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Encounter and compatibility filters, linking personality to parasitism in roe deer (*Capreolus capreolus*)

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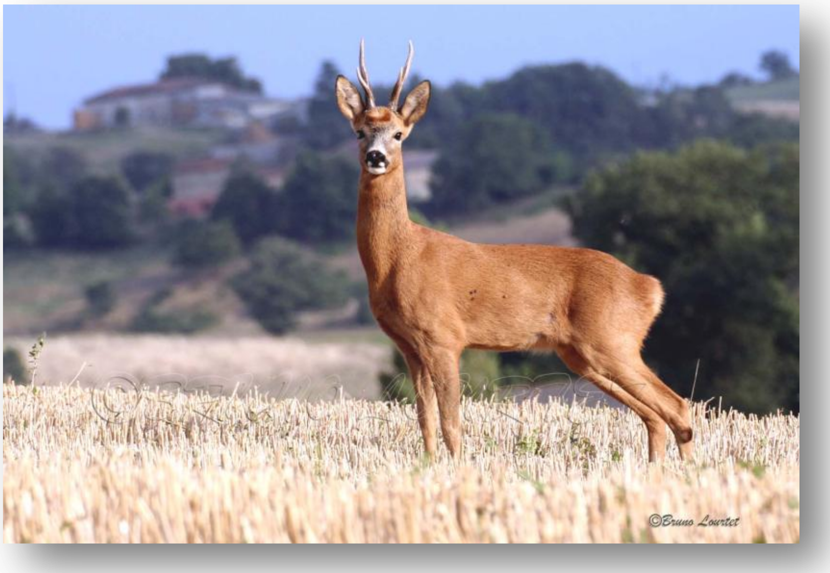

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Sommaire

Abstract:

Studying relationships between hosts and parasites is of great interest, to understand function and dynamics of host populations. To avoid infection hosts can act on encounter and compatibility filter. The aim of this study was to see if personalities could influence parasite infection, by acting on encounter (here habitat) and compatibility filter (here immunology). Personalities have been taken through proactivity-reactivity axis, and average level of activity. The study was conducted on a population of roe deer monitored for several years. Infection by 3 different parasites has been explored: *Toxoplama gondii*, *Babesia.sp* and gastrointestinal nematodes. Correlations between parasite infection and personality have been revealed. Reactive individuals had a higher probability of getting infected by *Babesia.sp* than do proactive individuals. Path analyses revealed for infection by *Toxoplasma gondii* that proactivity-reactivity axis could act jointly on encounter and compatibility filter. Proactive individuals had a higher probability, by being in open habitats, of being infected by *Toxoplasma gondii*. They also showed to have a higher level of specific induced immune response. However some aspects of results suggest parasitic host changes. Perfecting this approach could improve parasites and diseases control.

Key words: Proactivity-reactivity axis, encounter filter, compatibility filter, roe deer (*Capreolus capreolus*), path analysis

Résumé:

La compréhension de la dynamique et du fonctionnement des populations, passe par l'étude de la relation hôte-parasite. Les hôtes peuvent éviter l'infection en jouant sur le filtre de rencontre et de compatibilité. Le but de cette étude était de voir si différents axes de la personnalité pouvait influencer l'infection en jouant sur ces deux filtres représentés ici par l'habitat et la réponse immunitaire. L'axe de proactivité-réactivité et l'activité moyenne ont été pris comme traits de personnalité. L'étude a été conduite sur une population de chevreuils suivis depuis des années. L'infection par 3 parasites est explorée : *Toxoplasma gondii, Babesia.sp* et des nématodes gastro-intestinaux. Cette étude a permis de mettre en évidence des liens entre personnalité et parasitisme chez le chevreuil. Les individus dits réactifs ont une plus grande chance d'être infecté par *Babesia.sp* alors que les individus dits proactifs sont plus susceptibles d'être infectés par *Toxoplasma gondii.* De plus l'utilisation de l'analyse de pistes à permis de mettre en évidence un effet de l'axe proactif-réactif sur le filtre de rencontre et de comptabilité conjointement. Ainsi des individus proactifs en étant plus en milieux ouverts, ont une plus grande probabilité d'être infecté par *Toxoplasma gondii.* Les proactifs sont notamment ceux qui développent plus fortement la branche spécifique induite de la réponse immunitaire. Certains aspects des résultats laissent penser à des modifications immunitaires et comportementales induites par les parasites. La personnalité pourrait, de ce fait, être prise en compte dans l'épidémiologie et le contrôle des maladies et parasites.

Mots-clefs : Axe proactif-réactif, filtre de rencontre, filtre de compatibilité, chevreuils (*Capreolus capreolus*), analyse de piste

Thanks

I declare on my honour the authenticity of these words.

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INTRODUCTION:

Parasites are largely ubiquitous on earth and all living organisms are involved in parasitism either as hosts or parasite themselves (Price 1980 cited in Combes 2001, Poulin & Morand 2000). Parasites are classified into macro- (e.g. tapeworms, nematodes, trematodes, ticks) and micro-parasites (e.g. bacteria, viruses, protozoan) and can be intra- or extracellular. They impact the demography and survival of host (Hudson *et al.* 1998). There is a great interest in studying the relationships between host and parasite, thus,to understand function and dynamics of host population. Studying those relationships has especially been done by exploring the intrinsic (host characteristic) and extrinsic (environmental) factors that influence parasite infections among host (Cardon *et al.* 2011, Brunner and Ostfeld 2006). Environmental factors such as climate and sorts of habitats influence the survival of parasites free-living stage and thus the risk of exposure for the host. The infection risk is related to the host ability to avoid or struggle against parasites. It could be done through encounter filter by killing vectors or parasite in environments and/or avoiding contact in the environment with infesting stage of parasites. Once parasites break through the encounter filter, defences occur through the compatibility filter thus by struggling against parasites (Combes 2010). A growing number of studies have focused on how behaviour could influence transmission by contact rates (Côté & Poulin 1995, Ezenwa 2004, Boyer *et al.* 2010). Others have explored links between behaviours, host’s physiology and how it could impact it susceptibility to parasites (Creel 2001, De Groot *et al.* 2001, Sapolsky 2005). Surprisingly, few studies have yet explored the two processes at the same time, possibly generating positive or negative co-variation between exposure and susceptibility to parasites. Behaviours by influencing a host’s exposure to parasites might also simultaneously increase or decrease physiological susceptibility (Hawley *et al.* 2011). In other words, behaviours might act on the encounter filter through contact rate and simultaneously act on the compatibility filter through susceptibility. Personalities, or inter-individual differences in behaviour, consistent over time and or across situations (Koolhass *et al.* 1999, Réale *et al.* 2007, Sih *et al.* 2004), may result in co-variation within individuals between exposure and susceptibility (Barber & Dingemanse 2010). Personality is expressed on several continuous axes (Réale *et al.* 2007):

- Shyness/ boldness: characterising an individual’s reactions to a perceived risk (e.g. predators)

- Exploration/ neophobia: defining an individual responses to a novel situation (e.g. food, habitat, object)

- Activity: the general level of activity, but not in response to a novel situation.

- Sociability: individual reactions towards conspecifics

-Aggressiveness: Tendency to attack other individuals.

Inter-individual differences in behaviour are also expressed with different response to stress called copying style (Koolhass 1999, 2008). It corresponds to “a coherent set of behavioural and physiological stress response which is consistent over time and which is characteristic to a certain group of individuals” (Koolhaas 1999). Two different copying styles are recognized as the proactive one and reactive one, based on different stress reaction patterns. Proactive individuals are those who cope actively with a source of stress by adopting flight or fight responses and by being more aggressive, whereas reactive individuals tend to freeze and bemore passive and thus have a lower reaction toward stress. It concerns as well differences in physiology as in neuro-endocrinology. Proactive individuals display a higher sympathetic response, lower parasympathetic response and Hypothalamus-Pituitary-adrenal (HPA) reactivity, while reactive individuals have a high parasympathetic response and HPA reactivity.

