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Contemporary variations of immune responsiveness during range expansion of two invasive rodents in Senegal

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1 (Abstract)

2 Biological invasions provide unique opportunities for studying life history trait changes over  
3 contemporary time scales. As spatial spread may be related to changes in parasite  
4 communities, several hypotheses (such as the evolution of increased competitive ability  
5 (EICA) or EICA-refined hypotheses) suggest immune changes in invasive species along  
6 invasion gradients. Although native hosts may be subject to similar changes in parasite  
7 selection pressures, their immune responses have been rarely investigated in invasion  
8 contexts. In this study, we evaluated immune variations for invasive house mice *Mus*  
9 *musculusdomesticus*, invasive black rats *Rattusrattus* and native rodents  
10 *Mastomyserythroleucus* and *Ma. natalensis* along well-characterised invasion gradients in  
11 Senegal. We focused on antibody-mediated (natural antibodies and complement) and  
12 inflammatory (haptoglobin) responses. One invasion route was considered for each invasive  
13 species, and environmental conditions were recorded. Natural-antibody mediated responses  
14 increased between sites of long-established invasion and recently invaded sites only in house  
15 mice. Both invasive species exhibited higher inflammatory responses at the invasion front than  
16 in sites of long-established invasion. The immune responses of native species did not change  
17 with the presence of invasive species. These patterns of immune variations do not support the  
18 EICA and EICA refined hypotheses, and they rather suggest a higher risk of exposure to  
19 parasites on the invasion front. Altogether, these results provide a first basis to further assess  
20 the role of immune changes in invasion success.

## 21 INTRODUCTION

22 Biological invasions, i.e., the successful establishment and spread of species outside their  
23 native range, are increasingly frequent worldwide mostly due to human activities. They often  
24 have detrimental consequences for the communities invaded (Kolar and Lodge 2001). Despite  
25 the increasing burgeoning interest in invasion science these last decades, several gaps exist in  
26 our knowledge and understanding of the factors and forces driving invasion success of non-  
27 native species in new areas (Facon et al. 2006; Lowry et al. 2013).

28 Parasitism is one factor likely to promote invasion success by influencing a range of host  
29 community interactions (Dunn and Hatcher 2015). As such, invasive species may lose their  
30 natural enemies, including micro- and macro-parasites, in their non-native ranges, with  
31 positive outcomes on invader fitness and demography (enemy release hypothesis, Colautti et  
32 al. 2004). Alternatively, invaders may introduce exotic parasites with detrimental effects on  
33 the survival, fecundity and/or regulation of indigenous host populations and provide  
34 opportunities for diseases to emerge (spill-over or novel weapon hypothesis, Strauss et al.  
35 2012). They may also acquire local parasites from their new environment, amplifying the  
36 impact of some of them at the expense of native species, with effects at both the individual  
37 and population scales (spillback hypothesis, Kelly et al. 2009). As immunity is costly in terms  
38 of energy and immunopathology (Klasing 2004, Raberg et al. 2002), these changes in parasite  
39 pressure have led to some predictions regarding potential variations of invaders' immune  
40 defences in the course of invasion. With regards to the enemy release hypothesis, the invaders  
41 are expected to reallocate energetic resources from unnecessary defence mechanisms to life  
42 history traits favouring invasion success, such as dispersal or enhanced reproductive output.  
43 This prediction, known as the Evolution of Increased Competitive Ability (EICA) hypothesis  
44 (Blossey and Notzold 1995), has been refined to take into account the widespread occurrence  
45 of parasitism (Lee and Klasing 2004). In addition to parasites that would not be lost during

46 invasion, invaders could be infected by local parasites (including the possibility of spill-back  
47 of parasites carried by native hosts). A drastic reduction of immune responses would make  
48 invaders highly susceptible to generalist pathogens encountered in recently invaded host  
49 communities (Roberts and Janovy 2010). Then, successful invaders should be those that  
50 dampen the most expensive and/or least effective immune defences instead favouring less  
51 costly and more efficient immune strategies with respect to parasites that are kept from the  
52 source area or those newly encountered on the invasion front (EICA-refined hypothesis, Lee  
53 and Klasing 2004). Such trade-off would allow promoting other life history traits without  
54 dampening invader defences.

55 The mechanisms potentially mediating these phenotypic variations include various  
56 processes: evolutionary ones as suggested in the name of this hypothesis, but also ecological  
57 changes. A major part of immune variation relies on differences in genetic background  
58 (reviewed in Ardia et al. 2011). Genetic drift and natural selection may therefore be key  
59 evolutionary processes by which invaders will evolve on invasion front after few generations  
60 (review in Charbonnel and Cosson 2011). Recently, it was also assessed that epigenetic  
61 modifications - defined as changes in phenotypes that persist through mitosis and even  
62 meiosis, but occur independently of changes in underlying DNA sequence - may also play  
63 pivotal roles in immune changes related to invasion process (Brown et al. 2015; Na et al.  
64 2016). But alternatively, another important source of immune variation during invasion  
65 process could be related to phenotypic plasticity, which is the tendency/ability for phenotypes  
66 to change across different environments within generations (reviewed in Gervasi et al. 2015).  
67 This mechanism has been widely reported using experimental works modifying  
68 environmental conditions (resource quality and availability, cross-fostering) or individual  
69 surveys throughout their life, in both vertebrates and invertebrates (Schulenburg et al. 2009).

70 Another complexity resides in the immune system itself. In vertebrates, it is a highly  
71 diverse network of organs, cells and molecules that are generally classified into innate and  
72 adaptive compartments, both including cellular (e.g., macrophages, lymphocytes) and  
73 humoral components (e.g., antibodies, peptides), which interact together but may have  
74 different costs and benefits for the organism (Klasing 2004). It has been assessed that innate  
75 responses associated with local and/or systemic inflammation incur high-energy expenditure  
76 and major physiological, behavioural and pathological costs (Sorci and Faivre 2009)  
77 compared to responses mediated by other effectors, such as both natural (innate) and specific  
78 (acquired) antibodies (Raberg et al. 2002, Klasing 2004). This has led to suggest that invasion  
79 success would therefore be associated with dampened costly systemic inflammatory response  
80 and stronger less costly antibody-mediated immunity (Lee and Klasing 2004). Alternatively,  
81 because of potential high infection risks encountered on invasion fronts, the invaders'  
82 capacity to mount rapid, non-specific immune responses could be essential for invasion  
83 success. It would be therefore possible to make different predictions than those of Lee and  
84 Klasing (2004) for immune changes along invasion gradients, such as an increase of immune  
85 investment, or the absence of immune trade-offs (Phillips et al. 2010).

86 The impacts that bioinvasions may have on immune responses in native species remain  
87 scarcely described and investigated. We can first consider that native species might be  
88 exposed to a lower infection risk as a result of dilution effects (the presence of a less  
89 competent host decreases the infection prevalence in the native host, Dunn 2009) or of  
90 density-dependent effects (the density of native hosts may decrease due to competition with  
91 invaders, and infection prevalence of parasites transmitted through contacts will consequently  
92 decreased too, Keesing et al. 2010). In this context, a decrease in immune investment could be  
93 ~~selected~~ expected (EICA hypothesis). Nevertheless, parasite spill-over from invasive to native  
94 species is frequent (Harris 2009) and can strongly affect native host communities, even

95 leading to local extinction, which favorsthe invasive host (Daszak et al. 2000, Prenter et al.  
96 2004). Such a strong impact of exotic infections on native species could result from the lack  
97 of efficient immune responses (either in terms of immune effectors or amounts of  
98 responsiveness). A dampened immune defense could result from direct competition with  
99 invaders through a lowered access to resources or from stress hormones such as  
100 glucocorticoids that may ultimately compromise immunity (Martin et al. 2010a). Strong  
101 immunopathologic effects affecting natives' fitness may also result from infections with  
102 exotic parasites (spill-over) or from amplified risk of infections with native parasites (spill-  
103 back) (Martin et al. 2010b).

104 Limitedempirical data are currently available concerning the ecoimmunology of invasions  
105 (Pedersen and Babayan 2011, White and Perkins 2012). Indeed, few studies have investigated  
106 differences in immune phenotypes along invasion gradients for a given invasive species, and  
107 even fewer have considered several immune pathways simultaneously (Cornet et al. 2016, but  
108 see Llewellyn et al. 2012, Brown et al. 2015). Therefore, it remains important to improve our  
109 knowledge ofsuch immune variations associated with invasion. It is a necessary pre-requisite  
110 before addressing the eco-evolutionary processes dictating invasion success.In this study, we  
111 focused on two invasive rodent species, the domestic mouse (*Mus musculusdomesticus*) and  
112 the black rat (*Rattusrattus*).Weanalyzed phenotypic variations in immune responsiveness  
113 occurring along their well-known invasion routes in Senegal. These murid rodents are  
114 exclusively commensal in the study area (i.e., living in/around human dwellings/man-made  
115 structures) and are worldwide significant invasive species (Global Invasive Species Database  
116 - <http://www.issg.org/database/>). Complete syntheses reporting description of their current  
117 invasion histories in Senegal (including data of historical inventories, molecular analyses and  
118 ecological longitudinal surveys) are provided elsewhere (Dalecky et al. 2015, Konecny et al.  
119 2013). Briefly, both species originated from Asia and have expanded their distribution range

120 worldwide, making use of human migration to colonize all the continents. They were first  
121 brought to West Africa by European explorers and settlers from the 15th century. Large and  
122 likely standing populations of rats and mice are reported in coastal colonial cities from the  
123 middle of the 19<sup>th</sup> century. At the beginning of the 20<sup>th</sup> century, they began to expand  
124 eastwards with the development of inland commercial transport. Their distribution currently  
125 covers much of North and Central Senegal for *M. m. domesticus* and South and Central  
126 Senegal for *R. rattus*. Recent longitudinal surveys (Granjon and Duplantier 2009, Dalecky et  
127 al. 2015) have documented how the ongoing expansion of both species has resulted in the  
128 extirpation of native rodents (mostly *Mastomyserythroleucus* and *Ma. natalensis*) from  
129 commensal habitats, these latter species being now found almost only in villages at the  
130 invasion front and in non-invaded areas.

