11 ±0,06	-1,88	-
Beta-globulin	0,78 ±0,20	3,9	***	-2,38 E-05 ±5,64 E-05	-0,42	-	2,71 E-05 ±5,74 E-05	0,47	-
Gamma-globulin	4,27 ±0,50	8,53	***	-0,04 ±0,12	-0,37	-	0,28 ±0,18	1,53	-
Haptoglobin	0,10 ±0,10	1,01	-	-0,01 ±0,03	-0,45	-	0,03 ±0,04	0,85	-
Hemagglutination	0,08 ±0,13	0,66	-	0,01 ±0,03	0,3	-	0,006 ±0,05	0,12	-
Hemolysis	-0,04 ±0,10	-0,41	-	0,07 ±0,03	2,76	**	-0,05 ±0,04	-1,12	-

Table S9. Set of models fitted to assess senescence patterns of immune traits including a fixed effect of the longevity of individuals, in males and females at Trois-Fontaines, and in females at Chizé. We do not have enough data on Chizé males to test these models. Model comparison was based on AIC, k is the number of parameters, ΔAIC is the difference of AIC between the candidate model and the model having the lowest AIC, and wi the AIC weight of each model. All models included individual identity, the year of capture and the cohort of individuals as random effects. The model with the lowest AIC is shown in italics; and models with very similar explanatory power (ΔAIC < 2), which included fewer terms, are shown in bold.

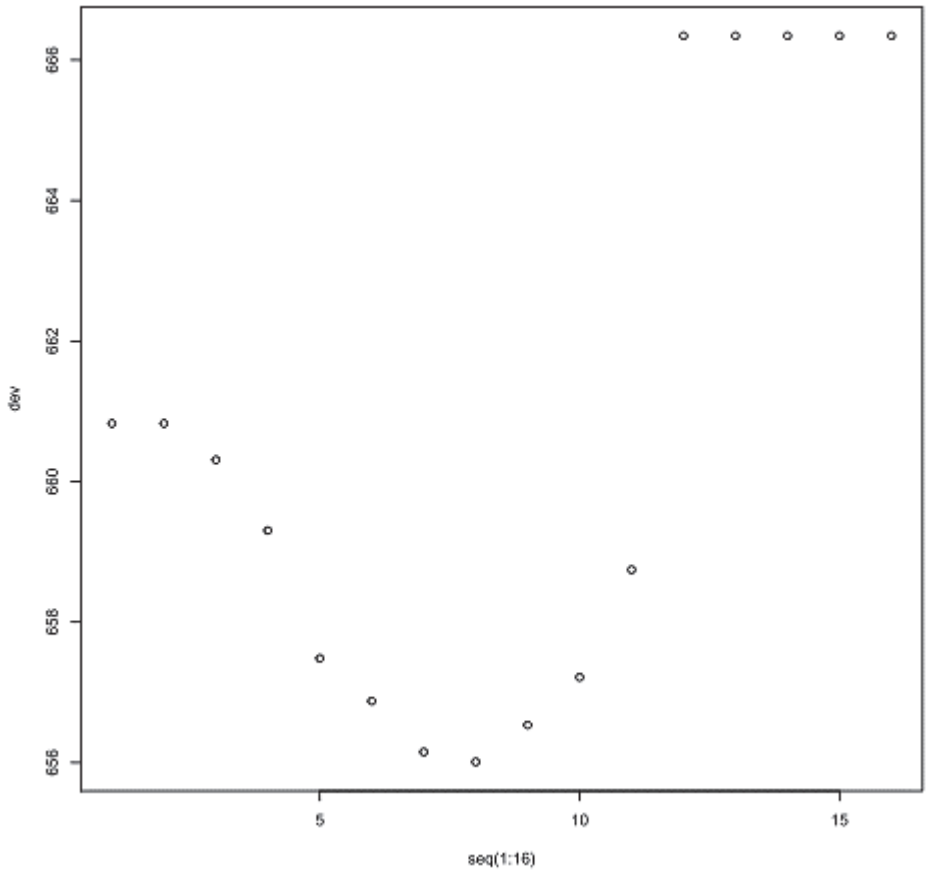
	TROIS FONTAINES MALES				TROIS FONTAINES FEMALES				CHIZE FEMALES			
Neutrophil count	k	AIC	DAIC	wi	k	AIC	DAIC	wi	k	AIC	DAIC	wi
nul	5	**340.60**	**0.67**	**0.14**	5	483.16	6.72	0.03	5	171.61	10.19	0.01
longevity	6	342.53	2.59	0.05	6	485.12	8.68	0.01	6	168.15	6.73	0.03
BM	6	342.02	2.08	0.06	6	485.12	8.67	0.01	6	173.49	12.07	0.00
longevity + BM	7	343.94	4.00	0.02	7	487.09	10.64	0.00	7	169.29	7.86	0.01
age (factor)	15	350.60	10.67	0.00	15	***476.45***	***0.00***	***0.09***	14	166.90	5.47	0.00
age (factor) + longevity	16	349.92	9.99	0.00	16	476.74	0.29	0.05	15	164.87	3.45	0.00
age (factor) + BM	16	352.45	12.52	0.00	16	478.17	1.72	0.02	15	168.89	7.46	0.00
age (factor) + longevity + BM	17	351.77	11.83	0.00	17	478.40	1.95	0.01	16	166.73	5.30	0.00
age (linear)	6	340.18	0.25	0.15	6	479.43	2.98	0.20	6	170.05	8.63	0.01
age (linear) + longevity	7	*339.93*	*0.00*	*0.14*	7	479.34	2.89	0.18	7	169.06	7.64	0.01
age (linear) + BM	7	341.79	1.86	0.05	7	480.91	4.46	0.08	7	171.89	10.47	0.00
age (linear) + longevity + BM	8	341.66	1.72	0.05	8	480.80	4.35	0.07	8	170.44	9.02	0.00
age (quadratic)	7	340.64	0.71	0.10	7	481.04	4.59	0.08	7	171.89	10.47	0.00
age (quadratic) + longevity	8	340.80	0.87	0.07	8	480.83	4.38	0.07	8	170.92	9.50	0.00
age (quadratic) + BM	8	342.64	2.71	0.03	8	482.22	5.77	0.04	8	173.73	12.31	0.00
age (quadratic) + longevity + BM	9	342.80	2.87	0.02	9	481.92	5.48	0.03	9	172.24	10.82	0.00
age (threshold)	6	341.82	1.89	0.07	6	485.14	8.70	0.01	6	164.35	2.93	0.19
age (threshold) + longevity	7	343.01	3.08	0.03	7	487.10	10.65	0.00	7	***161.42***	***0.00***	***0.54***
age (threshold) + BM	7	342.99	3.06	0.03	7	487.10	10.66	0.00	7	166.33	4.90	0.05
age (threshold) + longevity + BM	8	343.97	4.04	0.01	8	489.07	12.62	0.00	8	163.08	1.66	0.14
Eosinophil count	k	AIC	DAIC	wi	k	AIC	DAIC	wi	k	AIC	DAIC	wi
nul	5	***-115.72***	***0.00***	***0.28***	5	**-117.25**	**1.91**	**0.11**	5	***-36.65***	***0.00***	***0.31***
longevity	6	-113.73	1.99	0.09	6	*-119.16*	*0.00*	*0.26*	6	-34.70	1.96	0.08