There is more and more evidences for connections between behavioural, physiological and life-history traits and personality starts to be integrated in the Pace of Life Syndromes (Réale *et al*. 2010). Pace of life syndromes corresponds, to co-evolution between life history traits (e.g. reproduction, growth rate, and survival) and physiological traits (e.g. metabolism), due to trade-offs. The theory of Pace of Life Syndromes is thus associated to the classic concept of r and k strategy (Mac Arthur & Wilson 1967, Reznik *et al*. 2002).Thus it is reduced to an continuous axis going from fast-living species (high reproductive rates, low investment per offspring, low survival) to slow-living species (low reproductive rates, high investment per offspring, higher survival). At the individual level, some studies have shown correlation between life history and personality (Réale *et al.* 2009, Biro&Stamps 2008, Kontiainen *et al*. 2009), others with metabolic rate (Careau *et al.* 2008). As an example, Smith and Blumstein 2008 showed that boldness is associated with high reproductive success and low survival associated with a fast lifestyle. In return, high metabolic rate needs to supports fast lifestyle (Careau *et al*. 2008). Within personality framework, behavioural traits can be inter-correlated, as well and it is called behavioural syndromes. As an example aggressiveness is correlated with activity, boldness, fast exploration and proactivity (koolhaas 1999, Sih *et al*. 2004, Sih & Bell 2008, Réale *et al.* 2009). To summarize, according to Réale *et al.* 2010, fast-living individuals that tends to be more proactive, bold, active, explorative, and aggressive will have higher growth rate, shorter survival and earlier reproduction but also a low HPA axis reactivity, high sympathetic reactivity, high metabolism and conversely for reactive, shy, sociable, non aggressive, less active individuals.

In addition, others proposed to link personality, immunity and parasite (Koolhaas 1999, Koolhass 2008, Lee 2006) in the pace of life syndromes. Boyer *et al*. 2010 showed that more active and more explorative Siberian Chipmunks use a larger home range and are more infected by ticks, due to higher encounter rates.

Although in some systems, personality may also be immunomodulatory (Hessing *et a.l* 1995, Rantala & Kortet 2004, Ahtiainen *et al.* 2006). Dominant male crickets (more aggressive, bold and active Kortet & Hedrick 2007) showed more encapsulation rate and lytic activity (Rantala & Kortet 2004). Dominant wolf spiders showed higher lytic activity (Ahtiainen *et al*. 2006). Immune defences have different energetic costs (Sheldon and Verhulst 1996) with non specific and constitutive defences being fast to activate, cheap to develop but costly to use. Conversely, specific (direct against specific antigen) and induced defence are costly to develop but cheap to use once developed on a long term basis. In mammals an increase of 10% to over 50% in metabolic rate, have been estimated for the deployment of immune responses (Lochmiller & Deerenberg 2000). That is why, energetic balance of individual should be considered. Constitutive responses represent immune cells present before any contact with specific antigen stimulation (and parasite) as natural antibodies or phagocytic cells. Induced immune response corresponds to immunity that is developed due to a contact with an antigen for example expansions of B or T lymphocytes. Both constitutive and induced defences could be subdivided in cellular (lymphocytes, macrophage) and humoral (antibodies, complement proteins) immune responses. Trade-offs between cellular and humoral components of immunity exist (Johnsen & Zuk 1999). Lymphocytes helpers Th1 and Th2 are part of the induced response, although they are proved to be negatively correlated between each other due to their costs. Th1 cells struggle more against intracellular parasites and carry out cell mediated immunity and inflammatory cytokines, while Th2 cells struggle against extracellular parasites and carry out humoral mediated immunity and non inflammatory cytokines. Some studies showed that innate immunity, which relies on strong cellular response, is costly to use. In contrast, the cost of induced response is mainly due to its development (Klasing 2004).According to available energy some studies suggest that different immune components may be selected within and between species through pace of life syndromes (Lee 2006, 2008). In fact, Lee 2006 suggests that “fast-living “species or individuals due to investment in current reproduction more heavily than in survival, will have a higher non specific innate inflammatory (Th1 emphasis) immune response. She suggests, conversely, that slow living species or individuals, by favouring survival, will have a higher non inflammatory (Th2 emphasis) specific induced immune response. In neotropicals birds, slow life history species are linked to a higher antibody mediated response (induced immunity) whereas fast life history species are linked to higher non specific innate response (Lee, 2008). From an individual point of view, Hessing *et al.*1995 showed that reactive pigs have a higher humoral induced immune response while proactive pigs have a higher innate immune response.

The influence of behaviour on exposure versus susceptibility has largely been considered separately (Hawley *et al.* 2011). Thus individuals personality traits, associated with metabolism and immunity, may affect an individual parasite’s exposure and resistance (Kortet *et al.* 2010).

The aim of this study is to explore the links between personality, that is consistent individuals differences in behaviour (i.e. activity, reactivity to stress associated also to boldness), immune response, habitat use and parasitism. The study will be conducted on the European roe deer (*Capreolus capreolu*s), an omnipresent wild herbivore. These animals have an expanding range and are abundant due to global changes. They carry infectious diseases that can be transmitted to humans or domestics animals, through ticks’ vector or environmental transmission. That is why it makes it important to study the host-parasite relationships within roe deer

We supposed personalities could act indirectly through encounter filter (i.e. habitat use) and/or compatibility filter (i.e. immune response) on parasite infection.

Through compatibility filter:

Our assumptions is that proactive individuals (as bold and active), by having higher energy intake, invest more in non-specific innate and inflammatory immune response that require high instantaneous energetic investment. As this sort of immunity response struggles especially against intracellular parasite, we supposed that proactive individuals will be more parasitized by extracellular parasites. On the contrary, reactive (as shy and less active) individuals should invest more in specific induced, and non inflammatory immune response, that is less costly and gradually acquired. As this immune response struggles particularly against extracellular parasites, we expect that reactive individuals will be more parasitized by intracellular parasites.

Through encounter filter:

Proactive, bold and active individuals are supposed to be closer to habitations (open habitat) thus having higher probabilities of being infected by *Toxoplasma gondii* a protozoan parasite whose definitive hosts are felines (Dubey, 1998). Reactive, shy and less active individuals are supposed to live more in forest having, thus, a higher probability of infection by parasites transmitted by ticks, as ticks are more present in forest (Gilot *et al.* 1975).

MATERIALS AND METHODS:

The wild roe deer's monitoring is a long term study which takes place in Aurignac, a town located in Southwest France (N 43°27', E 0°85'). This site, of nearly 12 000 ha, has mixed landscapes composed of open fields, small woods patches (mean ± SD: 2.9 ± 7.6 ha) and two forest blocks (672 and 575 ha). Meadows and cultivated fields occupy respectively, 31.9 and 36.2 %, whereas woodland covers 19.3 % of the total study site (see Hewison *et al*. 2009 for details). The study site is partly consecrated to agriculture and livestock farming and is scattered by habitations and road networks.

The capture

The Capture-Mark and telemetry method has been applied each year for fifteen years (2001 to 2016) during winter. This method allows to estimate a deer density of 9.3 deer/100 ha in the open landscape (Hewison *et al.* 2007), and two at three times higher in the forest blocks (unpubl. data). Eleven capture sites were spread across the study area. Sites can be classified as closed and open landscapes. Roe deer are known to be highly sedentary (Strandgaard 1972, Hewison *et al*. 1998); it is supposed that individuals were consistent in their habitat use over years. An open habitat is defined just as what was not forest or hedgerows. It consists of crops, meadows, and scrubland (see Morellet *et al*. 2011, for more details).

Captures are conducted, each year in winter, thanks to CEFS employee, schools and volunteers. The day before the capture part of CEFS team, goes to stretch near 80 nets of two meters height and fifty meters long. Regularly along the nets wooden retention boxes are placed to receive captured roe deer. Around a hundred people are mobilized the day of capture. Roe deer are caught by drive-netting (Beasom *et al*. 1980).

During a capture some will wait discreetly near the stretched nets, while others, the beaters, walk over the site and push the deer in the nets. Once an animal is captured, intramuscular injunction of 0.3 mL (1.5mg) sedative (“acepromazine”) is administrated, in an ethical aim. Then the animal is restrained in a box before being manipulated. Then animals will be manipulated one by one to be marked but also to collect physical, physiological and behavioral samples. In case of a first time animal capture, it will be tagged by 2 ear tags (Tiptag). Then it will have VHF collar for juveniles, a GPS (Lotek 3300) or GSM (Lotek Small WildCell) collar for adult. GPS (and GSM) are programmed to function 11 months after what the collar drop-off and needs to be retrieved to collect the data. At the end the animals will be released in its capture site. Roe deer capture and marking procedures were approved by the French administration, taking into consideration animal welfare.