131 Immune phenotypes were described using effectors involved in natural antibody (NAb)-  
132 mediated and inflammatory immune pathways. They are main components of innate  
133 constitutive immunity acting in the earliest phase of immune defence against general  
134 challenges and new parasites (Rossi et al. 2013). First, NABs are humoral components of  
135 constitutive immunity, providing the first-line of non-cellular protection against antigens  
136 (Matson et al. 2005). They serve as recognition molecules capable of opsonising invading  
137 micro-organisms and initialising the Complement (Cp) enzyme cascade, which ends in cell  
138 lysis (Carroll and Prodeus 1998). They are unique among immunoglobulin molecules because  
139 their presence does not require prior exposure to exogenous antigens. The assessment of this  
140 immune pathway is particularly appealing because NABs should be less sensitive than  
141 acquired antibody responses to short-term variations in environmental conditions, nutritional  
142 status, or stress levels (Baumgarth et al. 1999). Moreover, Nab levels in the serum could be  
143 positively correlated with the ability to produce antibodies after a challenge (Matson et al.  
144 2005). Second, we used haptoglobin (Hp) concentration to assess the inflammatory state of



145 the rodents, as previously performed in wild birds (Martin et al. 2010a). Hp is a  
146 multifunctional hepatic acute-phase protein highly released in the blood during inflammation,  
147 with strong anti-inflammatory and anti-oxidant properties (Huntoon et al. 2008). It circulates  
148 at low concentrations in the blood of a range of animal species and its concentration is  
149 dependent on health status (Dobryszczyka 1997). Hp has been shown to be a distinctive trait of  
150 individuals (Matson et al. 2012). Both immune tests used here are simple, highly repeatable  
151 and non-specific. Moreover they do not require the recapture of animals or their maintenance  
152 in animal facilities. For these reasons, they enable to cope with the specific constraints of  
153 comparative immunological studies dealing with numerous samples of different non-model  
154 species caught in natural populations and sacrificed at the time of capture. All assays were  
155 performed at both long-established and recently invaded sites, for both invasive species, and  
156 for *Mastomys* species found either in sympatry with the invaders at the invasion front or alone  
157 at sites not yet invaded.

158 We specifically addressed the two following questions: 1) do the immune responsiveness  
159 patterns observed in rats and mice along invasion routes support the EICA or EICA refined  
160 hypotheses? We expected a decrease of immune responsiveness (Nabs and Hp) in recently  
161 invaded localities compared to long established ones under the EICA hypothesis, or a  
162 decrease of costly immune responsiveness (Hp) at the expense of less costly ones (NAbs)  
163 under the EICA-refined hypotheses; 2) do native species exhibit variations in immune  
164 responsiveness associated with the presence of invasive ones? Up to now, no framework has  
165 been developed with regard to this question, what prevents us to make any  
166 predictions. Altogether, these results enabled to discuss some of the general predictions of Lee  
167 and Klasing's paper (2004), which is one of the reference work when studying the potential  
168 links between immunology and invasion. Nevertheless, this study is descriptive and as such, it

169 was not designed to disentangle the ecological and evolutionary processes potentially  
170 underlying the immune patterns observed.

171

## 172 **MATERIALS AND METHODS**

### 173 **Ethical Statement**

174 Trapping campaigns within private properties were systematically realized with prior explicit  
175 agreement from relevant institutional (CBGP: 34 169 003) and local authorities. All animal-  
176 related procedures were carried out in accordance with relevant requirements of Senegalese  
177 and French legislation and following official ethical guidelines (Sikes et al. 2011).

### 178 **Rodent sampling and blood collection**

179 Field sampling was conducted separately along an invasion route for each invasive species  
180 (Figure 1). It was carried out during the dry season, in March-April 2013 for the ‘mouse’  
181 invasion route and from November 2013 to February 2014 for the ‘rat’ invasion route. The  
182 sampling sites belonged to one of three invasion status categories, defined on the basis of  
183 historical records and longitudinal surveys (see references in Dalecky et al. 2015, Konecny et  
184 al. 2013): (i) sites of long-established invasion, in which invasive populations have been  
185 recorded for centuries; (ii) sites at the invasion front (= sites recently invaded) in which  
186 invasive populations have been established for less than 30 years; and (iii) non-invaded sites  
187 in which invasive species have never yet been recorded or trapped. For each category, we  
188 sampled three to four sites (Figure 1). We used a standardised sampling protocol for all  
189 localities of the three categories of sites considered. It enabled to standardize the potential  
190 impacts of stress on immune responses due to animal capture and handling. The trapping  
191 procedures are described in detail elsewhere (Dalecky et al. 2015). At each site, we set at least  
192 120 traps within houses during one to three successive nights, with the aim to capture 20 adult  
193 rodents per species. Traps were checked and baited once a day with peanut butter

194 supplemented with fresh onions. Rodents captured at night were retrieved the following  
195 morning and then sacrificed by cervical dislocation within the following four hours. They  
196 were weighed to the nearest 0.5 g, sexed and dissected. Identification was based on  
197 morphometrics (head-body, tail, hind foot and ear lengths) and genetics (ZFY2 gene-based  
198 RFLP for identification to the subspecies level for *M. musculus*; cytochrome b gene-based  
199 RFLP for species characterisation in the genus *Mastomys*). As suggested by Granjon and  
200 Duplantier (2009), rodents were considered to be adults on the basis of body weight and  
201 reproductive status. Blood samples were collected by cardiac puncture after the animals were  
202 euthanized. They were kept on ice for 24 hours and then centrifuged. The floating serum  
203 supernatant fraction was removed, frozen in liquid nitrogen, and then stored in a freezer (-  
204 20°C) in Dakar (Senegal). Samples were transferred in dry ice from Dakar to Montpellier  
205 (France) in accordance with the regulations enforced in Senegal and France.

## 206 **Environmental data**

207 Because environmental variations may drive differences in immune responses between rodent  
208 populations independently of the invasion status categories, we described relevant climatic  
209 and commensal habitat parameters for all sampling sites and included them in further  
210 statistical analyses. We focused on these factors and did not include vegetation information  
211 because house mice and black rats are strictly commensal in Senegal, and because both  
212 invasive species expand their range through human trade and transport rather than by  
213 individual dispersal in the wild (Dalecky et al. 2015). Means and standard deviations of  
214 climatic data collected between 1997 and 2012 were used (data available on  
215 <http://www.ncdc.noaa.gov/cdo-web/datasets> for temperatures, and [http://richardis.univ-  
216 paris1.fr/precip/rainser1.html](http://richardis.univ-paris1.fr/precip/rainser1.html) for rainfall with GPCP-1DD as the source of data). We  
217 considered rainfall data in mm (for each year: cumulated annual rainfall, cumulated rainfall  
218 during the rainy season, minimum and maximum monthly rainfall during the rainy season)

219 and temperature data in degrees Celsius (for each year: monthly mean, monthly mean  
220 minimum and maximum, minimum of monthly mean minimum and maximum of monthly  
221 mean maximum). Commensal habitat characteristics were recorded during trapping sessions.  
222 In particular, we recorded for each sampled room the material used (sand, banco, cement,  
223 sheet metal, fibers) for each part of the building (floor, wall, ceiling), the type of room  
224 (dwelling house, shop, storehouse, kitchen) and for each site the inhabited area surface  
225 estimated using Google Earth Pro 7.1.

### 226 **Hemagglutination-hemolysis (HA-HL) assay**

227 The goal of this assay was to evaluate components of humoral innate immunity through the  
228 ability of plasma to agglutinate and lyse foreign cells through NAbs-Cp system. We  
229 characterised the serum agglutination of heterologous red blood cells (RBC) due to NAbs  
230 (HA assay) and RBC hemolysis due to NAbs-mediated Cp activation (HL assay), with a  
231 slightly modified version of the protocol described by Matson et al. (2005) for mammal  
232 species. Briefly, we used chicken RBCs as target cells, and serum samples from a rabbit  
233 immunised against chicken RBCs as positive controls. All the HA/HL assays from an  
234 invasion route were carried out using the same RBC suspension. We diluted twofold serially  
235 (from 1/2 to 1/128) ten rodent serum samples per plate with 10 $\mu$ l in every well. The plates  
236 were read blindly following Rossi et al. (2013) and scores were attributed for each sample  
237 (column) as the log<sub>2</sub> of the last dilution exhibiting each phenomenon (HA, HL). To exclude  
238 potential observer effects, all images were scored the same day by the same trained observer;  
239 thus correction for observer effect was not necessary in the subsequent analyses. It was  
240 possible to assign a half-point score in cases of ambiguity. High scores of HA reflected high  
241 concentrations of NAbs in the blood. Levels of HL reflected both the NAbs and complement  
242 activities.

### 243 **Haptoglobin (Hp) assay**

244 Hp was quantified from 10 $\mu$ l of serum with a commercially available colorimetric  
245 immunoassay kit (“PHASE” Haptoglobin Assay, TP-801, Tridelta), according to the  
246 manufacturer’s instructions. Absorbance at 650 nm was determined with a spectrophotometer,  
247 both before and after the addition of the final reagent triggering the colorimetric reaction. We  
248 used the pre-scan absorbance to correct for differences in plasma colour and cloudiness,  
249 including the initial redness of the serum(i.e., the initial serum hemolysis) that can hamper the  
250 assay if not taken into account (Matson et al. 2012). Serum Hp concentration (mg/ml) was  
251 estimated by comparing the difference in absorbance (final – pre-scan) to a calibration curve.

## 252 **Statistical analyses**

253 In order to assess whether environmental features differed between the three categories of  
254 sampled sites, we carried out a two-stage Principal Component Analysis (PCA) with sites as  
255 observations, independently for climatic features (using 16-years mean values and their  
256 respective standard deviations) and commensal habitat characteristics (proportion data). As  
257 many indicators appeared highly correlated ( $r > 0.75$ ), we kept only one variable in each set of  
258 highly correlated variables, based on both contributions to the axis construction and quality of  
259 representation. We then performed a final PCA with remaining variables. We tested  
260 statistically whether data were structured according to invasion status categories using a  
261 Between/Within-groups Analysis (BWA). Monte-Carlo tests of permutations (999  
262 permutations) were applied to analyse the statistical significance of the groups graphically  
263 observed on the PCAs.