BM	6	-114.43	1.30	0.12	6	-115.49	3.68	0.04	6	-34.93	1.73	0.09
longevity + BM	7	-112.43	3.29	0.04	7	-117.85	1.31	0.11	7	-32.96	3.70	0.02
age (factor)	15	-107.01	8.71	0.00	15	-106.59	12.57	0.00	14	-25.16	11.50	0.00
age (factor) + longevity	16	-105.19	10.54	0.00	16	-106.91	12.25	0.00	15	-23.16	13.50	0.00
age (factor) + BM	16	-106.12	9.60	0.00	16	-104.92	14.24	0.00	15	-23.16	13.50	0.00
age (factor) + longevity + BM	17	-104.32	11.40	0.00	17	-105.24	13.93	0.00	16	-21.16	15.49	0.00
age (linear)	6	-114.13	1.59	0.11	6	-115.98	3.18	0.05	6	-34.65	2.00	0.08
age (linear) + longevity	7	-112.28	3.45	0.03	7	-117.35	1.82	0.09	7	-32.71	3.94	0.02
age (linear) + BM	7	-112.96	2.77	0.05	7	-114.39	4.77	0.02	7	-32.93	3.73	0.02
age (linear) + longevity + BM	8	-111.17	4.55	0.02	8	-116.02	3.15	0.04	8	-30.96	5.69	0.00
age (quadratic)	7	-112.41	3.31	0.04	7	-114.06	5.10	0.02	7	-33.87	2.79	0.03
age (quadratic) + longevity	8	-110.52	5.20	0.01	8	-115.94	3.22	0.04	8	-31.91	4.75	0.01
age (quadratic) + BM	8	-112.07	3.65	0.02	8	-112.41	6.75	0.01	8	-32.16	4.49	0.01
age (quadratic) + longevity + BM	9	-110.22	5.50	0.01	9	-114.43	4.73	0.01	9	-30.18	6.47	0.00
age (threshold)	6	-113.93	1.80	0.10	6	-116.15	3.01	0.06	6	-36.54	0.12	0.20
age (threshold) + longevity	7	-111.94	3.79	0.03	7	-117.28	1.88	0.09	7	-34.74	1.91	0.05
age (threshold) + BM	7	-112.52	3.21	0.04	7	-114.39	4.78	0.02	7	-34.72	1.93	0.05
age (threshold) + longevity + BM	8	-110.52	5.21	0.01	8	-115.93	3.23	0.04	8	-32.91	3.74	0.01
Basophil count	k	AIC	DAIC	wi	k	AIC	DAIC	wi	k	AIC	DAIC	wi
nul	5	***-94.96***	***0.00***	***0.21***	5	**-241.77**	**0.52**	**0.16**	5	**-54.71**	**1.79**	**0.18**
longevity	6	-93.35	1.60	0.08	6	-240.59	1.71	0.08	6	-53.23	3.27	0.06
BM	6	-93.00	1.95	0.07	6	-240.18	2.11	0.06	6	-53.06	3.44	0.06
longevity + BM	7	-91.40	3.56	0.02	7	-239.15	3.14	0.03	7	-51.65	4.85	0.02
age (factor)	15	-81.48	13.48	0.00	15	-229.73	12.56	0.00	14	-50.54	5.96	0.00
age (factor) + longevity	16	-81.48	13.48	0.00	16	-227.79	14.50	0.00	15	-48.65	7.85	0.00
age (factor) + BM	16	-79.62	15.34	0.00	16	-229.44	12.86	0.00	15	-48.57	7.93	0.00
age (factor) + longevity + BM	17	-79.60	15.36	0.00	17	-227.46	14.83	0.00	16	-46.65	9.84	0.00
age (linear)	6	-93.95	1.01	0.11	6	-239.98	2.31	0.06	6	-54.49	2.01	0.11
age (linear) + longevity	7	-94.31	0.65	0.10	7	-238.60	3.69	0.02	7	-52.50	4.00	0.03
age (linear) + BM	7	-92.05	2.91	0.03	7	-238.45	3.84	0.02	7	-52.83	3.66	0.03
age (linear) + longevity + BM	8	-92.46	2.50	0.03	8	-237.16	5.13	0.01	8	-50.83	5.66	0.01
age (quadratic)	7	-92.15	2.80	0.04	7	*-242.29*	*0.00*	*0.15*	7	*-56.50*	*0.00*	*0.20*
age (quadratic) + longevity	8	-92.42	2.54	0.03	8	-240.34	1.95	0.05	8	-54.52	1.97	0.05
age (quadratic) + BM	8	-90.54	4.41	0.01	8	-241.23	1.06	0.08	8	-54.73	1.76	0.05
age (quadratic) + longevity + BM	9	-90.83	4.13	0.01	9	-239.32	2.97	0.02	9	-52.74	3.75	0.01
age (threshold)	6	-93.81	1.15	0.10	6	-241.35	0.94	0.11	6	-54.32	2.18	0.11
age (threshold) + longevity	7	-94.07	0.89	0.09	7	-240.86	1.44	0.07	7	-53.41	3.09	0.04
age (threshold) + BM	7	-91.84	3.11	0.03	7	-239.82	2.47	0.04	7	-52.73	3.77	0.03
age (threshold) + longevity + BM	8	-92.08	2.88	0.03	8	-239.60	2.69	0.03	8	-51.95	4.55	0.01