The total capture’s time for an animal, to its drop in the net from its releasing, is measured.

Roe deer physical and physiological samples

In winter, animals are easily sexed (figure1) by the presence of antlers for the males. Individuals age is estimated thanks to its dentition; indeed the third premolar goes to tricuspid from bicuspid by being adult. Afterwards, determination of age for adults is made by looking the teeth wear, although it is not really accurate That is why, individuals will be classified in three age categories: adult (more than 2 years old), yearling (animal is in its second year) and juvenile (less than one year old).Then animal are weighted, at the nearest 0.1 kg, by calculating the difference between the boxes containing the animals and the empty boxes. Body mass (weight) is known to be tremendously influenced by age and sex. Thus, we generated a standardize measure of body mass. It consists of subtracting the weights mean for an age and sex specific class to the individual values. Then it is possible to compare weight across individuals, thus varying from -4.2 to +4.9 kg.

During manipulation blood, feces samples and vaginal swabs are made. Blood samples permit to determine physiological parameters of individual, but also its infection by pathogens. They are done by jugular veins puncture (10 mL).The sample must be turn upside down around 10 times to avoid the formation of blood clot. There are conserved at 4°C and minimum two hours after the puncture, centrifugation is made during 20 minutes at 3600g. It allows reaping the serum and the plasma. At the end plasma, serum and vaginal swabs are sent to different laboratories to explore physiological and parasitological parameters (See Morau, 2015 for further details).

Physiological data collected

To explore immune response two kinds of tests are done, descriptive one to measure the level of immune parameters, and functional one based on the immune reaction during experimental stimulation. Thanks to blood samples, the distribution of white blood cells can be explored as a descriptive test. White blood cells concentration reflects global levels of immune cells. They can be classified into innate immune response cells (neutrophils and monocytes) and induced immune response cells (lymphocytes and eosinophils). Monocytes play a key role in chronic inflammatory response (Auffray 2009 cited in Jégo, 2015) while neutrophils play a role in acute inflammatory response. Lymphocytes include both T and B cells and thus involve anti-bodies production. Eosinophils reflect activation of Th2 responses (Jolles *et al.* 2008), activated by extracellular parasites.

Concentrations of each different sort of white blood cells are obtained thanks to global white blood cells count (measured by automaton at the ENVT) and their associated proportions (measured by manual reading at the ENVT). Those descriptive tests have the inconvenient of being dependant from the exposure of individuals to infection. Conversely, functional tests are not dependant of previous individuals experiences. Hemagglutination-hemolysis test is realized as a functional test and could be classified as innate humoral immunity. (Proposed by Matson *et al*. 2005 and adapted to mammals in Gilot-fromont *et al*. 2012). This test permits to observe the serum capacity to agglutinate and to lyse cells with an antigen never encountered before. It reflects circulating natural antibodies, which does not require previous exposure to a particular antigen to be present. When natural antibodies recognize antigens, a veil is formed and its concentration can be measured. When lysis occurs on red blood corpuscle, by being destroyed, it releases hemoglobin and its concentration can be measured (Matson *et al.* 2005).

See table 1 for further details

Parasite data collected

Infections by both extra- and intra- cellular parasites are checked since 2008, between early January and February, so that confounding seasonal variations in parasite presence were minimised. Yet, parasites infections are highly dependent of the neighborhood, the humidity and temperature changing throughout the years. Thus, to control annual variation, we generated annual prevalence in the population (Pi) corrected for co-linearity (subtracting the individuals) (see equation).

$Pi = \frac{I-i}{N-1}$, i stands for the individual, I the number of infected individuals, N the total number of individuals (infected and non-infected).

Feces samples allow to search for the presence of 8 nematodes, 1 tapeworm and 1 protozoan (see table). Fecal eggs counts based on McMaster methods permit to investigate nematodes abundance. It is a quantitative method based on floating. Once water is added to feces it is placed on microscope slides and facilitates the count of eggs by grams of feces (Raynaud 1970-cited in Jego 2015). Extracted serum analyzed by ELISA method (detailed in Sevila, 2015) allows the detection of antibodies against 3 protozoa, 4 bacteria and 5 viruses. The investigations of those pathogens were different according to years. Vaginal swabs allow determining the presence of an abortive bacterium (*Chlamydophilia sp)*. Expecting nematodes, each pathogens were measured as presence/absence. It has been decided to only explore *Toxoplasma gondii*, *babesia.sp*, and gastrointestinal nematodes, due to their high prevalence (>30%). See table 2 and 3, for further details.

Toxoplasma gondii (*T.gondii*) is an intracellular protozoan parasite. It is a heteroxenous parasite, with several intermediate hosts (i.e. roe deer, rats, humans) and having for definitive host felines. Its transmission to intermediate host is done by indirect contact (ingestion of aliments or water soiled by infected feces) (see figure 2). For intermediate host among wild mammals, symptoms are a bit perceptible (Gilot-fromont *et al*. 2012) and may cause abortion.

Babesia.sp is a protozoan parasite, transmitted by tick's saliva (see figure 3). Its pathogenecity are not well known although, it is suspected to cause mortality incidence.

Gastrointestinal nematodes are monoxenous parasites with environmental phases and indirect transmission by ingestion of aliments soiled by infected feces (see figure 4). They impact nutriments absorption provoking diarrhea and anemia and are hence correlated with body condition (Body *et al.* 2011, Stien *et al*. 2002)

Personality traits

As the study tries to link parasitism to personality, different personality's traits will be taken into account in a multi-dimensional approach, across different contexts or situations (behavioural syndromes). It will be taken across a context of acute stress that could be seen to as the proactive-reactive axis. It will also be taken in an activity context. Those personality traits are known to be consistent in roe deer (with a repeatability of respectively 0.21, 0.36) (Bonnot *et al*. 2014, Monestier 2016).

Proactivity-reactivity axis is measured thanks to behavioral observations made during captures. Behaviors are observed at different steps: in the net, in the wooden retention box, during marking and at release. During the whole manipulation and at releasing time, observations are made by the same person, thus limiting errors' measures. In the net, it is observed if animals struggle (encoded 0/0.5/1). In the wooden retention box it is observed if animals have turned upside down (encoded 0/1). During handling it is observed if animals struggle (encoded 0/0.5/1). At release the speed of flight behavior (encoded 0/0.5/1) and the scrub collar is observed (encoded 0/1). (See ethogram.1 for details) Behavioral scores are obtained as the mean of these 5 different behavioral items. Mean's behavioral score permits to take into account some individuals having only one missing values out of the five values. The score is a gradient from 0 to 1, from reactivity to pro-activity. The more high the score is, the more the reaction towards stress will be too.

Activity is approximated with individuals' mobility, thanks to localization data. Localization data are obtained through GPS and GSM collars that recorded individuals coordinates (x and y fixes) each hour during April. Differential corrections to improve fixes accuracy are realised (Adrados *et al*. 2002). From these localization data, mean average speed (m/s) is calculated, for each individual as the distance between two successive locations (each hour) divided by the 3600s fix interval. Average speed determines the mobility of individuals.

Statistical data

One of the pre-required was to see if immune response could vary among the roe deer population. Once the pre-required is confirmed, statistical analyses are conducted through 3 steps. At first general models were fitting to explore relations between parasite infection, personalities, immune response, habitat and potential confounding variables. Then, according to these pre-selected models, different models based on our biological hypotheses are tested, thanks to path analysis (Shipley 2000, 2009). To finish, model selection procedure based on AICc re-arranged for path analysis are conducted (Cardon *et al.* 2011, Shipley *et al.* 2013).

Parasite studied will be *T.gondii, Babesia.sp,* gastrointestinal nematodes. Analyses concerning *T.gondii* and gastrointestinal nematodes were conducted from 2009 to 2014. Analyses concerning *Babesia.sp* were conducted from 2012 to 2014 due to its low prevalence the other years (see table 2) (N=46).