264 Variations in Hp concentration (log-transformed), HA and HL values were analysed  
265 independently, using linear models, in R software v.3.1.0 (R Core Team 2015). The ‘mouse’  
266 and ‘rat’ invasion routes were investigated separately. The starting models included a factor  
267 combining the species and site status on the invasion route, hereafter referred to as ‘specific  
268 invasion status’ and classified into four categories : A) invasive species alone in sites of long-

269 established invasion; B) invasive species at recently invaded sites; C) native species at  
270 recently invaded sites and D) native species at non-invaded sites. Combining the two factors  
271 'species' and 'site category' into a single one (called "specific invasion status" hereafter)  
272 enabled to avoid confounding effects between host species and invasion on immune  
273 responses. Individual factors (sex, body mass and age class) may greatly affect immune  
274 system parameters. We therefore included these factors and their 2-ways interactions with  
275 specific invasion status as fixed effects. We also added the descriptors of environmental  
276 variations as potential predictors in the full models. Therefore, coordinates of each site on the  
277 two first PCA axes for both climatic and commensal habitat data were included. Finally, we  
278 had to include several additional factors specific to the different experimental protocols used:  
279 (i) a 'plate' factor in the HA model to take into account both the chronology in which the  
280 plates were analysed and the involvement of two different experimenters, and (ii) HA scores  
281 in the HL model as HL directly depends on the presence of antibody-antigen complex  
282 revealed by HA. For both the HA and HL models, we also included the initial level of serum  
283 hemolysis as initial hemolysis expected to interfere with the interpretation of HA and HL  
284 results. A value was then systematically given to the serum prior dilution, ranging from 1 (not  
285 red) to 8 (dark red) according to the intensity of the initial serum redness in order to correct  
286 for non-visibility and over-interpretation of the phenomena during the plate reading. The  
287 Akaike information criterion with correction for samples of finite size (AICc) was used for  
288 model selection. Models with all possible combinations of the terms included in the starting  
289 model were generated with the MuMIn v.1.10.5 R package (dredge function, Barton (2016)).  
290 Models with a  $\Delta AICc < 2$  with respect to the model with the lowest AICc were selected and  
291 the most parsimonious of these models was chosen. The significance of explanatory variables  
292 and their interactions was determined by deletion testing and log-likelihood ratio tests. The  
293 assumptions of each final model were checked graphically, by an analysis of their residuals.

294 Post-hoc tests for multiple comparisons were carried out with Tukey's test (95% family-wise  
295 confidence level).

296

## 297 **RESULTS**

298 We analysed serum samples from 646 individuals belonging to four rodent species captured at  
299 23 sites (Table 1, Figure 1). The dominant species in the native rodent communities were *Ma.*  
300 *erythroleucus* along the 'mouse' invasion route and *Ma. erythroleucus* or *Ma. natalensis*  
301 along the 'rat' invasion route. *Mastomysnatalensis* was found specifically at the invasion front  
302 of *R. rattus*, coinciding geographically with the limited distribution area of this native species  
303 in Senegal. *Mastomyserythroleucus* was occasionally captured in sites of long-established  
304 invasion (n = 15) and in non-invaded sites beyond the invasion front in western Senegal (n =  
305 3) along the 'rat' invasion route. These individuals were too few in number for more detailed  
306 analysis. No significant difference was detected in HA ( $F_{1,49} = 0.18, p = 0.71$ ), HL ( $F_{1,49} =$   
307  $2.14, p = 0.28$ ) or Hp ( $F_{1,53} = 1.27, p = 0.38$ ) levels between *Mastomys* species at sites recently  
308 invaded by *R. rattus*. *Mastomysnatalensis* and *Ma.erythroleucus* were therefore considered as  
309 a single taxon, native *Mastomys sp.*, in further statistical analyses on the 'rat' invasion route.

### 310 **Environmental data**

311 We found significant climatic differences along 'mouse' and 'rat' (Monte-Carlo tests,  $p < 0.05$ )  
312 invasion routes. Along the 'mouse' invasion route, climate seemed cooler and drier in sites of  
313 long-established invasion compared to the other sites. Along the 'rat' invasion route, climate  
314 seemed cooler and wetter in sites of long-established invasion compared to the other sites.  
315 These results are well represented on the second axis of mouse and rat PCAs (Figure S1a,  
316 Figure S2a, supplementary materials). The Between/Within analysis on commensal habitat  
317 characteristics revealed a significant segregation between invaded and non-invaded sites  
318 along the 'mouse' invasion route (Figure S1b, supplementary materials), suggesting more

319 traditional buildings in non-invaded sites. Along the ‘rat’ invasion route, no significant  
320 difference in commensal habitat characteristics was detected (Monte-Carlo test,  $p > 0.05$ ) with  
321 regard to site invasion status (Figure S2b, supplementary materials). However, these results  
322 must be taken cautiously as this study was not designed to investigate the influence of  
323 environmental characteristics on immune responsiveness.

#### 324 **Variation in HA/HL estimates**

325 ‘*Mouse*’ invasion route - The most parsimonious model explaining variation in HA included  
326 the climate (component 1:  $F_{1,290} = 5.83$ ,  $p < 0.0001$ ; component 2:  $F_{1,290} = 16.81$ ,  $p < 0.0001$ ),  
327 plate factor ( $F_{1,290} = 11.27$ ,  $p = 0.0163$ ), specific invasion status ( $F_{3,290} = 12.48$ ,  $p < 0.0001$ ,  
328 Figure 2) and its interaction with sex ( $F_{3,290} = 4.75$ ,  $p = 0.0029$ ) (Table S1, supplementary  
329 materials). Sex accounted for differences in HA levels only at non-invaded sites, with higher  
330 values recorded for males than females. Mice from sites of long-established invasion had the  
331 lowest HA values. No significant difference in mean HA was detected in *Ma. erythroleucus*  
332 between recently invaded and non-invaded sites (post-hoc Tukey’s test,  $p = 0.9999$ ), nor  
333 between *Ma. erythroleucus* and *M. m. domesticus* at the invasion front (post-hoc Tukey’s test,  
334  $p = 0.9999$ ).

335 The most parsimonious model explaining variation in HL included the initial degree of serum  
336 hemolysis ( $F_{1,293} = 99.82$ ,  $p < 0.0001$ ), HA score ( $F_{1,293} = 50.72$ ,  $p < 0.0001$ ), sex ( $F_{1,293} = 9.87$ ,  
337  $p = 0.0018$ ) and specific invasion status ( $F_{3,293} = 26.56$ ,  $p < 0.0001$ , Figure 2) (Table S1,  
338 supplementary materials). High values of HL were recorded for high values of HA and initial  
339 serum hemolysis, and for males compared to females. Mice from sites of long-established  
340 invasion had lower HL values compared with other invasion statuses. On invasion front, *M.*  
341 *m. domesticus* had lower values of HL than *Ma. erythroleucus* from recently invaded (post-  
342 hoc Tukey’s test,  $p < 0.0001$ ) and non-invaded sites (post-hoc Tukey’s test,  $p = 0.0006$ ). No



343 significant difference in mean HL between recently invaded and non-invaded sites was  
344 detected in *Ma. erythroleucus* (post-hoc Tukey's test,  $p = 0.8723$ ).

345 '*Rat*' invasion route - The most parsimonious model explaining variation in HA included the  
346 initial degree of serum hemolysis ( $F_{1,299} = 12.74$ ,  $p = 0.0004$ ), age ( $F_{1,299} = 9.40$ ,  $p = 0.0024$ ),  
347 body mass ( $F_{1,299} = 4.93$ ,  $p = 0.0270$ ) and climate ( $F_{1,299} = 12.91$ ,  $p = 0.0004$ ) (Table S2,  
348 supplementary materials). Heavier and adult rodents had higher HA levels than lighter and  
349 juveniles, respectively. Serum hemolysis was negatively correlated with HA. No significant  
350 difference was found according to specific invasion status ( $F_{3,299} = 1.41$ ,  $p = 0.2399$ ).

351 The most parsimonious model explaining variations in HL included the initial degree of  
352 serum hemolysis ( $F_{1,300} = 57.76$ ,  $p < 0.0001$ ), HA score ( $F_{1,300} = 355.92$ ,  $p < 0.0001$ ) and  
353 commensal habitat characteristics (component 1:  $F_{1,300} = 21.82$ ,  $p < 0.0001$ ) (Table S2,  
354 supplementary materials). HL values were positively correlated with both HA scores and with  
355 initial serum hemolysis. Specific invasion status had no significant effect on HL values  
356 ( $F_{3,297} = 0.82$ ,  $p = 0.4862$ ).

### 357 **Variation in Hp estimates**

358 '*Mouse*' invasion route - The most parsimonious model explaining variation in Hp  
359 concentration included climate (component 1:  $F_{1,183} = 13.31$ ,  $p = 0.0003$ ; component 2:  $F_{1,183} =$   
360  $22.74$ ,  $p < 0.0001$ ) and specific invasion status ( $F_{3,183} = 5.22$ ,  $p = 0.0017$ ) (Table S1,  
361 supplementary materials; Figure 2). Mice sampled from the invasion front had Hp levels  
362 twice those of mice from sites of long-established invasion (post-hoc Tukey's test,  $p =$   
363  $0.0039$ ). No significant difference in mean Hp concentration was found between *Ma.*  
364 *erythroleucus* from recently invaded and non-invaded sites (post-hoc Tukey's test,  $p =$   
365  $0.9307$ ). Furthermore, Hp levels were similar in *Ma. erythroleucus* and *M. m. domesticus*  
366 sampled in recently invaded sites (post-hoc Tukey's test,  $p = 0.4349$ ).

367 'Rat' invasion route - The most parsimonious model best explaining variation in Hp included  
368 climate ( $F_{3,308} = 12.62$ ,  $p = 0.0004$ ; Table S2, supplementary materials ) only. However, the  
369 specific invasion status was included in about half of the models within  $\Delta AICc < 2$  (in seven  
370 of the 16 selected) with *R. rattus* from sites of long-established invasion having lower Hp  
371 concentrations than rodents trapped in recently invaded sites (*R. rattus*: post-hoc Tukey's test,  
372  $p = 0.0362$ ; *Mastomys* sp.: post-hoc Tukey's test,  $p = 0.0058$ ; Figure 3). At the invasion front,  
373 Hp levels were similar in invasive and native species (post-hoc Tukey's test,  $p = 0.74$ ). No  
374 significant difference was detected in mean Hp between *Mastomys* sp. from recently invaded  
375 and non-invaded sites (post-hoc Tukey's test,  $p = 0.2163$ ).

376

## 377 **DISCUSSION**

378 Most studies investigating the role of immunity in the context of biological invasions have  
379 focused on comparisons between sympatric invasive and native species or between  
380 phylogenetically related species with contrasted levels of invasion success (Lee et al. 2006,  
381 Martin et al. 2010a). Because differences in immune investment may be due to intrinsic  
382 species characteristics, sampling designs focusing on interspecific comparisons may not be  
383 entirely appropriate for evaluating immune defences – invasion issues. Comparing several  
384 invasive (areas in which invaders are well established *vs* newly colonised areas) and native  
385 (non-invaded *vs* recently invaded areas) populations along a well-defined invasion route  
386 appears to be a more relevant approach, as it allows overcoming specific differences and  
387 taking into account the invasion history as a spatio-temporal continuum. Moreover, potential  
388 immune changes in native communities should be considered as a full issue that may  
389 ultimately impact the invasion process. Several predictions have been proposed with regard to  
390 immunity and invasion (Lee and Klasing 2004, Phillips et al. 2010). Our results did not fully  
391 support the major one, which is the EICA-refined hypothesis. Antibody-mediated defences

392 were found to increase along the invasion route, but for *M. m. domesticus* only (Fig. 2; Table  
393 S1, Supplementary materials). Moreover, the inflammatory response was found to be stronger  
394 in invasive populations at the invasion front than in populations from sites of long-established  
395 invasion, for both invasive species (Fig. 2; Fig. 3; Table S1, Table S2, Supplementary  
396 materials). Finally, we did not observe any difference in the immune effectors surveyed in  
397 native species between invaded and non-invaded localities.