Monocyte count	k	AIC	DAIC	wi	k	AIC	DAIC	wi	k	AIC	DAIC	wi
nul	5	***75.23***	***0.00***	***0.28***	5	***53.02***	***0.00***	***0.23***	5	***13.75***	***0.00***	***0.36***
longevity	6	77.23	2.00	0.09	6	55.01	1.98	0.07	6	15.35	1.60	0.11
BM	6	76.96	1.73	0.10	6	54.60	1.58	0.09	6	15.29	1.55	0.12
longevity + BM	7	78.96	3.73	0.03	7	56.57	3.54	0.03	7	16.58	2.84	0.04
age (factor)	15	87.33	12.09	0.00	15	57.63	4.61	0.00	14	17.59	3.85	0.00
age (factor) + longevity	16	88.84	13.61	0.00	16	58.47	5.44	0.00	15	18.49	4.74	0.00
age (factor) + BM	16	89.32	14.09	0.00	16	59.30	6.27	0.00	15	19.58	5.83	0.00
age (factor) + longevity + BM	17	90.84	15.61	0.00	17	60.18	7.15	0.00	16	20.44	6.70	0.00
age (linear)	6	77.01	1.77	0.10	6	54.24	1.22	0.11	6	15.73	1.98	0.09
age (linear) + longevity	7	78.94	3.71	0.03	7	56.10	3.08	0.04	7	17.13	3.39	0.03
age (linear) + BM	7	78.75	3.52	0.03	7	55.76	2.73	0.04	7	17.27	3.53	0.03
age (linear) + longevity + BM	8	80.66	5.43	0.01	8	57.66	4.63	0.01	8	18.22	4.48	0.01
age (quadratic)	7	77.90	2.67	0.05	7	55.60	2.58	0.05	7	17.72	3.97	0.02
age (quadratic) + longevity	8	79.74	4.50	0.02	8	57.57	4.55	0.01	8	19.13	5.38	0.01
age (quadratic) + BM	8	79.89	4.65	0.01	8	57.17	4.15	0.02	8	19.26	5.52	0.01
age (quadratic) + longevity + BM	9	81.72	6.49	0.00	9	59.16	6.13	0.01	9	20.22	6.48	0.00
age (threshold)	6	76.20	0.97	0.14	6	53.58	0.55	0.15	6	15.64	1.90	0.10
age (threshold) + longevity	7	77.99	2.76	0.05	7	55.54	2.51	0.05	7	17.29	3.55	0.03
age (threshold) + BM	7	78.10	2.87	0.05	7	55.13	2.11	0.06	7	17.22	3.48	0.03
age (threshold) + longevity + BM	8	79.90	4.66	0.01	8	57.11	4.09	0.02	8	18.57	4.82	0.01
Lymphocyte count	k	AIC	DAIC	wi	k	AIC	DAIC	wi	k	AIC	DAIC	wi
nul	5	***226.82***	***0.00***	***0.25***	5	***272.35***	***0.00***	***0.20***	5	***89.75***	***0.00***	***0.24***
longevity	6	228.63	1.81	0.09	6	273.19	0.84	0.11	6	90.04	0.29	0.15
BM	6	227.54	0.72	0.15	6	274.34	1.99	0.06	6	90.20	0.46	0.13
longevity + BM	7	229.37	2.55	0.05	7	275.13	2.78	0.04	7	90.74	1.00	0.07
age (factor)	15	236.94	10.12	0.00	15	280.33	7.98	0.00	14	104.28	14.54	0.00
age (factor) + longevity	16	238.93	12.11	0.00	16	282.34	9.99	0.00	15	102.37	12.63	0.00
age (factor) + BM	16	238.69	11.87	0.00	16	282.00	9.65	0.00	15	103.68	13.94	0.00
age (factor) + longevity + BM	17	240.66	13.85	0.00	17	283.85	11.50	0.00	16	102.09	12.35	0.00
age (linear)	6	228.46	1.64	0.09	6	273.21	0.86	0.11	6	91.65	1.91	0.06
age (linear) + longevity	7	230.45	3.63	0.03	7	274.86	2.51	0.04	7	90.36	0.61	0.08
age (linear) + BM	7	229.29	2.47	0.05	7	275.14	2.79	0.04	7	92.13	2.38	0.03
age (linear) + longevity + BM	8	231.27	4.45	0.01	8	276.78	4.43	0.01	8	91.31	1.57	0.03
age (quadratic)	7	230.44	3.62	0.03	7	273.15	0.80	0.10	7	93.65	3.90	0.02
age (quadratic) + longevity	8	232.42	5.60	0.01	8	274.98	2.63	0.03	8	92.28	2.54	0.02
age (quadratic) + BM	8	231.10	4.28	0.02	8	275.08	2.73	0.03	8	94.11	4.37	0.01
age (quadratic) + longevity + BM	9	233.09	6.27	0.00	9	276.89	4.54	0.01	9	93.30	3.56	0.01
age (threshold)	6	228.32	1.50	0.10	6	273.21	0.86	0.11	6	91.49	1.74	0.07
age (threshold) + longevity	7	230.32	3.50	0.03	7	274.85	2.50	0.04	7	91.91	2.17	0.04
age (threshold) + BM	7	228.80	1.98	0.06	7	275.14	2.79	0.04	7	91.94	2.20	0.04
age (threshold) + longevity + BM	8	230.78	3.96	0.02	8	276.77	4.42	0.01	8	92.60	2.86	0.02
Alpha1-globulin	k	AIC	DAIC	wi	k	AIC	DAIC	wi	k	AIC	DAIC	wi
nul	5	178.43	4.16	0.05	5	205.46	10.36	0.00	5	82.01	10.09	0.00
longevity	6	180.11	5.84	0.02	6	206.29	11.19	0.00	6	83.11	11.19	0.00
BM	6	***174.27***	***0.00***	***0.34***	6	203.19	8.09	0.01	6	76.43	4.50	0.05
longevity + BM	7	175.90	1.63	0.13	7	203.08	7.97	0.01	7	76.26	4.33	0.04
age (factor)	15	186.48	12.21	0.00	15	210.80	15.70	0.00	14	81.66	9.74	0.00
age (factor) + longevity	16	188.47	14.20	0.00	16	212.59	17.49	0.00	15	83.12	11.19	0.00
age (factor) + BM	16	180.70	6.43	0.00	16	208.62	13.51	0.00	15	79.34	7.41	0.00
age (factor) + longevity + BM	17	182.69	8.42	0.00	17	210.37	15.26	0.00	16	79.83	7.90	0.00
age (linear)	6	180.13	5.86	0.02	6	203.20	8.10	0.01	6	76.79	4.86	0.05
age (linear) + longevity	7	182.05	7.78	0.01	7	205.16	10.06	0.00	7	78.28	6.36	0.02
age (linear) + BM	7	175.70	1.43	0.14	7	199.41	4.30	0.03	7	**72.36**	**0.43**	**0.29**
age (linear) + longevity + BM	8	177.66	3.39	0.04	8	201.41	6.30	0.01	8	*71.92*	*0.00*	*0.24*
age (quadratic)	7	181.52	7.25	0.01	7	197.06	1.96	0.11	7	77.02	5.10	0.03
age (quadratic) + longevity	8	183.46	9.19	0.00	8	198.80	3.70	0.04	8	78.26	6.34	0.01
age (quadratic) + BM	8	177.65	3.38	0.04	8	195.36	0.26	0.21	8	73.17	1.25	0.13
age (quadratic) + longevity + BM	9	179.61	5.34	0.01	9	197.01	1.91	0.08	9	72.77	0.85	0.10
age (threshold)	6	179.56	5.30	0.02	6	197.65	2.54	0.09	6	84.01	12.09	0.00
age (threshold) + longevity	7	181.51	7.24	0.01	7	199.64	4.54	0.03	7	85.11	13.19	0.00
age (threshold) + BM	7	175.99	1.72	0.12	7	***195.10***	***0.00***	***0.28***	7	78.23	6.30	0.02
age (threshold) + longevity + BM	8	177.79	3.52	0.04	8	196.90	1.80	0.10	8	78.12	6.19	0.01