1. Immune response

As seven parameters were used to explore immune response, analyzing all possible relationships would lead to redundancies and high type I error. Thus, a Principal Component Analysis (PCA) is first conducted, on scaled variables (N=171). PCA’s axis scores are saved and used as synthetic variable of immune response, in the further analyses.
This analysis has been conducted with the function “dudi.pca” of the package (ade4)

2. Pre-models: exploring the link between habitat, immunology, personality and parasitism

According to our hypothesis, encounter filter (here represented by habitats) and compatibility filter (here represented by immune response) as personality acting on them ,could influence parasite infection. Firstly, to see if these variables could effectively influence parasitism, several generalized linear models (binomial) (function “glm” of “lme4” package) were done for each parasite, as dependant variables. However, Gastrointestinal Nematodes parasite abundance (dependant variable) was log (n+25) scaled (Sévila, 2015) and, thus, linear models were used The habitat, immune responses and personality have been taken as independent variables. Parasites infection could be influenced by age, sex, body mass (Jégo, 2015, Sévila, 2015) and annual prevalence in the population (Pi). Therefore, it has been integrated as confounding variables.

Analyses were conducted on two set of behavioral traits: behavioral score (set 1) and average speed (set 2) for the different parasites.

Models construct are:

Parasite~ mass+ sex+ PCA Axis+ Pi +habitat+ age + behavioral score (set 1)

Parasite~ mass + sex + age+ PCA Axis + Pi+ habitat+ average speed (set 2)

Once models are constructed, model selection based Akaike's Information Criterion corrected for small sample size (AICc; Burnham and Anderson 2002) were conducted. Akaike weights were used to select the model with the most support among models constructed a priori. The model having the lowest AICc value reflects the best compromise between precision and complexity. To have a clearer view on the impact of independent variables, prediction graphs were made thanks to function "visreg" of the library (visreg).

Once correlations among those five variables are determined, path analyses are conducted to determine direct and indirect effect of interest retained variables on parasite infection.

3. Path analyses: determining between indirect and direct effects.

Path analyses are statistical tools that allow testing for direct and indirect relationships in the cause-effect linkages between several variables (Shipley 2002). They required a priori designation of plausible hypotheses of causal relationships between independent and dependent variables (Thomas *et al*. 2007). Path analysis were conducted to see if personality could influence indirectly parasite infections through encounter (here habitat) and/or compatibility filter (here immunology). Binary variables (parasite infection and habitat) prevent multivariate normality and avoid the use of classical structural equations models (Shipley 2000). That is why the path models using Shipley's d-separation test is used (Shipley 2000, 2003, and 2009). Directional-separation test are based on Direct Acyclic Graph: a classical box and arrow diagram (without feedback). This graph permits to determine independence claims: variables not directly linked by an arrow and their causal parents (direct arrow). With the conditional independence of X and Y knowing variables Z1 and Z2, we obtained the null probability (P) that the slope of X was zero in a generalized linear model whose fixed structure is Y~Z1 + Z2 + X.

The whole of K independence claims is called a basis set. The final step is to combine these separate tests of independence into a combined test of the entire model. This is done using Fisher's C statistic (Shipley 2000, 2009).

$C = -2\sum ln(Pn)$, using the K independence claim, (P) is the null probability associated to the each independence claim.

This statistic follows a chi-squared distribution of 2k degrees of freedom if all of the independence claims in the model hold in the data. Lack of significant difference ($p>0.05$) between the observed and predicted pattern of independencies provides supports for the path model. The different models tested in relation with our hypotheses are detailed in figure 5.

As several models could be supported by the data, AIC model selection is then applied to consider the best model. Adapted AIC for path analysis were used (Cardon *et al.* 2011, Shipley 2013) and was calculated as following:

AICc = C+2q*[n/n-q-1), where C is the C-statistics, K the number of independence claims, q the number of free parameters and n the sample size. It was calculated thanks to the function "d-sep.AIC" (Fourtune 2016 -publication in process).

Once the best model is determined, path coefficients are calculated by regressing each variable only with its direct causes (Shipley 2009). It corresponds to partial regression coefficients and permits to appreciate the sense of relation between variables. Confounding variables susceptible to influence the studied variables were taken into consideration for correcting path coefficients, but were not included into our path analyses to simplify it. For immune response age, sex, body mass and capture's stress were taken into account. For habitat, age and sex were taken into account. For parasite infection, confounding variables of pre-restrained models were taken into account.

We standardized path coefficients of continuous variables. Statistical analyses were conducted on the software R 3.2.4

RESULTS:

Variability in immune response:

Following the Kaiser-Guttman criterion, the first two components were the only suitable components to retain (see figure 6 A). Immune response (Axis 1) explained 33.015% of total inertia. Neutrophils and White blood cells looks to covariate positively ($r=0.38$), while lymphocytes is opposed to them ($r= -0.37$ for WBC, $r=-0.98$ for neutrophils). Eosinophils, by their intermediate positions are represented by both Axis 1 and 2 and covariate positively with lymphocyte (r=0.16) and hemagglutination (r=0.15) and negatively with neutrophils (r=-0.26) and WBC(r=-0.12). Correlations with other variables were weak for eosinophils. Concerning Axis 2 (explaining 23.04% of total inertia), hemmaglutination and hemolysis covariate positively (r=0.47), but also with monocytes (r=0.15 for HA, r=0.19 for HL). Immune response (Axis 1) and immune response (Axis 2) are then retained for the rest of the study as synthetic variables and will be named respectively immune response 1 and immune response 2. Thus, positive values for immune response 1 represent high level of neutrophils and WBC whereas negative values represent high level of lymphocytes. Negative values of Axis 2 represent high values of hemagglutination and hemolysis and high concentration of monocytes (see figure 6 B). Immune response 1(axis 1) represents more a continuum of induced from constitutive defenses while immune response 2 (axis2) represents more humoral innate response.

Pre-models: exploring the link between habitat, immunology, personality and parasitism

1. Parasitism by *Toxoplasma gondii*

Models built with behavioural score to express personality (set 1: n=148):

On 256 models tested thanks to dredge function, 9 models had a difference in AICc below 2 units (see table 4). The 9 models are potentially interpretable but here, for the purpose of path analysis, we decided to choose the one with the lowest AICc. Model with the lowest AICc (179.3, df= 5), explained 17% of residual deviance, and has 17% of probability to be the best model.

Behavioural score, immune response 1 and habitat are positively correlated with the probability of individual to be infected by *T.gondii*. Confounding effect of Pi (annual prevalence in the population) is also positively correlated with individual probability of infection. Probability of being infected by *T.gondii* increased with the behavioural score and the immune response 1 of the individual. Thus, individuals that highly react to stress and individuals who use an immune based on lymphocytes have a higher probability of being infected. In addition, individuals caught in open habitats have a higher probability of being infected by *T.gondii* than those captured in closed forest habitats. Finally the more the prevalence in the population is important the more the probability of being infected by *T.gondii is* increased (figure 7).

Thus behavioural score, immune response 1 and habitat are retained for the path analysis. Confounding variables Pi won't be included in the path selection, but path coefficients will be calculated taking account for this effect.

Models built with mobility (average speed) to express personality (set2: n=74):

On 256 models explored, 6 models had a difference in AICc below 2 units (Table 6). The 6 models are potentially interpretable but here in the aim of path analysis, model with the lowest AICc (95.4, df = 6) was retained. It has a probability of 27% to be effectively the best model. It explains 24% of deviance and includes mobility, habitat, and immune response 1 as interest variable (see figure 10 for details). The probability of being infected by *T.gondii* decreases when the average speed of individuals increases. The probability of being infected by *T. gondii* increased with the body mass. Immune response 1 and habitat have the same effect found in the previous model with 148 individuals (see figure 8).

Hence all the variables of interest (mobility, habitat, immune response 1) are retained and path analyses can be conducted. Confounding factors as body mass and Pi will not be integrated in the Path analysis but will be taken into account for the path coefficient.