398

### 399 **Effects of methodological biases and individual characteristics on immune variations**

400 We carefully considered experimental factors in our statistical models as they could have  
401 biased our results. The initial levels of serum hemolysis and HA scores were found to  
402 significantly influence HA/HL results irrespective of the invasion route (Table S1, Table S2,  
403 Supplementary materials). Higher values lead to higher HA/HL scores, regardless to the  
404 species and the invasion status of sites. These results were expected as Cp lysis is activated by  
405 the formation of antigen-NAb complexes (Matson et al. 2005). Furthermore, the initial serum  
406 hemolysis is part of the HL score and may represent the total final HL in extreme cases where  
407 NAb-mediated lysis did not occur in a serum. These results highlight the crucial importance to  
408 incorporate such 'experimental' factors as potential predictors in the explanatory models.

409 Immune responses could also be biased due to stress resulting from capture and handling  
410 procedures. However, the immune effectors studied here are not strongly affected by stressors.

411 In particular, it has recently been shown that Nabs and Cp are insensitive to capture and  
412 handling stress (Buehler et al. 2008). Besides, Nab production seems to be largely  
413 independent of internal or external stimuli (Ochsenbein and Zinkernagel 2000). In addition,  
414 we minimized the potential biases due to different effects of stress between localities of  
415 colonization statuses by using a consistent standardized capture protocol among sampling  
416 sites. This design aimed at preventing any differences in methodology-related stressors

417 between rodent populations. Finally, a strong argument showing that capture and handling  
418 procedures had a very few impact on the immune patterns observed is that native rodents,  
419 which are also subject to these methodological stresses, exhibited no immune variation  
420 between non-invaded sites and invasion fronts. This result suggests that the invasion front  
421 itself is not an area generating specific stress reactions. Altogether, these elements argue in  
422 favour of a limited impact of stress on the immune variations observed here.

423 We found a significant influence of age, body mass and sex on some immune responses that  
424 were higher in adults, heavier individuals and males, respectively (Table S1, Table S2,  
425 Supplementary materials). Age and body mass influenced significantly NAb levels for both  
426 native and invasive rodents on the 'rat' invasion route. These findings were congruent with  
427 other works (Rossi et al. 2013) and corroborated the common trend of an increased immune  
428 capacity with host growth and condition. Our result of male mice showing higher HL levels  
429 than females corroborates the life-history theory predicting that females should invest more in  
430 specific immune pathways than in constitutive immunity, which would thus be expected to be  
431 downregulated (Lee 2006). Moreover, other factors including ecological, physiological, social  
432 and behavioural ones, probably interacting in a complex way, may contribute to the observed  
433 patterns of sexual differences observed in rodent immunity. For instance, it has been shown in  
434 voles that social environment and steroid hormones affect in a complex way sex differences in  
435 immune function (Klein et al. 1997). This question therefore remains a challenging area of  
436 research in evolutionary ecology.

437

### 438 **Impact of environmental conditions on immune patterns**

439 As *M. m. domesticus* and *R. rattus* are likely to encounter new environments along their  
440 respective invasion route, immune variations observed in these invasive species may be  
441 considered as responses to environmental factors (reviewed in Colautti and Lau 2015). It has

442 been previously shown that environmental parameters can affect the immune functions of  
443 rodents (see Beldomenico et al. 2008). Climate and commensal habitat structure may  
444 influence rodent immune responses, for instance through resource availability or community  
445 composition, and were thus considered in our statistical models. Nevertheless, these  
446 environmental features could not explain alone the patterns observed. Climatic data differed  
447 between sites of long- and recently-established invasion for both invasive mice and rats but no  
448 variation was observed in HA and HL levels for *R. rattus*, and climate was not a relevant  
449 predictor to explain variations of HL in *M. m. domesticus*. Likewise, habitat structure did not  
450 differ between sites of long- and recently-established invasion along the ‘mouse’ invasion  
451 route, although immune responses significantly varied. Conversely, habitat structure differed  
452 between non-invaded sites and those recently invaded by the house mouse, but no immune  
453 variation was observed in native populations along this invasion transect.

454

#### 455 **Absence of immune patterns supporting the EICA and EICA refined hypotheses**

456 Variations in immune responses between long- and recently-established invasive populations  
457 did not support the predictions expected under the EICA and EICA refined hypotheses, which  
458 are respectively a general decrease of immune responsiveness or opposite changes between  
459 energetically costly immune pathways (decrease of responses) and less costly ones (increase  
460 of responses). Instead, this study revealed increased inflammatory and/or humoral responses in  
461 expanding invasive populations of mice and rats compared to long established populations  
462 (Fig. 2; Fig. 3).

463 These results questioned the relevance of the physiological and ecological interactions that are  
464 at the basis of EICA expectations, namely parasitism and immune costs. The risks of exposure  
465 to parasites and subsequent infections are supposed to be an important component of the  
466 novel environment encountered during invasion, which may explain these immune variations

467 along invasion routes. Nevertheless, by contrast with the enemy release emphasized in the  
468 EICA hypothesis, invaders may experience novel parasite pressure as they may be exposed  
469 and/or infected with novel local parasites when pathobiome communities differ between  
470 native and invasive species (see Diagne et al. in press; Galan et al. 2016 for examples). The  
471 overall infection risk in recently invaded areas could thus increase on invasion front. In  
472 particular, the ability to mount strong inflammatory responses may prevent ‘naïve host  
473 syndrome’ in invasive species or within the invasion front, a severe adverse effect of parasites  
474 on hosts with which they have no co-evolutionary history (Mastitsky et al. 2010). Besides,  
475 larger responses for Hp in invaders would be consistent with efforts to attenuate  
476 inflammation. Indeed, although the secretion of Hp is enhanced in inflammatory states, Hp  
477 has strong anti-inflammatory and anti-oxidative properties. During infection, Hp is best  
478 characterized as a protein that protects hosts against “all the dangers of the acute phase  
479 response” (Dobryszcka 1997). Within 24h of infection, circulating Hp concentration  
480 increases considerably, to replenish hemoglobin stores and damp down inflammatory  
481 responses and thus, their detrimental effects. High Hp levels may therefore be of benefit to  
482 invaders due to a lessening of the negative impacts of immunity.

483 The extent to which the presumed costs of immune defences (high for inflammation, low for  
484 antibody-mediated responses, Lee and Klasing 2004) can be generalized to all biological  
485 models and ecological conditions must also be questioned. Immune strategies have been  
486 shown to differ significantly between species, or even between races of domesticated species  
487 (Mendes et al. 2006). Moreover, the estimation of these costs may be specifically dependent  
488 on the response examined for a given pathway, and/or the condition/energetics parameters  
489 chosen. However trade-offs between inflammation and humoral responses involving immune  
490 effectors other than those investigated here cannot be excluded. We must keep in mind that the  
491 indicators studied here only reflect one part of the immune capacities to fight infections and

492 may not be generalizable to other effectors. Although it is very difficult to measure immune  
493 costs in the wild, a full study dedicated to this issue should provide crucial information on  
494 immune trade-offs during invasion process.

495

#### 496 **Stronger patterns of immune variations in mouse compared to rat invasive species**

497 Although environmental parameters and interspecific differences might be obvious reasons to  
498 explain contrasted immune variations observed between rats and mice along their respective  
499 invasion routes, different abilities in direct competition with native communities may also be  
500 proposed. Direct competition is often put forward as an explanation for the replacement of  
501 native species by exotic rodents (Drake and Hunt 2009). The black rat and house mouse  
502 strongly differ in their competitive interactions with native rodents. *R. rattus* has been shown  
503 to be aggressive to intruders, physically eliminating experimentally introduced conspecific  
504 individuals from an insular population (Smith and Banks 2014). They are much larger than  
505 *Mastomys* species and would therefore be at an advantage in direct competition. On the  
506 contrary, *M. m. domesticus* is known to perform poorly in direct competition with several  
507 native rodents (Gomez Villafane et al. 2013). Variation in immune phenotype may therefore  
508 be a less important strategy for the invasion success of *R. rattus* than for *M. m. domesticus*.  
509 Invasion success mediated by immune variations in house mice would account for  
510 observations that this species is less parasitized than rats, for zoonoses for instance (Blackwell  
511 1981, Meerburg et al. 2009). This hypothesis needs to be tested, and it would also be  
512 interesting to investigate whether it is observed in other cases of biological invasions.

513

#### 514 **No variation of immune response in native species**

515 The questions of what happens in native species and whether immune responses change in  
516 invaded compared to non-invaded areas remain scarcely explored. In this work, native

517 *Mastomys* species exhibited similar immune responses in non-invaded and recently invaded  
518 sites, along both the ‘mouse’ and ‘rat’ invasion routes (Fig. 2; Fig. 3). This pattern could  
519 result from the matching between environmental conditions (especially parasite pressure,  
520 resource availability, etc.) and the development of adequate immune responses by native  
521 rodents that have co-evolved with their natural environment. However, we found significant  
522 environmental differences between non-invaded sites and invasion fronts for both invasion  
523 routes. It is also likely that novel epidemiological conditions are established during invasion  
524 as the introduction of invaders directly affects host community composition and may also  
525 modify the infection risk of native species (Dunn and Hatcher 2015, Diagne et al. in revision,  
526 Galan et al. unpublished data). As such, the infection risk may be either amplified by spill-  
527 over and/or spill-back mechanisms, or decreased when invaders are not competent to maintain  
528 and transmit local parasites (dilution effect). Thus, the absence of immune variations in native  
529 species could reflect their inability to adapt their immune phenotypes.

530

531 In summary, our data provide no support for (i) the predictions of the EICA-refined  
532 hypothesis that antibody-mediated immunity should be favoured over inflammation during  
533 invader range expansion, and (ii) our expectations on immune changes experienced by native  
534 species when co-occurring with invaders. Whether immune changes could occur due to a  
535 higher risk of exposition to/infection with novel parasites at the invasion front and a greater  
536 ability of invasive species, such as *M. m. domesticus* in particular, to adjust their immune  
537 phenotypes during invasion are hypotheses that need to be confirmed experimentally in the  
538 future. Such studies could also enable to assess the respective roles of evolutionary and  
539 ecological processes in driving these phenotypic immune variations.

540



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548

549

## REFERENCES

550 Ardia, D. R., H. K. Parmentier, and L. A. Vogel. 2011. The role of constraints and limitation  
551 in driving individual variation in immune response. *Functional Ecology* 25: 61-73.