Alpha2-globulin	k	AIC	DAIC	wi	k	AIC	DAIC	wi	k	AIC	DAIC	wi
nul	5	**304.11**	**1.30**	**0.10**	5	**415.30**	**1.78**	**0.12**	5	***186.72***	***0.00***	***0.28***
longevity	6	305.50	2.69	0.04	6	417.27	3.75	0.04	6	188.72	2.00	0.08
BM	6	304.64	1.83	0.06	6	*413.52*	*0.00*	*0.26*	6	186.80	0.08	0.20
longevity + BM	7	305.98	3.17	0.03	7	415.47	1.95	0.09	7	188.72	2.00	0.06
age (factor)	15	307.12	4.30	0.00	15	426.64	13.12	0.00	14	197.56	10.84	0.00
age (factor) + longevity	16	306.35	3.54	0.00	16	428.55	15.03	0.00	15	199.56	12.84	0.00
age (factor) + BM	16	308.39	5.58	0.00	16	423.82	10.30	0.00	15	197.87	11.15	0.00
age (factor) + longevity + BM	17	307.72	4.90	0.00	17	425.67	12.14	0.00	16	199.81	13.09	0.00
age (linear)	6	304.60	1.78	0.06	6	417.03	3.51	0.05	6	188.72	2.00	0.08
age (linear) + longevity	7	*302.81*	*0.00*	*0.13*	7	418.88	5.36	0.02	7	190.72	4.00	0.02
age (linear) + BM	7	305.23	2.41	0.04	7	415.39	1.86	0.09	7	188.79	2.07	0.05
age (linear) + longevity + BM	8	303.65	0.84	0.07	8	417.21	3.69	0.03	8	190.71	3.99	0.01
age (quadratic)	7	306.40	3.58	0.02	7	418.95	5.43	0.02	7	190.37	3.65	0.02
age (quadratic) + longevity	8	304.79	1.97	0.04	8	420.82	7.30	0.01	8	192.36	5.64	0.01
age (quadratic) + BM	8	307.22	4.41	0.01	8	417.19	3.67	0.03	8	190.71	3.99	0.01
age (quadratic) + longevity + BM	9	305.61	2.80	0.02	9	419.05	5.53	0.01	9	192.61	5.89	0.00
age (threshold)	6	303.67	0.86	0.10	6	416.66	3.14	0.05	6	188.54	1.82	0.08
age (threshold) + longevity	7	303.62	0.80	0.09	7	418.66	5.14	0.02	7	190.51	3.79	0.02
age (threshold) + BM	7	303.54	0.72	0.09	7	414.62	1.10	0.13	7	188.69	1.97	0.06
age (threshold) + longevity + BM	8	302.97	0.16	0.10	8	416.61	3.09	0.04	8	190.67	3.95	0.01
Haptoglobin	k	AIC	DAIC	wi	k	AIC	DAIC	wi	k	AIC	DAIC	wi
nul	5	253.52	3.99	0.06	5	326.27	23.95	0.00	5	74.93	6.47	0.02
longevity	6	255.23	5.70	0.02	6	327.37	25.05	0.00	6	76.13	7.68	0.01
BM	6	252.96	3.43	0.07	6	327.98	25.66	0.00	6	76.91	8.45	0.00
longevity + BM	7	254.73	5.20	0.02	7	328.58	26.26	0.00	7	78.12	9.66	0.00
age (factor)	15	258.96	9.44	0.00	15	318.38	16.07	0.00	14	74.26	5.80	0.00
age (factor) + longevity	16	260.96	11.43	0.00	16	320.35	18.03	0.00	15	72.51	4.05	0.00
age (factor) + BM	16	259.62	10.09	0.00	16	319.58	17.26	0.00	15	75.36	6.90	0.00
age (factor) + longevity + BM	17	261.59	12.06	0.00	17	321.55	19.23	0.00	16	74.42	5.96	0.00
age (linear)	6	254.61	5.08	0.03	6	316.54	14.23	0.00	6	71.47	3.02	0.07
age (linear) + longevity	7	256.61	7.08	0.01	7	318.01	15.70	0.00	7	69.55	1.09	0.13
age (linear) + BM	7	254.18	4.65	0.03	7	316.73	14.41	0.00	7	72.98	4.53	0.02
age (linear) + longevity + BM	8	256.17	6.64	0.01	8	318.34	16.02	0.00	8	71.54	3.08	0.03
age (quadratic)	7	253.16	3.63	0.05	7	308.66	6.34	0.02	7	70.79	2.33	0.07
age (quadratic) + longevity	8	255.11	5.58	0.01	8	310.66	8.34	0.01	8	*68.46*	*0.00*	*0.15*
age (quadratic) + BM	8	254.89	5.36	0.02	8	310.28	7.96	0.01	8	72.70	4.24	0.02
age (quadratic) + longevity + BM	9	256.85	7.32	0.00	9	312.28	9.96	0.00	9	70.26	1.80	0.04
age (threshold)	6	***249.53***	***0.00***	***0.37***	6	***302.32***	***0.00***	***0.51***	6	**69.66**	**1.20**	**0.17**
age (threshold) + longevity	7	251.51	1.98	0.11	7	304.32	2.00	0.17	7	69.18	0.73	0.16
age (threshold) + BM	7	251.00	1.47	0.15	7	303.78	1.46	0.22	7	71.66	3.20	0.05
age (threshold) + longevity + BM	8	253.00	3.47	0.04	8	305.77	3.45	0.07	8	70.68	2.22	0.05
Gamma-globulin	k	AIC	DAIC	wi	k	AIC	DAIC	wi	k	AIC	DAIC	wi
nul	5	484.59	12.28	0.00	5	664.61	10.59	0.00	5	307.48	2.09	0.11
longevity	6	486.05	13.74	0.00	6	663.23	9.21	0.00	6	307.69	2.30	0.08
BM	6	478.62	6.31	0.02	6	665.98	11.96	0.00	6	308.45	3.06	0.05
longevity + BM	7	479.64	7.32	0.01	7	664.30	10.29	0.00	7	309.21	3.82	0.03
age (factor)	15	491.07	18.75	0.00	15	667.35	13.34	0.00	14	313.67	8.29	0.00
age (factor) + longevity	16	492.33	20.01	0.00	16	669.33	15.31	0.00	15	315.63	10.24	0.00
age (factor) + BM	16	478.20	5.88	0.00	16	668.22	14.20	0.00	15	315.43	10.04	0.00
age (factor) + longevity + BM	17	479.78	7.46	0.00	17	670.20	16.18	0.00	16	317.42	12.03	0.00
age (linear)	6	481.22	8.90	0.01	6	654.78	0.76	0.14	6	***305.39***	***0.00***	***0.25***
age (linear) + longevity	7	482.56	10.24	0.00	7	656.77	2.75	0.04	7	307.31	1.92	0.07
age (linear) + BM	7	***472.32***	***0.00***	***0.40***	7	654.94	0.92	0.11	7	306.25	0.86	0.11
age (linear) + longevity + BM	8	473.49	1.17	0.18	8	656.92	2.90	0.04	8	308.25	2.86	0.03
age (quadratic)	7	482.95	10.63	0.00	7	654.94	0.92	0.11	7	306.22	0.83	0.12
age (quadratic) + longevity	8	484.19	11.87	0.00	8	656.86	2.84	0.04	8	308.19	2.80	0.03
age (quadratic) + BM	8	472.86	0.55	0.24	8	655.62	1.60	0.07	8	307.27	1.88	0.05
age (quadratic) + longevity + BM	9	474.22	1.91	0.10	9	657.55	3.53	0.02	9	309.27	3.88	0.01
age (threshold)	6	483.28	10.96	0.00	6	***654.02***	***0.00***	***0.20***	6	309.42	4.03	0.03
age (threshold) + longevity	7	485.06	12.75	0.00	7	655.94	1.93	0.07	7	309.53	4.14	0.02
age (threshold) + BM	7	477.86	5.54	0.03	7	654.76	0.74	0.12	7	310.45	5.06	0.01
age (threshold) + longevity + BM	8	479.29	6.98	0.01	8	656.63	2.61	0.04	8	311.15	5.76	0.01