2. Parasitism by *Babesia.sp*

Models built with Behavioural score to express personality (set 1: n=46)

On 256 models explored, 8 models were retained with a difference in AICc below 2 units (table 6). The model with the lowest AICc (45.6, df=6) is retained, with 17% of probability to effectively be the best model, with six degree of freedom and explained 41% of deviance. It represents the effect of mass, sex, behavioural score, Pi and Habitat. Probability of being infected by *Babesia.sp* decreased when individuals had high behavioural score (proactive) and were in open habitats. Males have a higher probability of being infected by *Babesia.sp* like individuals with low body mass. Probability of an individual to be infected by *Babesia.sp* increased with the annual prevalence of *Babesia.sp* in the population. See figure 9.

As the immune responses (1 and 2) were not retained, no path analyses will be conducted to see if personality has an indirect effect on infection through habitat or immune responses.

Models built with mobility (average speed) to express personality (set 2: n=19):

On 256 models explored, 9 models were retained with a difference in AICc below 2 units (table 7). The model with lowest AICc (22.7,df=2) is retained with only the animal sex variable susceptible to have an effect on being infected by *Babesia.sp*. This model has a probability of 19% to be effectively the true model and explained 22% of deviance. Male have higher probability of being infected by *Babesia.sp* than females.

3. Parasitism with gastrointestinal nematodes

Model built with behavioural score to express personality (set 1: n=149)

On 256 models explored 8 models with delta AIC below 2 units were retained (table 8). The model retained is the one with lowest AICc (421.1, df=4) and have a probability of 23% to be the best model. The more the body mass is low the more gastrointestinal nematodes are abundant. The more the immune response 1 is high the more gastrointestinal nematodes are abundant (Figure 10). Nonetheless with a R squared of 0.03, it is probably indicating that chosen variables of interest are not the best ones.

Model built with mobility (speed) to express personality (set 2: n=75):

On 256 models explored 16 have a difference in AICc below 2 units, however the model with the lowest AICc is the null model. Thus, it reveals that other selected models do not have any support, and that the explanatory variables seem not to be the adequate one. No variables of interest for the path analyses have thus been retained

Path analyses and model selection

1. Personality expressed as Behavioural score (n=148):

Seven path models were tested according to our hypothesis. Only one model was rejected due to C-statistics not following a chi squared distribution ($p<0.05$). Model g, modelling an indirect effect of behavioural score on immune response and infection by toxoplasma gondii through habitat, was rejected ($p<0.01$, see table 9). The six others models were notrejected because their C-values did not statistically differed from a chi-square distribution (table 9). Among them, four models have a delta AIC lower than two and thus were retained. There were 2 models with the lowest AICc (22.14): model b and c. It represents an indirect effect of proactivity (behavioural score) on infection, through immune response (model b) and through both immune response and habitat (model c). Figure 11 shows the standardized path coefficient (+/- SE) and the direction of action for the relationships between behavioural score and infection by T.*gondii*. Proactive (high behavioural score) individuals were more in open habitats and have a higher probability of being infected by T.gondii. More proactive individuals have less immune response 1, although immune response 1 increases with the probability of infection.

2. Personality expressed as mobility (n=79)

On seven path models tested, no models were rejected with the C-statistics, however only two models had a difference in AICc lower of two units. Model a, is the one with the lowest AICc (19.14) (table 10). It corresponds to the effect of individuals' mobility on infection by *T.gondii* through the habitat and the direct effect of immune response on *T.gondii*. No direct individuals mobility effects on immune response have been retained. In Figure 12, there are standardized path coefficients (+/-SE) and the direction of their action in linking individuals mobility to infection by *T.gondii*. More mobile individuals were more in open habitats and had a higher probability of being infected by *T.gondii*. More mobile individuals were not linked to immune response. Finally immune response 1 increases with the probability of being infected.

DISCUSSION:

The aim of this study was to explore the link between personality and parasitism. We expected that personality could act indirectly on parasite infection through habitat use (encounter filter) as well as on immune response (compatibility filter). However, we first needed to verify that immune response could vary within the roe deer population. We expected contrasts between constitutive versus induced immune response and cellular versus humoral components.

Contrast in immune response

Our results showed a contrast in immune response with a first axis accounting for cellular immunity and the second axis account for humoral immunity. Moreover, the results suggest a compromise between non-specific innate inflammatory cellular response (neutrophils and overall white blood cell increase) and induced cellular response (lymphocytes). Variation in lymphocyte could be attributed as part of induced (lymphocyte B &T helper) and innate responses (Lymphocyte T cytotoxic). As variations in lymphocytes cannot be separated, this variable is not a straightforward indicator of induced response. Some additional indicators should been taken into account in the future, as gamma-globuline known to be strictly part of induced immune response.

Second PCA axis explained more innate humoral immunity with hemagglutination (HA) and hemolysis (HL) ability. Indeed, HA-HL ability is part of innate response but coming from the humoral components, explaining their presences on others axes. Monocytes co-varying with HA-HL, is a cell-mediated innate response and thus is supposed to co-vary with neutrophils. However, monocytes do not contribute greatly on describing immunity on PCA Axis2. This could be due to their relative scarcities in the blood formula. Eosinophils were also not interpretable due to their intermediate position on the axes. There are indicators of Th2 responses, known to be humoral adaptive components of immune response. This could explain their intermediate position between axis 1 (innate/induced, cellular defence) and 2 (humoral innate defence.)

Similar studies have been done on roe deer in between different populations, individuals with high body conditions were having more non-specific inflammatory innate immunity while pattern was not clear for induced immunity (Gilot-fromont *et al.* 2012). In the future, further indicators of immunity should be taken into account to discriminate easily the different immune response between individuals. Nonetheless, we were able to discriminate contrasted immune response, in between non-specific innate inflammatory cellular response (neutrophils and overall white blood cell increase), induced response (lymphocytes) and humoral innate response. This allows us to test our predictions about inter-individual differences in compatibility filter.

Link between personality and parasitism

We expected that proactive individuals, by using more open habitat will be more infected by *T.gondii*, a parasite excreted by felines (Dubey 1998). Conversely reactive individual, inhabiting more closed forest habitats, will be more infected by *Babesia.sp*, a protozoan transmitted by ticks which are more present in forests (Gilot *et al.* 1975). We also expected that proactive individuals would invest more in non-specific innate and inflammatory cellular immunity, that do better against intracellular parasites. On the contrary, reactive individuals would invest more in specific induced and non inflammatory immunity that do better against extracellular parasites.

As expected, we found a link between parasitism and habitat use (encounter filter), immunity (compatibility filter) and personality for *T.gondii*. A link between parasitism, habitat use and personality was found for *Babesia.sp*, but no link has been found for gastrointestinal nematodes. Considering these results, path analyses were used to understand if personality could act jointly on the encounter and the compatibility filter.

In the wild, multiple infections are a common phenomenon. Co-infecting parasites will influence each others. Parasites could benefit of already infected host which are more vulnerable (Schmid-Hempel 2011). It is an interesting aspect, however this study is a first approach and to simplify it parasites were treated separately.

1. *Toxoplasma gondii*:

Correlations with parasitism were found for 2 personality traits, a response to stressful events (proactivy- reactivy axis) and an average level of activity (mobility). Proactive and less mobile individuals were having higher probability of being infected by *T.gondii*. Path analyses showed that both personalities' traits could act through encounter filter and/or compatibility filter on parasite infection.

The action of personality on compatibility filter was only relevant for the proactivity-reactivity axis. While proactivity-reactivity axis is also link to encounter filter, this relation seems less powerful. Indeed this model cannot be discriminated from a model with just action of personality through compatibility filter. This could probably due to a representation of habitat too simplified (open/closed), more detailed measures should be taken into account for further studies. However this model stays one of the best models. Thus, proactive individuals by being more in open habitats had a higher probability of being infected by *T.gondii*. It is in line with higher exposure to cats' faeces in open habitats (Sévila 2015). Proactive individuals have less non specific inflammatory innate response, while this immune response was higher with high probability of infection. These results should be interpreted keeping in mind that correlation between immune response and pathogen resistance is pathogen-dependant (Adamo, 2004). Moreover neutrophils, included in non specific inflammatory innate response, are known to be part of inflammatory defence against *T.gondii* (da Silva *et al.* 2009). The effect of personality and infection by *T.gondii* should probably be considered jointly to explain immune response. However, the negative relationships between pro-activity and non specific inflammatory innate response are contrary to our expectations. It is challenging to measure immunity in the wild and acute stress, like capture event, can modify the leukocytes counts and ratio (i.e. lymphocytes, neutrophils) (Davis *et al.* 2008). Thus, it may not reflect the real immune response.