552 Barton K. (2016). MuMIn: Multi-Model Inference. R package version 1.15.6.  
553 <https://CRAN.R-project.org/package=MuMIn>.

554 Baumgarth, N., et al. 1999. Innate and acquired humoral immunities to influenza virus are  
555 mediated by distinct arms of the immune system. - *Proceedings of the National Academy of*  
556 *Sciences* 96: 2250-2255.

557 Blackwell, J. M. 1981. The role of the house mouse in diseases and zoonoses. - In: Berry, R.  
558 J. (ed.) *Biology of the house mouse*. Academic Press, pp. 591-609.

559 Blossey, B. and Notzold, R. 1995. Evolution of increased competitive ability in invasive  
560 nonindigenous plants - a hypothesis. - *Journal of ecology* 83: 887-889.

561 Brown, G. P., et al. 2015. Invader immunology: invasion history alters immune system  
562 function in cane toads (*Rhinella marina*) in tropical Australia. - *Ecology Letters* 18: 57-65.

563 Carroll, M. C. and Prodeus, A. P. 1998. Linkages of innate and adaptive immunity. - Current  
564 Opinions in Immunology 10: 36-40.

565 Charbonnel, N. and Cosson, J.-F. 2011. Molecular epidemiology of disease resistance genes  
566 with perspectives for researches on biological invasions and hybrid zones. In New frontiers of  
567 Molecular Epidemiology of Infectious Diseases, Morand et al. Eds, 332p.

568 Colautti, R. I. and Lau, J. A. 2015. Contemporary evolution during invasion: evidence for  
569 differentiation, natural selection, and local adaptation. - Molecular Ecology 24: 1999-2017.

570 Colautti, R. I., et al. 2004. Is invasion success explained by the enemy release hypothesis? -  
571 Ecology letters 7: 721-733.

572 Cornet, S., Brouat, C., Diagne, C., & Charbonnel, N. 2016. Eco- immunology and  
573 bioinvasion: revisiting the evolution of increased competitive ability hypotheses. Evolutionary  
574 Applications. DOI: 10.1111/eva.12406

575 Dalecky, A., et al. 2015. Range expansion of the invasive house mouse *Mus musculus*  
576 *domesticus* in Senegal, West Africa: a synthesis of trapping data over three decades, 1983–  
577 2014. - Mammal Review.

578 Daszak, P., et al. 2000. Emerging infectious diseases of wildlife - threats to biodiversity and  
579 human health. - Science 287: 443-449.

580 Diagne, C. A., et al. in revision. Parasites and invasions: changes in gastrointestinal helminth  
581 assemblages in invasive and native rodents in Senegal. – In revision (Available on BioRxiv).

582 Dobryszcka, W. 1997. Biological functions of haptoglobin-new pieces to an old puzzle. -  
583 Eur J Clin Chem Clin Biochem 35: 647-654.

584 Drake, D. and Hunt TL. 2009. Invasive rodents on islands: integrating historical and  
585 contemporary ecology. - Biol Invasions 11: 1483-1487.

586 Dunn, A. M. 2009. Parasites and Biological Invasions. - In: Webster, J. P. (ed.) Advances in  
587 Parasitology, Vol 68: Natural History of Host-Parasite Interactions, pp. 161-184.

588 Dunn, A. M. and Hatcher, M. J. 2015. Parasites and biological invasions: parallels,  
589 interactions, and control. - TRENDS in Parasitology 31: 189-199.

590 Facon, B., et al. 2006. A general eco-evolutionary framework for understanding bioinvasions.  
591 - Trends in Ecology & Evolution 21: 130-135.

592 Gervasi, S. S., D. J. Civitello, H. J. Kilvitis, and L. B. Martin. 2015. The context of host  
593 competence: a role for plasticity in host–parasite dynamics. TRENDS in Parasitology 31: 419-  
594 425.

595 Gómez-Díaz, E., J. A. Morris-Pocock, J. González-Solís, and K. D. McCoy. 2012.  
596 Transoceanic host dispersal explains high seabird tick diversity on Cape Verde islands.  
597 Biology Letters 8: 616-619.

598 Gomez Villafane, I. E., et al. 2013. Differences in population parameters of *Rattus norvegicus*  
599 in urban and rural habitats of central Argentina. - Mammalia 77: 187-193.

600 Granjon, L. and Duplantier, J. M. 2009. Les rongeurs de l'Afrique sahélo-soudanienne. -  
601 Publications scientifiques du Muséum.

602 Harris, D. B. 2009. Review of negative effects of introduced rodents on small mammals on  
603 islands. - Biological Invasions 11: 1611–1630.

604 Huntoon, K. M., et al. 2008. The acute phase protein haptoglobin regulates host immunity. - J.  
605 Leukoc. Biol. 84: 170-181.

606 Keesing, F., et al. 2010. Impacts of biodiversity on the emergence and transmission of  
607 infectious diseases. - Nature 468: 647-652.

608 Kelly, D., et al. 2009. Parasite spillback: a neglected concept in invasion ecology? - Ecology  
609 90: 2047-2056.

610 Klasing, K. C. 2004. The costs of immunity. - Current Zoology (formerly Acta Zoologica  
611 Sinica) 50: 961 - 969.

612 Klein, S. L., et al. 1997. Social environment and steroid hormones affect species and sex  
613 differences in immune function among voles. - Hormones and Behavior 32: 30-39.

614 Kolar, C. S. and Lodge, D. M. 2001. Progress in invasion biology: predicting invaders. -  
615 Trends in Ecology & Evolution 16: 199-204.

616 Konecny, A., et al. 2013. Invasion genetics of the introduced black rat (*Rattus rattus*) in  
617 Senegal, West Africa. - Molecular Ecology 22: 286-300.

618 Lee, K. A. and Klasing, K. C. 2004. A role for immunology in invasion biology. - Trends in  
619 ecology and evolution 19: 523-529.

620 Lee, K. A., et al. 2006. Contrasting adaptive immune defenses and blood parasite prevalence  
621 in closely related Passer sparrows. - Oecologia 150: 383-392.

622 Llewellyn, D., et al. 2012. Reduced investment in immune function in invasion-front  
623 populations of the cane toad (*Rhinella marina*) in Australia. - Biological Invasions 14: 999-  
624 1008.

625 Lowry, E., Rollinson, E. J., Laybourn, A. J., Scott, T. E., Aiello-Lammens, M. E., Gray, S.  
626 M., & Gurevitch, J. 2013. Biological invasions: a field synopsis, systematic review, and  
627 database of the literature. Ecology and Evolution, 3: 182-196.

628 Martin, L. B., et al. 2010a. Variation in inflammation as a correlate of range expansion in  
629 Kenyan house sparrows. - Oecologia 164: 339-347.

630 Martin, L. B., et al. 2010b. The effects of anthropogenic global changes on immune functions  
631 and disease resistance. - *Annals of the New York Academy of Sciences* 1195 : 129-148.

632 Mastitsky, S. E., et al. 2010. Parasites of exotic species in invaded areas: does lower diversity  
633 mean lower epizootic impact? . - *Diversity and Distribution* 16: 798-803.

634 Matson, K. D., et al. 2012. Baseline haptoglobin concentrations are repeatable and predictive  
635 of certain aspects of a subsequent experimentally-induced inflammatory response. - *Comp.*  
636 *Biochem. Physiol., Part A Mol. Integr. Physiol* 162: 7-15.

637 Matson, K. D., et al. 2005. A hemolysis–hemagglutination assay for characterizing  
638 constitutive innate humoral immunity in wild and domestic birds. - *Developmental &*  
639 *Comparative Immunology* 29: 275-286.

640 Meerburg, B. G., et al. 2009. Rodent-borne diseases and their risks for public health. - *Critical*  
641 *reviews in microbiology* 35: 221-270.

642 Mendes, L., et al. 2006. Variation in the innate and acquired arms of the immune system  
643 among five shorebird species. - *Journal of Experimental Biology* 209: 284-291.

644 Na, R., & Gijzen, M. 2016. Escaping Host Immunity: New Tricks for Plant Pathogens. *PLoS*  
645 *Pathogens*, 12(7), e1005631.

646 Ochsenbein, A. F., and Zinkernagel, R. M. 2000. Natural antibodies and complement link  
647 innate and acquired immunity. - *Immunology today* 21: 624-630.

648 Pedersen, A. B. and Babayan, S. A. 2011. Wild immunology. - *Molecular Ecology* 20: 872-  
649 880.

650 Phillips, B. L., et al. 2010. Life-history evolution in range-shifting populations. - *Ecology* 91:  
651 1617-1627.

652 Prenter, J., et al. 2004. Roles of parasites in animal invasions. - Trends in Ecology &  
653 Evolution 19: 385-390.

654 R Core Team. 2015. A language and environment for statistical computing. Vienna, Austria.  
655 2014.

656 Raberg, L., et al. 2002. Basal metabolic rate and the evolution of the adaptive immune system.  
657 - Proceedings of the Royal Society of London B. 269: 817-821.

658 Roberts, L. S. and Janovy, J. 2010. Foundations of Parasitology. - Mcgraw Hill.

659 Rossi, S., et al. 2013. Innate immunity correlates with host fitness in wild boar (*Sus scrofa*)  
660 exposed to classical swine fever. -.

661 Saco, Y., et al. 2011. Serum acute phase proteins as biomarkers of pleuritis and cranio-ventral  
662 pulmonary consolidation in slaughter-aged pigs. - Research in veterinary science 91: 52-57.

663 Sapolsky R.M. 1987. Stress, social status, and reproductive physiology in free-living  
664 baboons. In: Crews DE (ed) Psychobiology of Reproductive Behavior: An Evolutionary  
665 Perspective. Prentice-Hall, Englewood Cliffs, NJ, p 291–322.

666 Schulenburg, H., J. Kurtz, Y. Moret, and M. T. Siva-Jothy. 2009. Introduction. Ecological  
667 immunology. Philosophical transactions of the Royal Society of London. Series B, Biological  
668 sciences 364:3-14.

669 Sikes, R. S., et al. 2011. Guidelines of the American Society of Mammalogists for the use of  
670 wild mammals in research. - Journal of Mammalogy 92: 235-253.

671 Smith, H. M. and Banks, P. B. 2014. Disease and competition, not just predation, as drivers of  
672 impacts of the black rat (*Rattus rattus*) on island mammals. - Global Ecology and  
673 Biogeography 23: 1485-1488.

674 Soler, L., et al. 2013. Response of salivary haptoglobin and serum amyloid A to social  
675 isolation and short road transport stress in pigs. *Research in veterinary science* 95: 298-302.

676 Sorci, G. and Faivre, B. 2009. Inflammation and oxidative stress in vertebrate host-parasite  
677 systems. - *Philosophical Transactions of the Royal Society B-Biological Sciences* 364: 71-83.