Beta-globulin	k	AIC	DAIC	wi	k	AIC	DAIC	wi	k	AIC	DAIC	wi
nul	5	398.12	4.04	0.05	5	475.24	12.41	0.00	5	**203.02**	**0.74**	**0.22**
longevity	6	400.10	6.02	0.01	6	477.03	14.20	0.00	6	204.97	2.68	0.06
BM	6	398.86	4.78	0.03	6	473.45	10.62	0.00	6	205.01	2.73	0.06
longevity + BM	7	400.86	6.78	0.01	7	474.93	12.10	0.00	7	206.90	4.62	0.02
age (factor)	15	400.27	6.18	0.00	15	480.45	17.62	0.00	14	203.94	1.66	0.00
age (factor) + longevity	16	400.99	6.91	0.00	16	482.45	19.62	0.00	15	204.01	1.73	0.00
age (factor) + BM	16	400.13	6.05	0.00	16	476.66	13.83	0.00	15	205.05	2.77	0.00
age (factor) + longevity + BM	17	400.99	6.91	0.00	17	478.66	15.83	0.00	16	205.66	3.38	0.00
age (linear)	6	***394.08***	***0.00***	***0.30***	6	467.55	4.72	0.03	6	*202.28*	*0.00*	*0.24*
age (linear) + longevity	7	395.28	1.20	0.14	7	469.53	6.70	0.01	7	203.33	1.04	0.10
age (linear) + BM	7	395.07	0.99	0.15	7	462.89	0.06	0.26	7	204.27	1.98	0.06
age (linear) + longevity + BM	8	396.19	2.11	0.07	8	464.88	2.05	0.08	8	205.30	3.01	0.02
age (quadratic)	7	396.03	1.95	0.09	7	468.08	5.25	0.02	7	204.01	1.73	0.07
age (quadratic) + longevity	8	397.19	3.11	0.04	8	470.04	7.21	0.01	8	204.90	2.62	0.03
age (quadratic) + BM	8	396.83	2.75	0.05	8	464.38	1.55	0.11	8	205.97	3.68	0.02
age (quadratic) + longevity + BM	9	398.02	3.94	0.02	9	466.33	3.50	0.03	9	206.90	4.62	0.01
age (threshold)	6	399.72	5.64	0.02	6	466.00	3.17	0.06	6	205.02	2.74	0.06
age (threshold) + longevity	7	401.65	7.57	0.01	7	467.84	5.01	0.02	7	206.97	4.68	0.02
age (threshold) + BM	7	400.84	6.76	0.01	7	***462.83***	***0.00***	***0.27***	7	207.01	4.73	0.02
age (threshold) + longevity + BM	8	402.83	8.75	0.00	8	464.66	1.82	0.09	8	208.90	6.62	0.00
Hemagglutination	k	AIC	DAIC	wi	k	AIC	DAIC	wi	k	AIC	DAIC	wi
nul	5	**296.65**	**0.55**	**0.17**	5	**394.98**	**0.67**	**0.13**	5	**177.97**	**0.78**	**0.15**
longevity	6	298.52	2.42	0.06	6	396.89	2.58	0.05	6	179.89	2.70	0.04
BM	6	298.64	2.54	0.05	6	394.72	0.41	0.14	6	*177.19*	*0.00*	*0.16*
longevity + BM	7	300.50	4.40	0.02	7	396.72	2.41	0.04	7	179.19	2.00	0.04
age (factor)	15	308.61	12.51	0.00	15	403.23	8.92	0.00	14	189.00	11.80	0.00
age (factor) + longevity	16	309.24	13.14	0.00	16	405.00	10.69	0.00	15	189.37	12.18	0.00
age (factor) + BM	16	310.54	14.44	0.00	16	402.71	8.39	0.00	15	188.33	11.14	0.00
age (factor) + longevity + BM	17	311.16	15.06	0.00	17	404.53	10.21	0.00	16	189.10	11.91	0.00
age (linear)	6	297.27	1.17	0.10	6	396.95	2.64	0.04	6	178.31	1.12	0.09
age (linear) + longevity	7	297.50	1.40	0.08	7	398.65	4.33	0.02	7	178.54	1.35	0.06
age (linear) + BM	7	299.25	3.15	0.03	7	396.48	2.17	0.05	7	177.29	0.10	0.11
age (linear) + longevity + BM	8	299.42	3.32	0.02	8	398.32	4.01	0.02	8	178.04	0.85	0.05
age (quadratic)	7	299.13	3.02	0.03	7	396.81	2.50	0.04	7	180.31	3.12	0.02
age (quadratic) + longevity	8	299.26	3.16	0.03	8	398.73	4.42	0.01	8	180.42	3.23	0.02
age (quadratic) + BM	8	301.12	5.02	0.01	8	397.25	2.94	0.03	8	179.23	2.04	0.03
age (quadratic) + longevity + BM	9	301.26	5.15	0.01	9	399.21	4.89	0.01	9	179.84	2.65	0.01
age (threshold)	6	*296.10*	*0.00*	*0.19*	6	394.56	0.25	0.15	6	178.84	1.65	0.07
age (threshold) + longevity	7	296.61	0.50	0.12	7	395.72	1.41	0.07	7	179.79	2.60	0.03
age (threshold) + BM	7	298.05	1.95	0.06	7	*394.31*	*0.00*	*0.15*	7	177.97	0.78	0.08
age (threshold) + longevity + BM	8	298.53	2.43	0.04	8	395.94	1.63	0.06	8	179.31	2.12	0.03
Hemolysis	k	AIC	DAIC	wi	k	AIC	DAIC	wi	k	AIC	DAIC	wi
nul	5	**283.08**	**0.69**	**0.18**	5	356.51	5.26	0.10	5	*147.69*	*0.10*	*0.26*
longevity	6	285.04	2.66	0.06	6	357.90	6.65	0.04	6	149.37	1.78	0.09
BM	6	284.43	2.05	0.08	6	357.87	6.62	0.04	6	149.69	2.10	0.07
longevity + BM	7	286.40	4.02	0.02	7	358.96	7.71	0.02	7	151.37	3.77	0.02
age (factor)	15	293.90	11.52	0.00	15	**352.06**	**0.81**	**0.10**	14	150.47	2.87	0.00
age (factor) + longevity	16	295.75	13.37	0.00	16	*351.25*	*0.00*	*0.11*	15	152.44	4.85	0.00
age (factor) + BM	16	293.97	11.58	0.00	16	354.01	2.76	0.03	15	151.40	3.80	0.00
age (factor) + longevity + BM	17	295.86	13.48	0.00	17	353.17	1.92	0.03	16	153.30	5.70	0.00
age (linear)	6	284.77	2.39	0.06	6	357.28	6.02	0.06	6	149.69	2.10	0.07
age (linear) + longevity	7	286.73	4.35	0.02	7	355.70	4.45	0.11	7	151.24	3.65	0.02
age (linear) + BM	7	286.12	3.73	0.03	7	358.91	7.66	0.02	7	151.69	4.10	0.02
age (linear) + longevity + BM	8	288.07	5.69	0.01	8	357.04	5.79	0.05	8	153.23	5.64	0.01
age (quadratic)	7	284.79	2.41	0.05	7	358.97	7.71	0.02	7	149.95	2.35	0.05
age (quadratic) + longevity	8	286.79	4.41	0.02	8	357.65	6.40	0.04	8	151.77	4.18	0.01
age (quadratic) + BM	8	284.65	2.27	0.05	8	360.33	9.08	0.01	8	151.94	4.35	0.01
age (quadratic) + longevity + BM	9	286.64	4.26	0.01	9	358.81	7.56	0.02	9	153.77	6.18	0.00
age (threshold)	6	*282.38*	*0.00*	*0.21*	6	357.16	5.91	0.06	6	**147.59**	**0.00**	**0.21**
age (threshold) + longevity	7	284.18	1.80	0.07	7	356.56	5.30	0.07	7	149.09	1.49	0.07
age (threshold) + BM	7	283.38	0.99	0.11	7	358.63	7.37	0.03	7	149.59	1.99	0.06
age (threshold) + longevity + BM	8	285.19	2.81	0.03	8	357.41	6.16	0.04	8	151.06	3.46	0.02