Monestier 2016 showed that more mobile individuals were also the proactive one. Thus we expected that both mobile and proactive individuals will have a higher probability of being infected by *T.gondii.* Contrary to our hypothesis, more mobile individuals had a lower probability of being infected by *T.gondii*. Nonetheless, path analyses revealed that mobile individuals were using more open habitat thus generating a higher probability of infection. Hence, indirect effect of mobility on infection and negative correlation between mobility and infection seems contradictory. This negative relationship could probably be due to a direct effect of *T.gondii* on mobility. Indeed, parasites are known to manipulate host behaviour (Biron *et al*. 2005, Thomas *et al*. 2010, 2010, Poulin 2013). It is particularly the case of *T.gondii* (Berdoy 2000, Webster 2007, Vyas 2007, Poirotte *et al.* 2016). Rats once infected by *T.gondii* have shown a higher level of activity, exploration and altered risk predation perception. It makes them being more vulnerable to predation attack by domestic cats, to ensure completion of parasite life cycle (Webster 2007). Roe deer is an intermediate host predated by lynx being the definitive host of *T.gondii*. It is possible that less mobility, for roe deer, makes them vulnerable to lynx attack and thus completing parasite life cycle. Moreover, according to Poulin 2013, parasites could decouple co-variation between personality traits. Thus behavioural traits that were co-varying positively could be co-varying negatively due to infection. Nonetheless, method used for our data (d-separation tests) did not permit to add the hypothesis of host behaviour manipulation. However, even though path analyses did not reject our supposed causal model, additional experimental studies are needed to test correlation identified here.

2. *Babesia.sp*:

Babesia.sp is a protozoan transmitted through ticks' vectors, which are more present in forests (Gilot *et al.* 1975). Reactive individuals are supposed to live more in closed habitats (forest) and thus, to encounter more Babesia's vector. As expected, reactive individuals had a higher probability of being infected by *Babesia.sp*. Moreover, results showed that being in closed forest habitats increased the probability of being infected by *Babesia.sp*. As shown thanks to path analyses on a larger sample size, the more the behavioural score is high the more individuals lived in open habitats. Thus, reactive individuals (low behavioural score) have a higher probability of being in closed habitats and to be infected by *Babesia.sp*. Proactivity-reactivity axis seems to act on infection by *Babesia.sp* through encounter filter (habitat) Probability of being infected by *Babesia.sp* does not appear to vary according to immune response. *Babesia.sp* being an intracellular parasite, we were expecting that individuals having higher probability of infection will have a higher non specific inflammatory innate response. A study led on mice has shown that immune differences were correlated with *Babesia.sp* resistance (Barnard *et al.* 1994). In fact aggressive, high ranked individuals were shown to have lower gamma-globuline (induced immunity) and reduced resistance to *Babesia.sp*. No similar results have been found, once again, representation of immune response in our study could be a bad representation of immunology. Even though immune response correlation has not been revealed, we have to consider that our sample size is small.

For these reasons the indirect effect of personality through encounter and compatibility filters on infection could have not been confirmed through path analyses.

Concerning the potential hypothesis of host behavioural manipulation by *Babesia.sp,* to our knowledge no studies have yet been done on it.

3. Gastrointestinal nematodes:

We supposed that proactive and mobile individuals invest in a non specific inflammatory innate immunity which struggles more against intracellular parasite. Hence we suppose they will be more infected by extracellular parasite. Gastrointestinal nematodes are extracellular parasites, however no link between personality and infection caused by them have been found. Gastrointestinal nematodes are the only quantitative variable that could characterize parasite abundance in this study. As found in the literature, the more an individual is parasitized the more his body mass is light (Jégo, 2015). As expected, individuals with a higher non specific inflammatory response were more infected by gastrointestinal nematodes. Immunocompetence has been linked to lower gastrointestinal nematodes load, in roe deer and goats (Navarro-gonzalez *et al.* 2011, Hoste *et al.* 2010). Nonetheless the best model to explain parasite abundance had a low explanatory power (R^2=0.03). The chosen variables do not explain sufficiently nematodes abundance and further variables are needed to be considered. In goats and sheep, gastrointestinal nematodes count is influenced by food selection with direct antihelmintic properties (Egea *et al.* 2014, Hoste *et al.* 2010). Thus, potential future variables such as food selection should be included in our model. Moreover gastrointestinal nematode is a large group including specific and non specific parasite, a more precise subdivision should be considered in further studies.

Conclusion:

As shown here, there is a correlation between personalities and parasitism in roe deer. Personality seems to act jointly on compatibility filter and encounter filter for *T.gondii*. Epidemiological studies take into account parasite distributions in a population and between populations. However individuals' differences in behaviour and immunology are not yet integrated in epidemiological study. Our measure of immune response should be ameliorated by incorporating more components of immunity to better distinguish, between innate versus induced responses and cellular versus humoral components. Finally our use of path analyses must be improved. Indeed it will be interesting to compare the strength of associations found thanks to the path coefficients, even with different kinds of variables.

This study was a first approach and it is difficult to attribute causality in these associations (Dunn *et al.* 2011). This is a common chicken-egg dilemma that only starts to be explored between host characteristics and parasites (Blanchet *et al*. 2009). It is known that personality can influence immunology (Koolhass 2008, Hessing *et al*. 1995) but that immunology can influence personality as well (Butler *et al.* 2012). Parasites are known to influence hosts behaviours but also immunology (Lafferty & Shaw 2013) and immunology is known to influence susceptibility to infection. The perfect model is probably a “pell-mell” of positive and negative feedbacks, possible to untangle by fixing each components through experimentations. Path analyses is more and more used to distinguish indirect versus direct effect (Boyer *et al.* 2010, Cardon *et al.* 2011, Costantini *et al.* 2012), or even to try to resolve the chicken eggs dilemma (Blanchet *et al.* 2009). Yet, this kind of analyses required having a particular set of data as continuous variables to consider complex patterns. Perfecting this approach could improve parasites and diseases control. Studies have already shown that concentrating efforts on individuals having an important role could diminish infections risks (Woolhouse 1997, Perkins *et al.* 2003 cited in Boyer *et al.* 2010).

REFERENCES :

ADRADOS, Christophe, GIRARD, Irène, GENDNER, Jean-Paul, *et al.* Global positioning system (GPS) location accuracy improvement due to selective availability removal. *Comptes Rendus Biologies*, 2002, vol. 325, no 2, p. 165-170.

AHTIAINEN, Jari J., ALATALO, Rauno V., KORTET, Raine, *et al.* Immune function, dominance and mating success in drumming male wolf spiders Hygrolycosa rubrofasciata. *Behavioral Ecology and Sociobiology*, 2006, vol. 60, no 6, p. 826-832.

AUFFRAY, Cedric, SIEWEKE, Michael H., et GEISSMANN, Frederic. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annual review of immunology*, 2009, vol. 27, p. 669-692.

BARBER, Iain et DINGEMANSE, Niels J. Parasitism and the evolutionary ecology of animal personality. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 2010, vol. 365, no 1560, p. 4077-4088.

BERDOY, Manuel, WEBSTER, Joanne P., et MACDONALD, D. W. Fatal attraction in rats infected with Toxoplasma gondii. *Proceedings of the Royal Society of London B: Biological Sciences*, 2000, vol. 267, no 1452, p. 1591-1594

BIRON, David, PONTON, Fleur, et THOMAS, Frédéric. Parasitic manipulation: where are we and where should we go?. *MS Medecine Sciences*, 2005, vol. 21, no 12, p. 1022-1023.

BEASOM, Samuel L., EVANS, Wain, et TEMPLE, Larry. The drive net for capturing western big game. *The Journal of Wildlife Management*, 1980, vol. 44, no 2, p. 478-480.

BIRO, Peter A. et STAMPS, Judy A. Are animal personality traits linked to life-history productivity?. *Trends in Ecology & Evolution*, 2008, vol. 23, no 7, p. 361-368.