678 Strauss, A., et al. 2012. Invading with biological weapons: the importance of disease-  
679 mediated invasions. - *Functional Ecology* 26: 1249-1261.

680 White, T. A. and Perkins, S. E. 2012. The ecoimmunology of invasive species. - *Functional*  
681 *ecology* 26: 1313-1323.

682 Zuk, M. and Stoehr, A. M. 2010. Sex differences in susceptibility to infection: an  
683 evolutionary perspective. *Sex hormones and immunity to infection*. Springer, pp. 1-17.

684

685 **Data archiving.**Data for this study will be available onDryad ([DataDryad.Org](http://DataDryad.Org)) after  
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687

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693 managed the extensive field work. KB, MK, YN, MD, AS, CB, NC and CD performed the  
694 field sampling. SP and EA were responsible for sample collection and the database.

695

696 **Table 1:** Sample size for each assay, by invasion status (LI = sites of long-established  
697 invasion; IF = invasion front; NI = non-invaded sites), sampled site (code in parentheses) and  
698 host species for a) the ‘mouse’ invasion route (166 *Mus musculus domesticus*,  
699 145 *Mastomys erythroleucus*) and b) the ‘rat’ invasion route (196 *Rattus rattus*,  
700 88 *Mastomys natalensis*, 50 *Mastomys erythroleucus*). Numbers in parentheses indicate sample  
701 size for males/females, respectively. ‘-’ indicates that no rodent was trapped or  
702 analysed. Sample sizes differ between immune assays because of the limited volume of some  
703 serum samples prevented to perform both assays.

704 a)

Invasion status	Sites	Hemagglutination-Hemolysis		Haptoglobin	
		<i>M. m. domesticus</i>	<i>Ma. erythroleucus</i>	<i>M. m. domesticus</i>	<i>Ma. erythroleucus</i>
LI	Dagathie (DAG)	19(10/9)	-	12(6/6)	-
	Mbakhana (MBA)	23(13/10)	-	12(8/4)	-
	Thilene (THL)	20(8/12)	-	13(3/10)	-
	Ndombo (NDB)	21(10/11)	-	15(7/8)	-
IF	Dodel (DOD)	23(9/14)	19(11/8)	10(3/7)	12(9/3)
	Aere Lao (AEL)	21(10/11)	19(12/7)	17(8/9)	11(7/4)
	Dendoudi (DEN)	17(12/5)	23(16/7)	10(6/4)	16(12/4)
	Lougue (LOU)	22(10/12)	22(15/7)	10(3/7)	14(11/3)
NI	Doumga Lao (DOL)	-	20(10/10)	-	14(7/7)
	Lambango (LAM)	-	20(9/11)	-	16(7/9)
	SaréMaoundé (SAM)	-	9(5/4)	-	5(3/2)
	DiomandouWalo (DIW)	-	13(9/4)	-	11(7/4)

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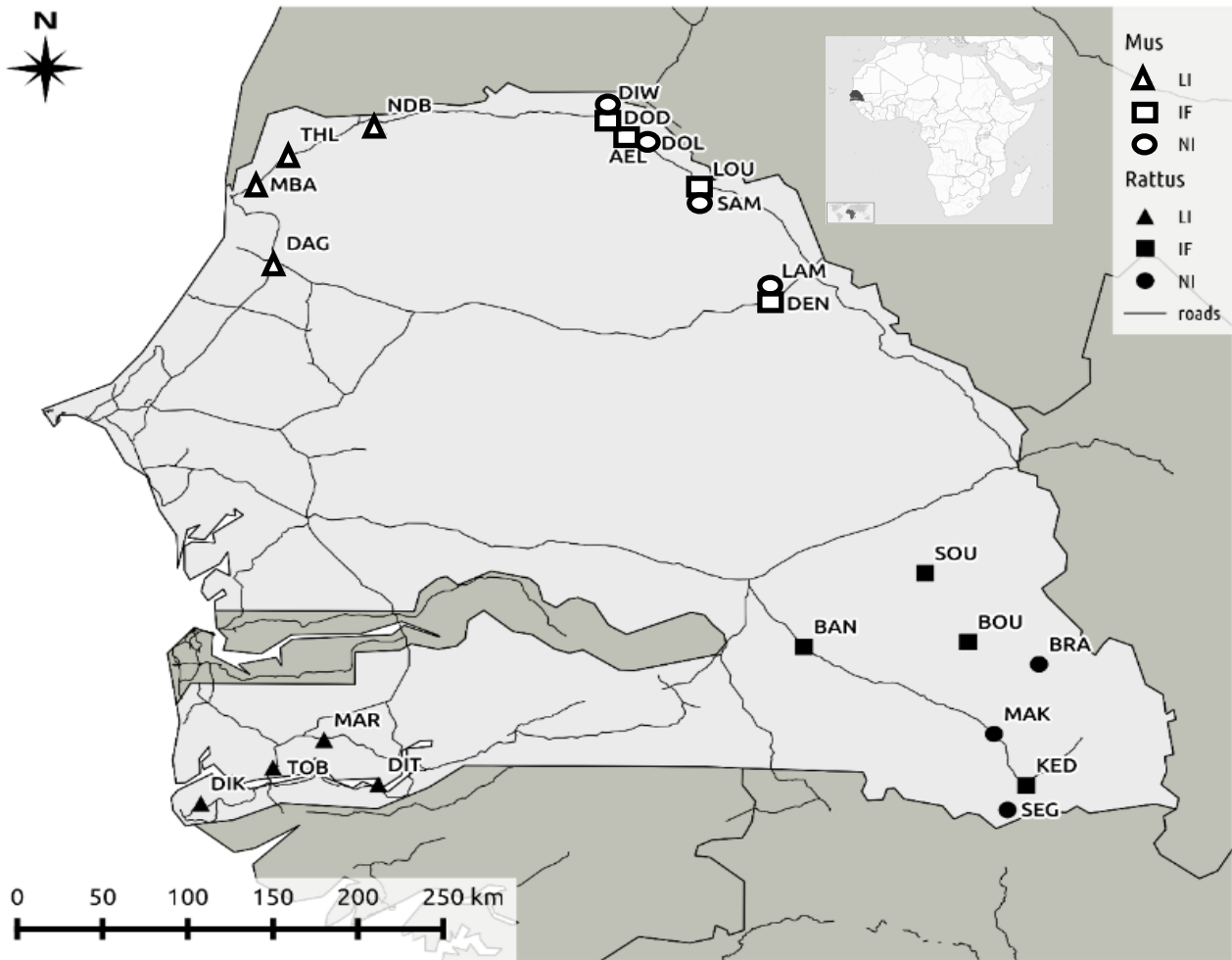
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Invasion status	Sites	Hemagglutination-Hemolysis			Haptoglobin		
		<i>R. rattus</i>	<i>Ma. erythroleucus</i>	<i>Ma. natalensis</i>	<i>R. rattus</i>	<i>Ma. erythroleucus</i>	<i>Ma. natalensis</i>
LI	Diakene Wolof (DIK)	24(9/15)	-	-	24(9/15)	-	-
	Diattacounda (DIT)	27(13/14)	-	-	27(13/14)	-	-
	Marsassoum (MAR)	26(13/13)	-	-	26(13/13)	-	-
	Tobor (TOB)	20(6/14)	-	-	20(6/14)	-	-
IF	BadiNieriko (BAN)	21(8/13)	11(5/6)	-	23(10/13)	12(6/6)	-
	Bountougoufara (BOU)	31(9/22)	12(7/5)	-	31(9/22)	13(8/5)	-
	Kedougou (KED)	22(9/13)	-	22(9/13)	22(9/13)	-	22(9/13)
	Soutouta (SOU)	22(11/11)	9(5/4)	-	23(12/11)	9(5/4)	-
NI	Bransan (BRA)	-	-	23(10/13)	-	-	23(10/13)
	Mako (MAK)	-	-	23(11/12)	-	-	26(13/13)
	Segou (SEG)	-	-	20(7/13)	-	-	21(8/13)

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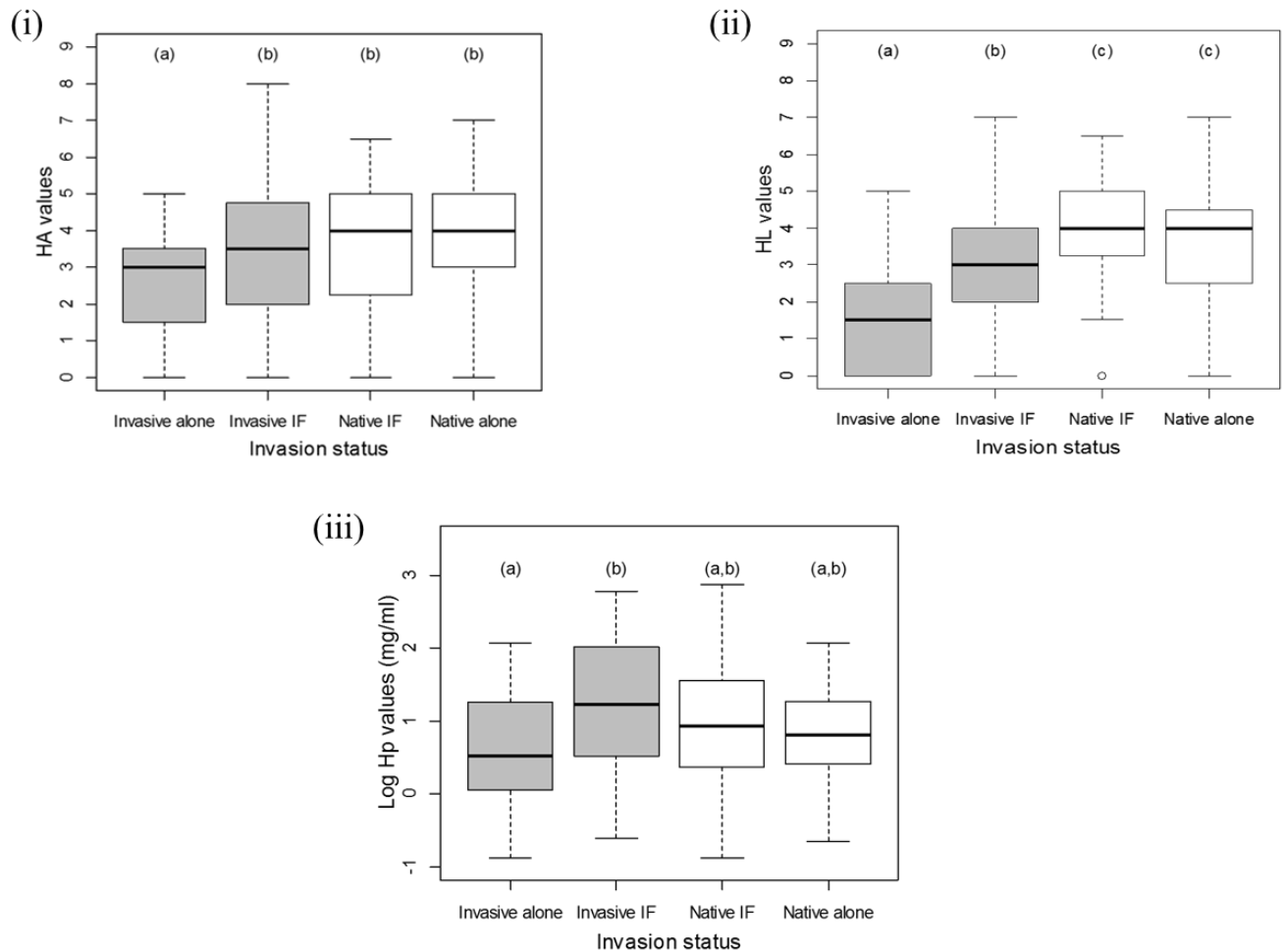