Fig. S1. **Selection of the best threshold for the "threshold model" by maximum likelihood (see Methods).** We used the deviance profiles of a continuous age model with a varying threshold and selected the age leading to the lowest deviance as the threshold age.



Appendix 5.1. Supporting information for the paper *Age-dependent associations between telomere length and environmental conditions in roe deer.*

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SUPPLEMETARY METHODS

Study population and sample preparation

Roe deer have been continuously monitored at the Trois-Fontaines (TF) and Chizé (CH) study sites using Capture-Mark-Recapture methods since 1975 and 1977, respectively. Trapping sessions occur at each site between January and March each winter (see [1] for further details), and each year, approximately 50% of the population are captured. Sex, body mass (to the nearest 50g) and hind foot length (to the nearest mm, measured from the heel to the tip of the hoof) are recorded, and age is determined for each individual through prior knowledge of their year of birth[2]. During the 2015/2016 field season, blood samples were collected from the jugular vein of known-age individuals (n=160). Within 30 min of sampling, whole blood was spun at approximately 3000 rpm for 10 min and the plasma layer drawn off and replaced by the same quantity of 0.9% w/v NaCl solution and spun again. The intermediate buffy coat layer, comprising mainly leukocytes (white blood cells) was collected into a 1.5-mL Eppendorf tube and stored at −80 °C until further use.

DNA extraction

Genomic DNA was extracted from white blood cells using Macherey-Nagel NucleoSpin® Blood QuickPure kit (Catalogue number 740569) at the CNRS, Lyon (France). All downstream processing of samples (e.g. quality control (QC), telomere measurement) was then carried out at the University of Edinburgh, Scotland (UK). DNA yield and purity was quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington DE, USA) and DNA integrity was assessed by running 200 ng total DNA on a 0.5% agarose gel and DNA bands scored on a scale of 1-5 by visual examination. Samples passed QC with a DNA yield of ≥20 ng/µl, an acceptable purity absorption range of 1.7 - 2.0 for the 260/280 nm ratio and > 1.8 for the 260/230 nm ratio, and a DNA integrity score of either 1 or 2 [3].

DNA extraction protocol

1. Lyse blood samples

Pipette 25 µL Proteinase K and up to 150 µL blood (leucocytes) + 50 µL PBS into 1.5ML microcentrifuge tubes. Incubate at room temperature for 1 min. Add 200 µL Lysis Buffer BQ1 to the samples and vortex the mixture vigorously (10-20s). Incubate samples at 70°C for 10 minutes.

2. Adjust DNA binding conditions

Vortex again the mixture and centrifuge few seconds at 11,000 x g. Add 200µL ethanol (96-100%) to each samples, vortex again, centrifuge again few seconds at 11, 000 x g.

3. Bind DNA

Add the samples to the NucleoSpin Blood QuickPure Columns placed in a collection tubes and centrifuge 1 min at 11,000 x g. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (up to 15,000 x g). Discard collection tube with flow-through.

4. Wash & dry silica membrane

Place the Nucleospin blood quickpure column into a new collection tube (2mL) and add 350 µL Buffer BQ2. Centrifuge 3 min at 11,000 x g. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (up to 15,000 x g). Discard collection tube with flow-through.

5. Elute highly pure DNA

Place the NucleoSpin blood quickpure column in a 1.5 mL microcentrifuge tube and add 50 µL prewarmed Buffer BE (70°C). Dispense buffer directly onto the silicq membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.

Telomere length measurement

We measured relative leukocyte telomere length (RTL) using a real-time quantitative PCR method (qPCR; [4]) which has previously been optimised and validated in sheep and cattle [3]. This method measures the total amount of telomeric sequence present in a DNA sample, relative to the amount of a non-variable copy number reference gene (beta-2-microglobulin (B2M)). For telomere reactions we used the following HPLC purified primers, Tel 1b (5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3') and Tel 2b (5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3') (from Epel et al., 2004). For B2M reactions, primers were supplied by Primer Design (Catalogue number: HK-SY-Sh-900, Southampton, UK).

Using an automated liquid handling robot (Freedom Evo-2 150; Tecan) we were able to load both the DNA samples and qPCR master mix in 384 well plates; allowing us to run both telomeric and B2M reactions in separate wells but on a single plate. A separate master mix for each primer set was prepared containing 5 µl LightCycler 480SYBR Green I Master Mix (Cat # 04887352001, Roche, West Sussex, UK), 0.5 µl B2M (300 nm) primer or 0.6 µl each tel primer (900 nm), and 2 ng of sample DNA. DNA was amplified in 10 µl reactions. Each plate included a non-treated control (water; NTC), a calibrator sample (2ng) on each row to account for plate to plate variation and robot pipetting error, as well as a 1:4 serial dilution starting at 10ng/µl to visually inspect the qPCR curves. The calibrator sample is DNA that has been extracted from a large quantity of blood obtained from a single wild roe deer. In this case, the calibrator was extracted using the Qiagen DNeasy Blood and Tissue kit (Cat# 69581, Manchester, UK), pooled and quality controlled in the same way as our DNA samples of interest. All samples, calibrators and NTC's were run in triplicate and all qPCR performed using a Roche LC480 instrument using the following reaction protocol: 10 min at 95 ºC (enzyme activation), followed by 50 cycles of 15 s at 95 ºC (denaturation) and 30 s at 58 ºC (primer annealing), then 30 s at 72 ºC (signal acquisition). Melting curve protocol was 1 min at 95 °C, followed by 30 s at 58 °C, then 0.11 °C/s to 95 °C followed by 10 s at 40 °C.