BLANCHET, Simon, MÉJEAN, Lionel, BOURQUE, Jean-François, *et al.* Why do parasitized hosts look different? Resolving the “chicken-egg” dilemma.*Oecologia*, 2009, vol. 160, no 1, p. 37-47.

BODY, Guillaume, FERTÉ, Hubert, GAILLARD, Jean-Michel, *et al.* Population density and phenotypic attributes influence the level of nematode parasitism in roe deer. *Oecologia*, 2011, vol. 167, no 3, p. 635-646.

BONNOT, Nadège, VERHEYDEN, Hélène, BLANCHARD, Pierrick, *et al.* Interindividual variability in habitat use: evidence for a risk management syndrome in roe deer?. *Behavioral Ecology*, 2015, vol. 26, no 1, p. 105-114.

BURNHAM, K. P., *et al.* dr AnderSon. 2002. Model selection and multimodel inference: a practical information-theoretic approach.

BOYER, Nelly, RÉALE, Denis, MARMET, Julie, *et al.* Personality, space use and tick load in an introduced population of Siberian chipmunks Tamias sibiricus. *Journal of Animal Ecology*, 2010, vol. 79, no 3, p. 538-547.

BRUNNER, Jesse L. et OSTFELD, Richard S. Multiple causes of variable tick burdens on small-mammal hosts. *Ecology*, 2008, vol. 89, no 8, p. 2259-2272.

BUTLER, Michael W., TOOMEY, Matthew B., MCGRAW, Kevin J., *et al.*Ontogenetic immune challenges shape adult personality in mallard ducks.*Proceedings of the Royal Society of London B: Biological Sciences*, 2012, vol. 279, no 1727, p. 326-333.

CARDON, Maxime, LOOT, Geraldine, GRENOUILLET, Gael, *et al.* Host characteristics and environmental factors differentially drive the burden and pathogenicity of an ectoparasite: a multilevel causal analysis. *Journal of Animal Ecology*, 2011, vol. 80, no 3, p. 657-667.

CAREAU, V., THOMAS, D., HUMPHRIES, M. M., *et al.* Energy metabolism and animal personality. *Oikos*, 2008, vol. 117, no 5, p. 641-653.

CÉZILLY, Frank, PERROT-MINNOT, Marie-Jeanne, et RIGAUD, Thierry. Cooperation and conflict in host manipulation: interactions among macro-parasites and micro-organisms. *Roles and mechanisms of parasitism in aquatic microbial communities*, 2015, p. 144.

COMBES, Claude. *Parasitism: the ecology and evolution of intimate interactions*. University of Chicago Press, 2001.

COMBES, Claude. *L'art d'être parasite: les associations du vivant*. Flammarion, 2010 (nouv ed)

COSTANTINI, David, FERRARI, Caterina, PASQUARETTA, Cristian, *et al.*Interplay between plasma oxidative status, cortisol and coping styles in wild alpine marmots, Marmota marmota. *Journal of Experimental Biology*, 2012, vol. 215, no 2, p. 374-383.

CÔTÉ, Isabelle M. et POULINB, Robert. Parasitism and group size in social animals: a meta-analysis. *Behavioral Ecology,* 1995, vol. 6, no 2, p. 159-165.

CREEL, Scott. Social dominance and stress hormones. *Trends in Ecology & Evolution*, 2001, vol. 16, no 9, p. 491-497.

da Silva, R. C., & Langoni, H. (2009). Toxoplasma gondii: host–parasite interaction and behavior manipulation. *Parasitology research*, *105*(4), 893-898.

DAVIS, A. K., MANEY, D. L., et MAERZ, J. C. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology*, 2008, vol. 22, no 5, p. 760-772.

DE GROOT, Johanna, RUIS, Marko AW, SCHOLTEN, Jan W., *et al.* Long-term effects of social stress on antiviral immunity in pigs. *Physiology & behavior,* 2001, vol. 73, no 1, p. 145-158.

DUBEY, J. P. Advances in the life cycle of Toxoplasma gondii. *International journal for parasitology,* 1998, vol. 28, no 7, p. 1019-1024.

DUNN, Jenny C., COLE, Ella F., et QUINN, John L. Personality and parasites: sex-dependent associations between avian malaria infection and multiple behavioural traits. *Behavioral Ecology and Sociobiology*, 2011, vol. 65, no 7, p. 1459-1471.

EGEA, A. Vanina, HALL, Jeffery O., MILLER, James, *et al.* Reduced neophobia: A potential mechanism explaining the emergence of self-medicative behavior in sheep. *Physiology & behavior*, 2014, vol. 135, p. 189-197.

EZENWA, Vanessa O. Host social behavior and parasitic infection: a multifactorial approach. *Behavioral Ecology*, 2004, vol. 15, no 3, p. 446-454.

FOURTUNE, Lisa., PAZ-VINAS,Ivan., PRUNIER, Jérome G., LOOT Géraldine, VEYSSIERE, Charlotte., BLANCHET Simon. Inferring causalities in landscape genetics: An extension of Wright's causal modeling to distance matrices. In revision for The American Naturalist.

GILOT, B., PAUTOU, G., et MONCADA, E. analyse de la vegetation appliquee a la detection des populations de tiques exophiles dans le sud-est de la France: l'exemple d'Ixodes ricinus (Linne 1758)(Acarina, Ixodoidea). *Acta tropica*, 1975.

GILOT-FROMONT, Emmanuelle, JÉGO, Maël, BONENFANT, Christophe, *et al.*Immune phenotype and body condition in roe deer: individuals with high body condition have different, not stronger immunity. *PloS one*, 2012, vol. 7, no 9, p. e45576.

HAWLEY, Dana M., ETIENNE, Rampal S., EZENWA, Vanessa O., *et al.* Does animal behavior underlie covariation between hosts' exposure to infectious agents and susceptibility to infection? Implications for disease dynamics.*Integrative and comparative biology*, 2011, p. icr062.

HESSING, M. J. C., COENEN, G. J., VAIMAN, M., *et al.* Individual differences in cell-mediated and humoral immunity in pigs. *Veterinary immunology and immunopathology*, 1995, vol. 45, no 1, p. 97-113.

HEWISON, A. J. M., VINCENT, J. P., et REBY, D. Social organisation of European roe deer. *The European roe deer: the biology of success. Scandinavian University Press, Oslo,* 1998, p. 189-219.

HEWISON, AJ Mark, ANGIBAULT, Jean-Marc, CARGNELUTTI, Bruno, *et al.* Using radio-tracking and direct observation to estimate roe deer Capreolus capreolus density in a fragmented landscape: a pilot study. *Wildlife Biology,* 2007, vol. 13, no 3, p. 313-320.

HOSTE, H., TORRES-ACOSTA, J. F. J., et AGUILAR-CABALLERO, A. J. Nutrition–parasite interactions in goats: is immunoregulation involved in the control of gastrointestinal nematodes?. *Parasite Immunology*, 2008, vol. 30, no 2, p. 79-88.

HOSTE, Hervé, SOTIRAKI, Smaragda, LANDAU, Serge Yan, *et al.* Goat–Nematode interactions: think differently. *Trends in parasitology*, 2010, vol. 26, no 8, p. 376-381.

JÉGO, Maël. Condition physique, immunocompétence et parasitisme dans des populations naturelles de chevreuils *(Capreolus capreolus)*. 2014. Thèse de doctorat. Université Claude Bernard-Lyon I.

JOHNSEN, Torgeir S. et ZUK, Marlene. Parasites and tradeoffs in the immune response of female red jungle fowl. *Oikos*, 1999, p. 487-492.

JOLLES, Anna E., EZENWA, Vanessa O., ETIENNE, Rampal S., *et al.* Interactions between macroparasites and microparasites drive infection patterns in free-ranging African buffalo. *Ecology*, 2008, vol. 89, no 8, p. 2239-2250.

KLASING KC. 2004 The costs of immunity. Acta Zoological Sinica 50, 961-969.

KONTIAINEN, Pekka, PIETIÄINEN, Hannu, HUTTUNEN, Kalle, *et al.* Aggressive Ural owl mothers recruit more offspring. *Behavioral Ecology*, 2009, p. arp062.

KOOLHAAS, J. M., KORTE, S. M., DE BOER, S. F., *et al.* Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews*, 1999, vol. 23, no 7, p. 925-935.