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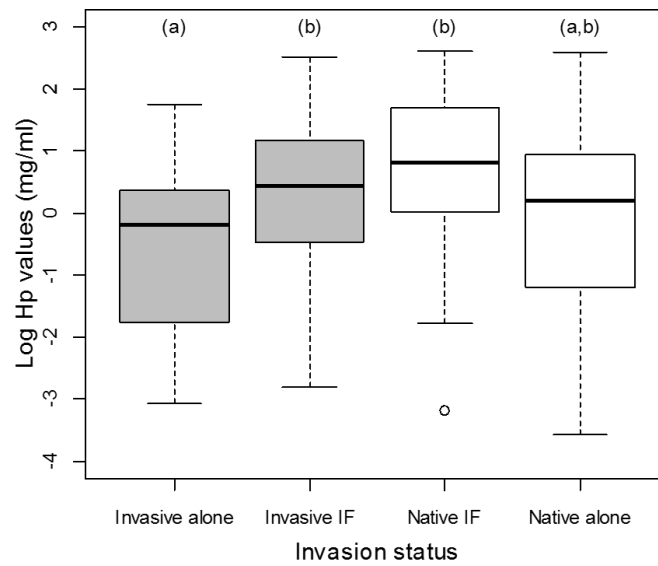
**Figure 1.** Sampling sites. Triangles, squares and circles correspond respectively to sites of long-established invasion (> 100 years), recently invaded sites (< 30 years) and non-invaded sites. Mouse invasion route (symbols in white): Dagathie (DAG), Mbakhana (MBA), Thilene (THL), Ndombo (NDB), Dodel (DOD), Aere Lao (AEL), Dendoudi (DEN), Lougue (LOU), Doumga Lao (DOL), Lambango (LAM), SaréMaoundé (SAM), DiomandouWalo (DIW). Rat invasion route (symbols in black): Diakene Wolof (DIK), Diattacounda (DIT), Marsassoum (MAR), Tobor (TOB), BadiNieriko (BAN), Bountougoufara (BOU), Kedougou (KED), Soutouta (SOU), Bransan (BRA), Mako (MAK), Segou (SEG).



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733 **Figure 2.** Effects of specific invasion status on the variation in (i) hemagglutination (HA), (ii)  
 734 hemolysis (HL) and (iii) haptoglobin (Hp) levels in the serum of *Mus musculus domesticus*  
 735 (*boxplots in grey*) and *Mastomys erythroleucus* (*boxplots in white*). The whiskers denote 1.5  
 736 Inter-Quartile Range. Legend: Invasive alone = *M. m. domesticus* in sites of long-established  
 737 invasion; Invasive IF = *M. m. domesticus* on invasion front (IF); Native IF = *Ma.*  
 738 *erythroleucus* on IF; Native alone = *Ma. erythroleucus* in non-invaded sites. The letters above  
 739 boxplots denote the significance of differences between specific invasion statuses: boxplots  
 740 with the same letter above are no significantly different while boxplots with different letters  
 741 above are significantly different.

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746 **Figure 3.** Effects of specific invasion status on the variation of serum haptoglobin (Hp)  
 747 concentration in *Rattusrattus* (boxplots in grey) and *Mastomysspp.* (boxplots in white). The  
 748 whiskers denote 1.5 Inter-Quartile Range. Legend: Invasive alone = *R. rattus* at sites of long-  
 749 established invasion; Invasive IF = *R. rattus* on invasion front (IF); Native IF = *Ma.*  
 750 *erythroleucus* (n = 34) and *Ma. natalensis* (n = 22) on IF; Native alone = *Ma. natalensis* in non-  
 751 invaded sites. The letters above boxplots denote the significance of differences between  
 752 specific invasion statuses: boxplots with the same letter above are no significantly different  
 753 while boxplots with different letters above are significantly different.

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## Supplementary materials

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**Table S1:** Results of the linear mixed-effect models for hemagglutination (HA), hemolysis (HL) and haptoglobin (Hp) variations along the *Mus musculus domesticus* invasion route. ‘i:j’ indicates the interaction between the factors ‘i’ and ‘j’. Significant effects are highlighted in bold. AICc: Akaike’s information criterion with correction for finite sample size.  $\Delta$ : difference between the model chosen and the model with the lowest AICc. N: total number of models selected as  $\Delta AICc < 2$  with respect to the model with the lowest AICc. S: number of models selected in which the factor was significant.

Assay	AICc ( $\Delta$ )	Factors in the model selected	F-value	<i>p</i> -value	S (N)
Hemagglutination (HA)	1187.4 (1.07)	<b>Plate</b>	11.27	0.0163	8 (8)
		<b>Climate (component 1)</b>	5.83	< 0.0001	7 (8)
		<b>Climate (component 2)</b>	16.81	< 0.0001	8 (8)
		<b>Invasion status</b>	12.48	< 0.0001	8 (8)
		Sex	0.0001	0.9931	8 (8)
		<b>Invasion status:Sex</b>	4.75	0.0029	8 (8)
Hemolysis (HL)	1014.8 (1.71)	<b>Initial hemolysis</b>	99.82	< 0.0001	5 (5)
		<b>HA</b>	50.72	< 0.0001	5 (5)
		<b>Sex</b>	9.87	0.0018	5 (5)
		<b>Invasion status</b>	26.56	< 0.0001	5 (5)
Haptoglobin (Hp)	464.7 (0.00)	<b>Climate (component 1)</b>	13.31	0.0003	5 (5)
		<b>Climate (component 2)</b>	22.74	< 0.0001	5 (5)
		<b>Invasion status</b>	5.22	0.0017	5 (5)

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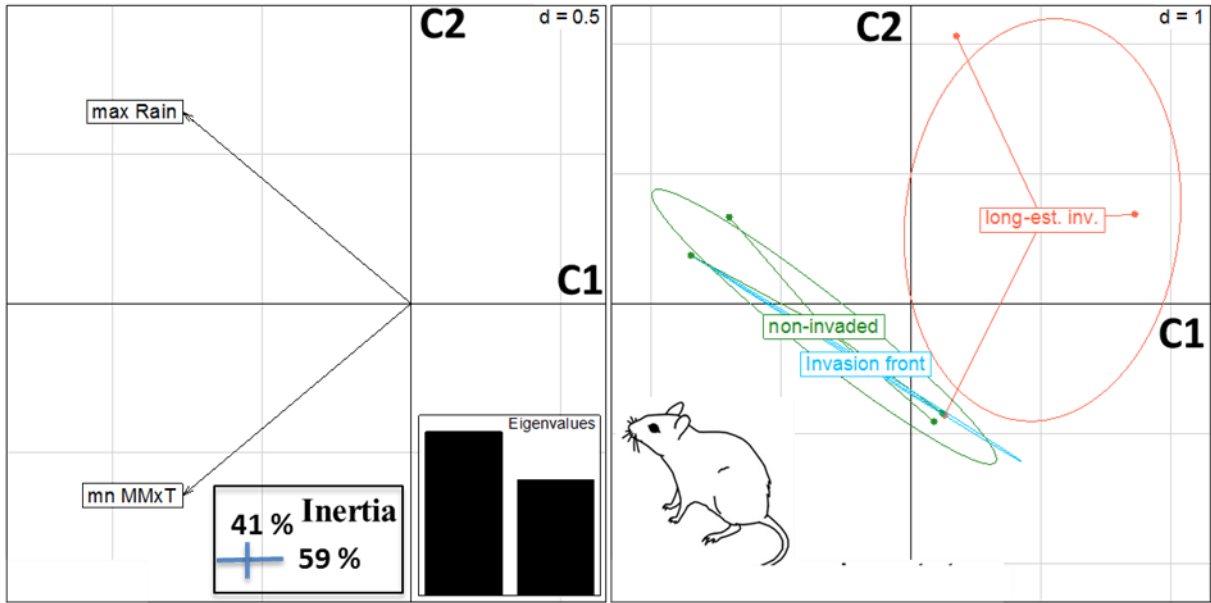
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**Table S2:** Results of the linear mixed-effect models for hemagglutination (HA), hemolysis (HL) and haptoglobin (Hp) variations along the *Rattus. rattus* invasion route. ‘i;j’ indicates the interaction between the factors ‘i’ and ‘j’. Significant effects are highlighted in bold. AICc: Akaike’s information criterion with correction for finite sample size.  $\Delta$ : difference between the model chosen and the model with the lowest AICc. N: total number of models selected as  $\Delta AICc < 2$  with respect to the model with the lowest AICc. S: number of models selected in which the factor was significant.