We used the LinRegPCR software package (version 2016.0; [5]) to correct for baseline fluorescence, set a window of linearity for each amplicon group and to calculate well-specific reaction efficiencies and Cq values. A constant fluorescence threshold was set within the window of linearity for each amplicon group, calculated using the average Cq across all three plates. The threshold values used were 0.140 and 0.203, and the average efficiency across all plates were 1.91 and 1.93 for the B2M and telomere amplicon groups, respectively. Samples were excluded from further analysis if the coefficient of variation (CV) across triplicate Cq values for either amplicon was > 5 %, or if at least one of their triplicate reactions had an efficiency that was 5 % higher or lower than the mean efficiency across all wells on that plate for the respective amplicon. Overall, thirteen samples failed quality control at either the DNA extraction or qPCR stage and were excluded from the study, leaving a total of 147 samples for further analyses.

We calculated relative telomere length (RTL) for each sample following Pfaffl et al 2001 as follows:

$$RTL = (E_{TEL}^{(CqTEL[Calibrator] - CqTEL[Sample])}) / (E_{B2M}^{(CqB2M[Calibrator] - CqB2M[Sample])})$$

Where E_{TEL} and E_{B2M} are the mean reaction efficiencies for the respective amplicon group across all samples on a given plate; CqTEL[Calibrator] and CqB2M[Calibrator] are the average Cqs for the relevant amplicon across all calibrator samples on the plate; and CqTEL[Sample] and CqB2M[Sample] are the average of the triplicate Cqs for the sample for each amplicon.

Additional models testing the effect of body mass on telomere length

To determine if and how variation in body condition within populations might influence our results, we generated metrics of body condition our best index of body size, by calculating the residuals from a

linear model for body mass including an index of body size, hind foot length (n=133; b=0.231 ± 0.013 SE, $p<0.001$). We then tested for an association between size-corrected mass and TL, independent of previously identified associations between TL, age, sex and population, by adding size-corrected body mass to the minimal model for TL. There was no evidence for a relationship between TL and size-corrected body mass, and its inclusion in the TL model did not alter the magnitude of the age-by-population interaction (Fig. 1B; Table S3).

SUPPLEMENTARY ETHICS

The protocol of capture and blood sampling of roe deer under the authority of the Office National de la Chasse et de la Faune Sauvage (ONCFS) was approved by the Director of Food, Agriculture and Forest (Prefectoral order 2009-14 from Paris).

SUPPLEMENTARY FUNDING

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Table S1. Linear models of telomere length in two populations of wild roe deer (N=139). (A) Maximal model including all terms and (B) minimal model after stepwise removal of non-significant effects. The reference groups for factors are females for sex and Chizé for population. Statistically significant terms in bold.

	a) RTL maximal model				*b) RTL minimal model*			
Explanatory variables	**Estimate**	**SE**	**F-Value**	**P Value**	**Estimate**	**SE**	**F**	**P**
Intercept	**1.100**	**0.048**	**23.146**	**<0.001**	**1.072**	**0.035**	**30.548**	**<0.001**
Age	-0.012	0.007	-1.623	0.107	-0.011	0.006	-1.660	0.106
Population (TF)	-0.048	0.061	-0.783	0.435	-0.007	0.047	-0.155	0.877
Sex (males)	-0.037	0.058	-0.643	0.521				
Age * Sex (males)	-0.004	0.010	-0.392	0.696				
Sex (males) * Population (TF)	0.060	0.060	0.996	0.321				
Age * Population (TF)	**0.026**	**0.009**	**2.718**	**0.007**	**0.022**	**0.009**	**2.509**	**0.013**

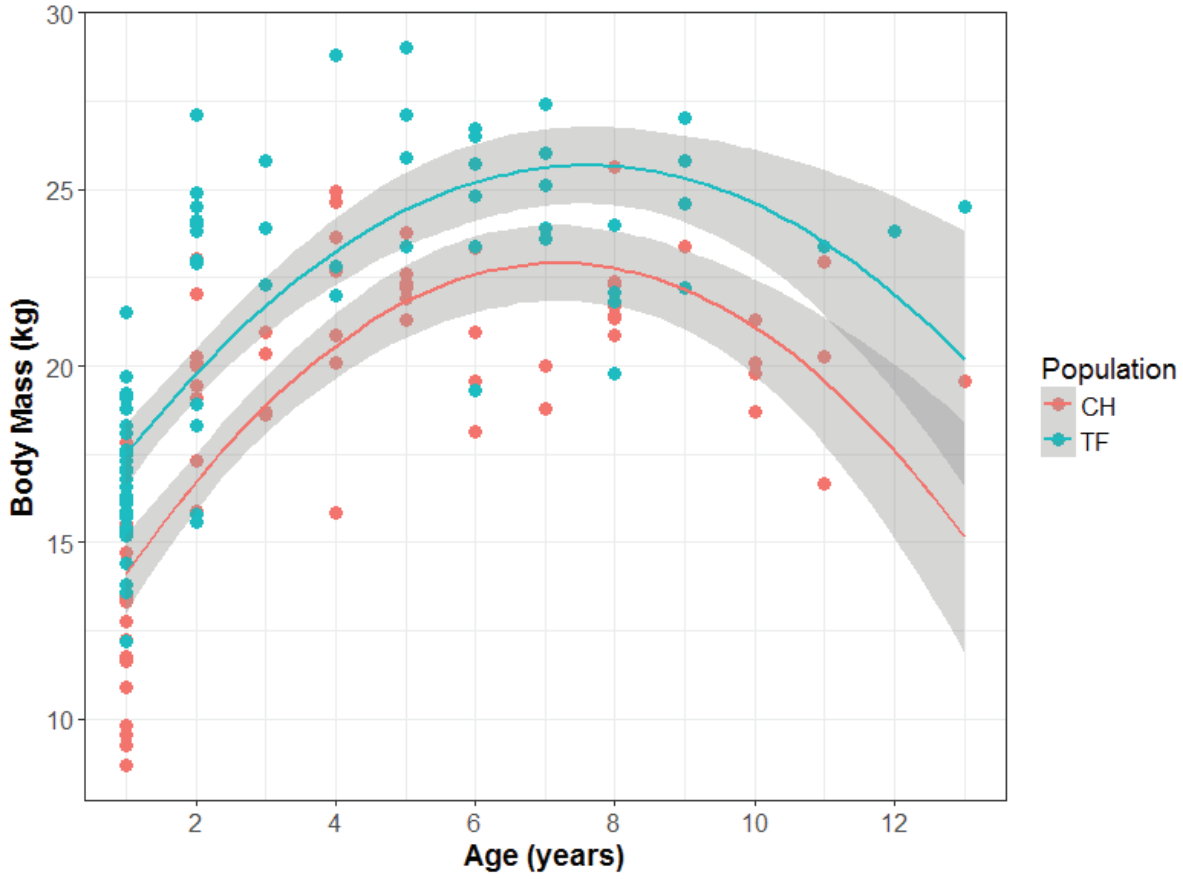
Table S2. Linear models of body mass (kg) in two populations of wild roe deer (N=139). (A) Maximal model including all terms (B) minimal model after stepwise removal of non-significant terms. The reference groups for factors are females for sex and Chizé for population. Statistically significant terms in bold.