KOOLHAAS, J. M. Coping style and immunity in animals: making sense of individual variation. *Brain, behavior, and immunity*, 2008, vol. 22, no 5, p. 662-667.

KORTET, Raine, HEDRICK, Ann V., et VAINIKKA, Anssi. Parasitism, predation and the evolution of animal personalities. *Ecology Letters*, 2010, vol. 13, no 12, p. 1449-1458.

KORTET, Raine et HEDRICK, A. N. N. A behavioural syndrome in the field cricket Gryllus integer: intrasexual aggression is correlated with activity in a novel environment. *Biological Journal of the Linnean Society*, 2007, vol. 91, no 3, p. 475-482.

LAFFERTY, Kevin D. et SHAW, Jenny C. Comparing mechanisms of host manipulation across host and parasite taxa. *The Journal of experimental biology*, 2013, vol. 216, no 1, p. 56-66.

LEE, Kelly A. Linking immune defenses and life history at the levels of the individual and the species. *Integrative and Comparative Biology*, 2006, vol. 46, no 6, p. 1000-1015.

LEE, K. A., WIKELSKI, M., ROBINSON, W. D., *et al.* Constitutive immune defences correlate with life-history variables in tropical birds. *Journal of Animal Ecology*, 2008, vol. 77, no 2, p. 356-363.

LOCHMILLER, Robert L. et DEERENBERG, Charlotte. Trade-offs in evolutionary immunology: just what is the cost of immunity?. *Oikos*, 2000, vol. 88, no 1, p. 87-98.

MACARTHUR, Robert H. et WILSON, Edward O. *Theory of Island Biogeography.(MPB-1).* Princeton University Press, 2015.

MATSON, Kevin D., RICKLEFS, Robert E., et KLASING, Kirk C. A hemolysis–hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Developmental & Comparative Immunology*, 2005, vol. 29, no 3, p. 275-286.

MONESTIER, Chloé. Inter-individual heterogeneity of demographic performance and personality in roe deer (*Capreolus capreolus*) 2016. Thèse de doctorat- Université Paul Sabatier Toulouse III

MORELLET, Nicolas, VERHEYDEN, Helene, ANGIBAULT, Jean-Marc, *et al.* The effect of capture on ranging behaviour and activity of the European roe deer Capreolus capreolus. *Wildlife Biology*, 2009, vol. 15, no 3, p. 278-287.

MURRAY, Kim et CONNER, Mary M. Methods to quantify variable importance: implications for the analysis of noisy ecological data. *Ecology*, 2009, vol. 90, no 2, p. 348-355.

NAVARRO-GONZALEZ, Nora, VERHEYDEN, Hélène, HOSTE, Hervé, *et al.* Diet quality and immunocompetence influence parasite load of roe deer in a fragmented landscape. *European Journal of Wildlife Research*, 2011, vol. 57, no 3, p. 639-645.

PERKINS, Sarah E., CATTADORI, Isabella M., TAGLIAPIETRA, Valentina, *et al.* Empirical evidence for key hosts in persistence of a tick-borne disease. *International journal for parasitology*, 2003, vol. 33, no 9, p. 909-917.

POIROTTE, Clémence, KAPPELER, Peter M., NGOUBANGOYE, Barthelemy,*et al.* Morbid attraction to leopard urine in Toxoplasma-infected chimpanzees.*Current Biology,* 2016, vol. 26, no 3, p. R98-R99.

POULIN, Robert et MORAND, Serge. The diversity of parasites. *Quarterly Review of Biology*, 2000, p. 277-293.
POULIN, Robert. Parasite manipulation of host personality and behavioural syndromes. *The Journal of experimental biology*, 2013, vol. 216, no 1, p. 18-26.

RANTALA, Markus J. et KORTET, Raine. Male dominance and immunocompetence in a field cricket. *Behavioral Ecology*, 2004, vol. 15, no 2, p. 187-191.

PRICE, Peter W. *Evolutionary biology of parasites*. Princeton University Press, 1980.

RAYNAUD, J. P. Etude de l'efficacité d'une technique de coproscopie quantitative pour le diagnostic de routine et le contrôle des infestations parasitaires des bovins, ovins, équins et porcins. *Annales de Parasitologie humaine et comparée*, 1970.

RÉALE, D., MARTIN, J., COLTMAN, D. W., *et al.* Male personality, life-history strategies and reproductive success in a promiscuous mammal. *Journal of evolutionary biology,* 2009, vol. 22, no 8, p. 1599-1607.

RÉALE, Denis, GARANT, Dany, HUMPHRIES, Murray M., *et al.* Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society of London B: Biological Sciences,* 2010, vol. 365, no 1560, p. 4051-4063.

REZNICK, David, BRYANT, Michael J., et BASHEY, Farrah. r-and K-selection revisited: the role of population regulation in life-history evolution. *Ecology,* 2002, vol. 83, no 6, p. 1509-1520.

SAPOLSKY, Robert M. The influence of social hierarchy on primate health. *Science*, 2005, vol. 308, no 5722, p. 648-652.

SCHMID HEMPEL, Paul et SCHMID-HEMPEL, Paul. *Evolutionary parasitologythe integrated study of infections, immunology, ecology, and genetics*. 2011.

SHELDON, Ben C. et VERHULST, Simon. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in ecology & evolution*, 1996, vol. 11, no 8, p. 317-321.

SIH, Andrew, BELL, Alison, et JOHNSON, J. Chadwick. Behavioral syndromes: an ecological and evolutionary overview. *Trends in ecology & evolution*, 2004, vol. 19, no 7, p. 372-378.

SEVILA, Julie. *Relation entre comportement spatial et parasitisme chez le chevreuil en milieu anthropisé*. 2015. Thèse de doctorat. Université de Toulouse, Université Toulouse III-Paul Sabatier.

SHIPLEY, B. Cause and correlation in biology Cambridge University Press. *Cambridge, UK*, 2000.

SHIPLEY, Bill. Analysing the allometry of multiple interacting traits. *Perspectives in Plant Ecology, Evolution and Systematics*, 2004, vol. 6, no 4, p. 235-241.

SHIPLEY, Bill. Confirmatory path analysis in a generalized multilevel context. *Ecology*, 2009, vol. 90, no 2, p. 363-368.

SHIPLEY, Bill. The AIC model selection method applied to path analytic models compared using a d-separation test. *Ecology*, 2013, vol. 94, no 3, p. 560-564.

SIH, Andrew et BELL, Alison M. Insights for behavioral ecology from behavioral syndromes. *Advances in the Study of Behavior*, 2008, vol. 38, p. 227.

STIEN, Audun, IRVINE, R. J., ROPSTAD, E., *et al.* The impact of gastrointestinal nematodes on wild reindeer: Experimental and cross-sectional studies. *Journal of Animal Ecology*, 2002, vol. 71, no 6, p. 937-945.

STRANDGAARD, H. *The roe deer (Capreolus Capreolus) population at Kalø: and the factors regulating its size*. Vildtbiologisk station, 1972.

THOMAS, D. W., SHIPLEY, B., BLONDEL, J., *et al.* Common paths link food abundance and ectoparasite loads to physiological performance and recruitment in nestling blue tits. *Functional Ecology*, 2007, vol. 21, no 5, p. 947-955.

THOMAS, Frédéric, POULIN, Robert, et BRODEUR, Jacques. Host manipulation by parasites: a multidimensional phenomenon. *Oikos*, 2010, vol. 119, no 8, p. 1217-1223.

VYAS, Ajai, KIM, Seon-Kyeong, GIACOMINI, Nicholas, *et al.* Behavioral changes induced by Toxoplasma infection of rodents are highly specific to aversion of cat odors. *Proceedings of the National Academy of Sciences*, 2007, vol. 104, no 15, p. 6442-6447.

WEBSTER, Joanne P. The effect of Toxoplasma gondii on animal behavior: playing cat and mouse. *Schizophrenia bulletin*, 2007, vol. 33, no 3, p. 752-756.

WOOLHOUSE, Mark EJ, DYE, C., ETARD, J.-F., *et al.* Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proceedings of the National Academy of Sciences*, 1997, vol. 94, no 1, p. 338-342.