Assay	AICc ( $\Delta$ )	Factors in the model chosen	F-value	p-value	S (N)
Hemagglutination (HA)	1210.3 (0.84)	<b>Serum hemolysis</b>	12.74	0.0004	8 (8)
		<b>Age</b>	9.40	0.0024	8 (8)
		<b>Body mass</b>	4.93	0.0270	8 (8)
		<b>Climate (component 1)</b>	12.91	0.0004	8 (8)
Hemolysis (HL)	765.6 (1.33)	<b>Serum hemolysis</b>	57.76	< 0.0001	16 (16)
		<b>HA</b>	355.92	< 0.0001	16 (16)
		<b>Commensal habitat (component 1)</b>	21.82	< 0.0001	16 (16)
Haptoglobin (Hp)	2170.0 (1.64)	<b>Climate (component 1)</b>	12.62	0.0004	10 (16)

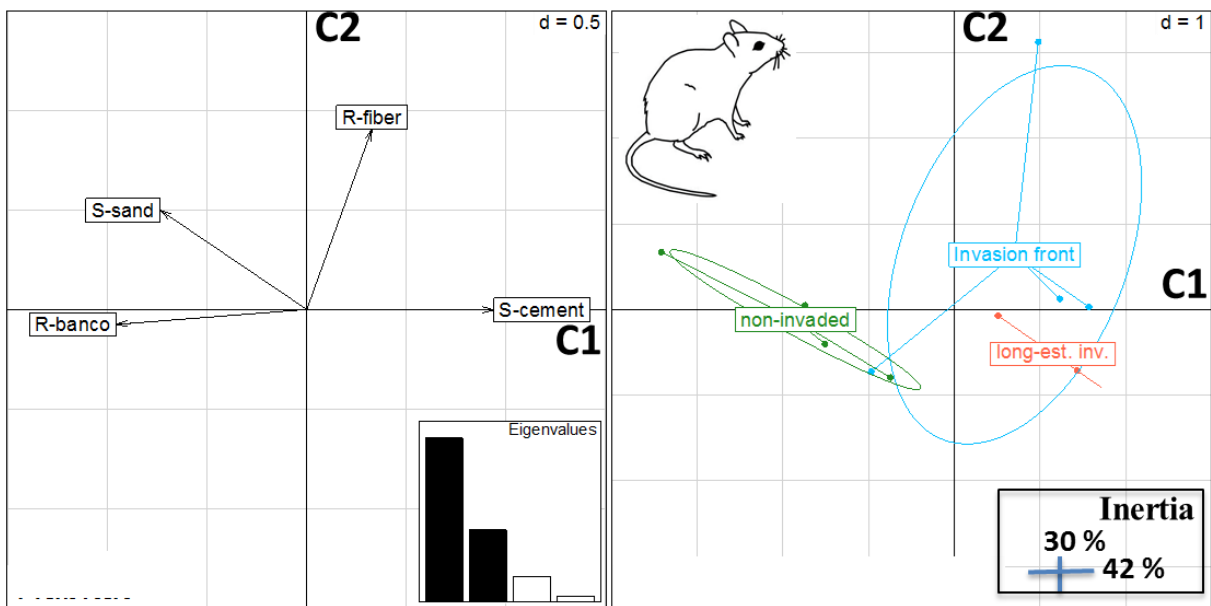
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(a)



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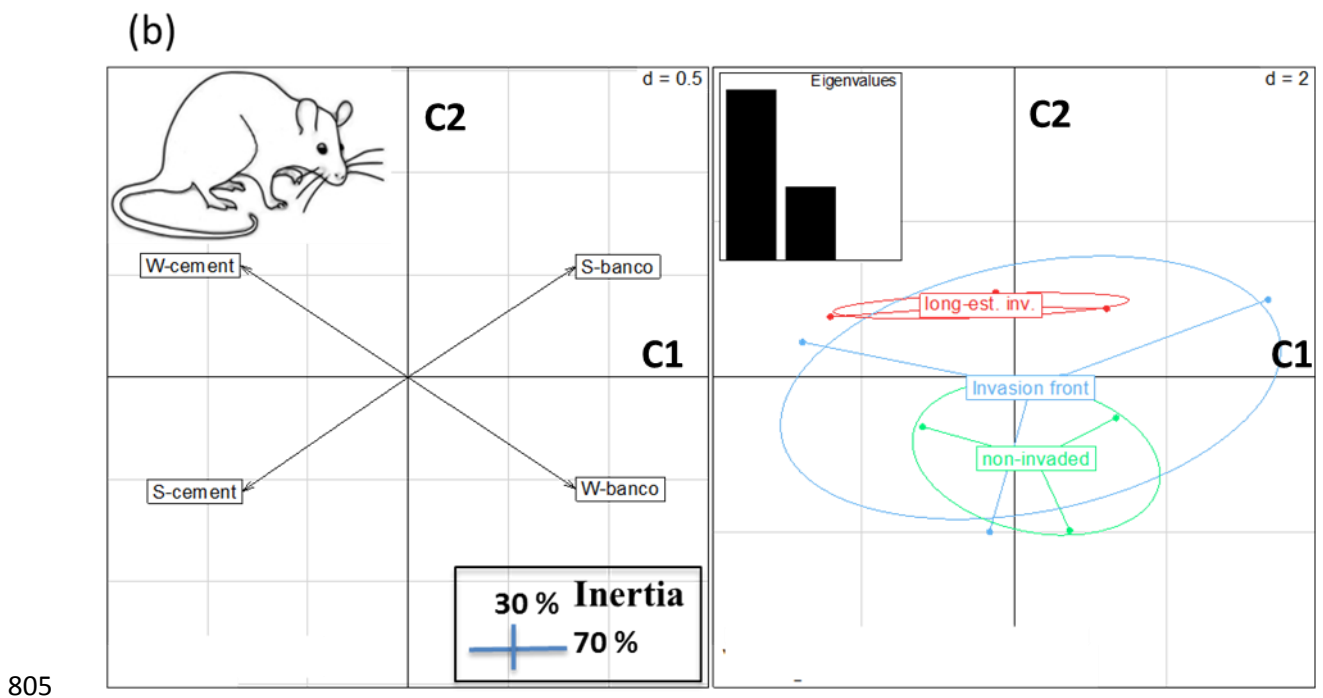
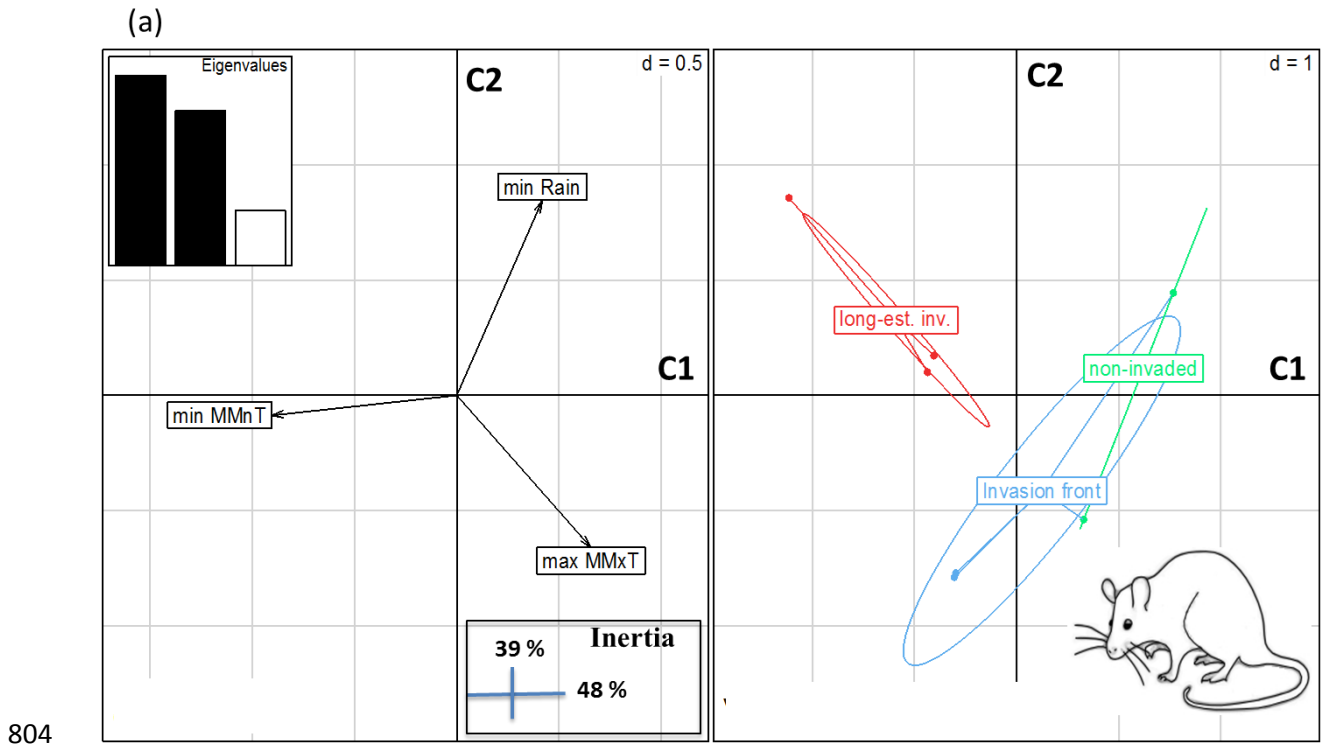


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789 **Fig. S2.** Principal component analysis (PCA) of climatic (a) and commensal habitat (b) data for  
790 categories of localities sampled along the house mouse invasion route. PCAs were based on the  
791 uncorrelated climatic (temperatures in °C, rainfall in mm, recorded between 1997 and 2012) and  
792 commensal habitat (b) variables remaining after a first PCA (left side). Between-within analysis  
793 showed significant classes (Monte-Carlo test,  $p < 0.05$ ; see figures right side). Legend: max  
794 rain: maximum monthly rainfall during rainy season (mean per year); mnMnTM: lowest

795 monthly minimum temperature (mean per year); S-cement: floor in cement;W-banco: wall in  
796 banco;S-sand: floor in sand;R-banco:: ceiling in banco; R-fiber: ceiling in fibers; localities of  
797 long-established invasion (red); localities of invasion front (blue); non-invaded localities (green).  
798 Temperature data were recorded from local weather stations closest to sampled localities and  
799 available on <http://www.ncdc.noaa.gov/cdo-web/datasets>; rainfall data were recorded from  
800 satellite products available on <http://richardis.univparis1.fr/precip/rainser1.html> with GPCP-  
801 1DD as data source. Commensal habitat data were recorded directly on the field during rodent  
802 sampling.

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806 **Fig. S3.** Principal component analysis (PCA) of climatic (a) and commensal habitat (b) data  
 807 for categories of localities sampled along the rat mouse invasion route. PCAs were based on  
 808 the uncorrelated climatic (temperatures in °C, rainfall in mm, recorded between 1997 and  
 809 2012) and commensal habitat (b) variables remaining after a first PCA (left side). Between-  
 810 within analysis showed significant classes (Monte-Carlo test,  $p < 0.05$ ; see figure right side)



811 only for climatic data. Legend: max MMxT: highest daily maximum temperature (mean per  
812 year); min MMnT: lowest daily minimum temperature (mean per year); min Rain: minimum  
813 monthly rainfall during rainy season (mean per year); W-cement: wall in cement; S-cement:  
814 floor in cement; W-banco: wall in banco; S-banco: floor in banco; localities of long-established  
815 invasion (red); localities of invasion front (blue); non-invaded localities (green).  
816 Temperature data were recorded from local weather stations closest to sampled localities and  
817 available on <http://www.ncdc.noaa.gov/cdo-web/datasets>; rainfall data were recorded from  
818 satellite products available on <http://richardis.univparis1.fr/precip/rainser1.html> with GPCP-  
819 1DD as data source. Commensal habitat data were recorded directly on the field during rodent  
820 sampling.

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