	a) Body mass maximal model				*b) Body mass minimal model*			
Explanatory variables	**Estimate**	**Std Error**	**F-Value**	**P Value**	**Estimate**	**Std Error**	**F-Value**	**P Value**
Intercept	**11.133**	**1.015**	**10.966**	**<0.001**	**11.479**	**0.804**	**14.279**	**<0.001**
Age	**2.773**	**0.431**	**6.428**	**<0.001**	**2.543**	**0.342**	**7.436**	**<0.001**
Age^2	**-0.175**	**0.037**	**-4.768**	**<0.001**	**-0.156**	**0.029**	**-5.339**	**<0.001**
Population (TF)	**3.818**	**1.188**	**3.215**	**0.002**	**3.161**	**0.454**	**6.963**	**<0.001**
Sex (males)	-0.795	1.212	-0.656	0.513	-0.539	1.043	-0.517	0.606
Age * Sex (males)	**1.293**	**0.520**	**2.487**	**0.014**	**1.238**	**0.511**	**2.424**	**0.017**
Age^2 * Sex (males)	**-0.126**	**0.048**	**-2.622**	**0.010**	**-0.121**	**0.047**	**-2.568**	**0.011**
Sex (males) * Population (TF)	0.301	0.942	0.319	0.750				
Age * Population (TF)	-0.463	0.509	-0.911	0.364				
Age^2 * Population (TF)	0.040	0.045	0.881	0.380				

Table S3. Linear models testing the effect of size-corrected body mass (n=133) on telomere length in two populations of wild roe deer. Residuals from models from a linear model of body mass containing only hind foot length as a covariate were added to the minimal model for RTL (see Table S1B). The reference groups for factors are females for sex and Chizé for population. Statistically significant terms in bold.

Explanatory variables	Estimate	Std Error	F-Value	P Value
Intercept	**1.072**	**0.037**	**28.923**	**<0.001**
Age	-0.010	0.007	-1.344	0.181
Population (TF)	-0.018	0.048	-0.379	0.705
Size-corrected body mass	-0.002	0.007	-0.331	0.741
Age * Population (TF)	**0.024**	**0.009**	**2.572**	**0.011**

Figure S1. Plot illustrating differences in body mass with age in the two study populations: the better environment at Trois Fountaines (TF, blue) and the poorer environment at Chize (CH, red). Raw data for CH (red) and TF (blue) are presented with a quadratic regression function (red and blue lines, respectively) along with their associated standard errors (grey shading).



Appendix 5.2. Supporting information for the supplementary analyses *Telomere length vary with level of inflammatory markers, but not with proportions of different leucocyte cell types, in two populations of a wild mammal. (In preparation)*

Table S1. Model selection table for relative telomere length. The best fitting models among the set of candidate models considering the inclusion of different leukocyte cell types (i.e. basophil, eosinophil, lymphocyte, monocyte, neutrophil), sex, population (Pop.), body mass (BM) and the interaction 'age x population' are reported. The selected model is indicated in bold.

	I	Age	Basophil	Eosinophil	Lymphocyte	Monocyte	Neutrophil	BM	Pop.	Sex	Age x Pop.	df	logLik	AIC	ΔAIC	w_i
1	**1.122**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**+**	**NA**	**NA**	**7**	**57.615**	**-100.504**	**0.000**	**0.031**
2	1.146	NA	NA	NA	NA	-0.072	NA	NA	+	NA	NA	8	58.451	-99.960	0.543	0.023
3	1.164	NA	NA	NA	NA	NA	-0.006	NA	+	NA	NA	8	58.125	-99.309	1.195	0.017
4	0.991	NA	NA	NA	NA	NA	NA	0.005	+	NA	NA	8	58.104	-99.266	1.237	0.017
5	1.118	NA	0.239	NA	NA	NA	NA	NA	+	NA	NA	8	57.965	-98.988	1.516	0.014
6	1.131	NA	NA	NA	NA	NA	NA	NA	+	+	NA	8	57.956	-98.972	1.532	0.014
7	1.115	NA	NA	0.069	NA	NA	NA	NA	+	NA	NA	8	57.903	-98.864	1.640	0.014
8	1.141	NA	NA	0.091	NA	-0.081	NA	NA	+	NA	NA	9	58.938	-98.693	1.811	0.012
9	1.147	NA	NA	NA	-0.011	NA	NA	NA	+	NA	NA	8	57.810	-98.680	1.824	0.012
10	1.108	0.003	NA	NA	NA	NA	NA	NA	+	NA	NA	8	57.778	-98.614	1.889	0.012
11	0.949	NA	NA	NA	NA	NA	NA	0.008	+	+	NA	9	58.857	-98.530	1.974	0.011
12	1.157	NA	NA	NA	NA	-0.074	NA	NA	+	+	NA	9	58.848	-98.513	1.991	0.011
13	1.143	NA	0.247	NA	NA	-0.073	NA	NA	+	NA	NA	9	58.827	-98.470	2.034	0.011

Table S2. Model selection table for relative telomere length. The best fitting models among the set of candidate models considering the inclusion of inflammatory markers levels (i.e. alpha1-, alpha2-, beta-globulin and haptoglobin), oxidative damages (i.e. malondialdehyde, MDA), sex, population (Pop.), body mass (BM) and the interaction 'age x population' are reported. The selected model is indicated in bold.

	I	Age	Alpha1-globulin	Alpha2-globulin	Beta-globulin	BM	MDA	Pop.	Sex	Age x Pop.	df	logLik	AIC	ΔAIC	w_i
1	**1.285**	**NA**	**-0.056**	**NA**	**NA**	**NA**	**NA**	**+**	**NA**	**NA**	**9**	**63.154**	**-107.224**	**0.000**	**0.053**
2	1.156	NA	-0.052	NA	NA	0.005	NA	+	NA	NA	10	63.541	-105.748	1.476	0.026
3	1.259	NA	-0.058	NA	NA	NA	0.004	+	NA	NA	10	63.479	-105.624	1.600	0.024
4	1.282	NA	-0.052	NA	NA	NA	NA	+	+	NA	10	63.436	-105.538	1.685	0.023
5	1.256	0.003	-0.052	NA	NA	NA	NA	+	NA	NA	10	63.435	-105.536	1.688	0.023
6	1.305	NA	-0.049	NA	-0.006	NA	NA	+	NA	NA	10	63.318	-105.303	1.921	0.020
7	1.295	NA	-0.056	-0.002	NA	NA	NA	+	NA	NA	10	63.169	-105.006	2.218	0.018