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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 19th century. At the beginning of the 20th 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 (Dobryczycka 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 10µl 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 log₂ 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 μ 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 Dobryzycka, 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) after
686 manuscript is accepted for publication.

687

688 **Authorship.**CD performed the HAHL analyses and wrote the first draft of the paper. EGF
689 participated in the interpretation and statistical analyses of HAHL data. SC, LH, SD
690 performed the Hp analyses and SC carried out the statistical analyses. OFG participated in the
691 multivariate analyses of environmental data. CB, AD and NC designed the sampling. NC
692 designed the immunological experiments and formulated the hypotheses tested. AD and KB
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)

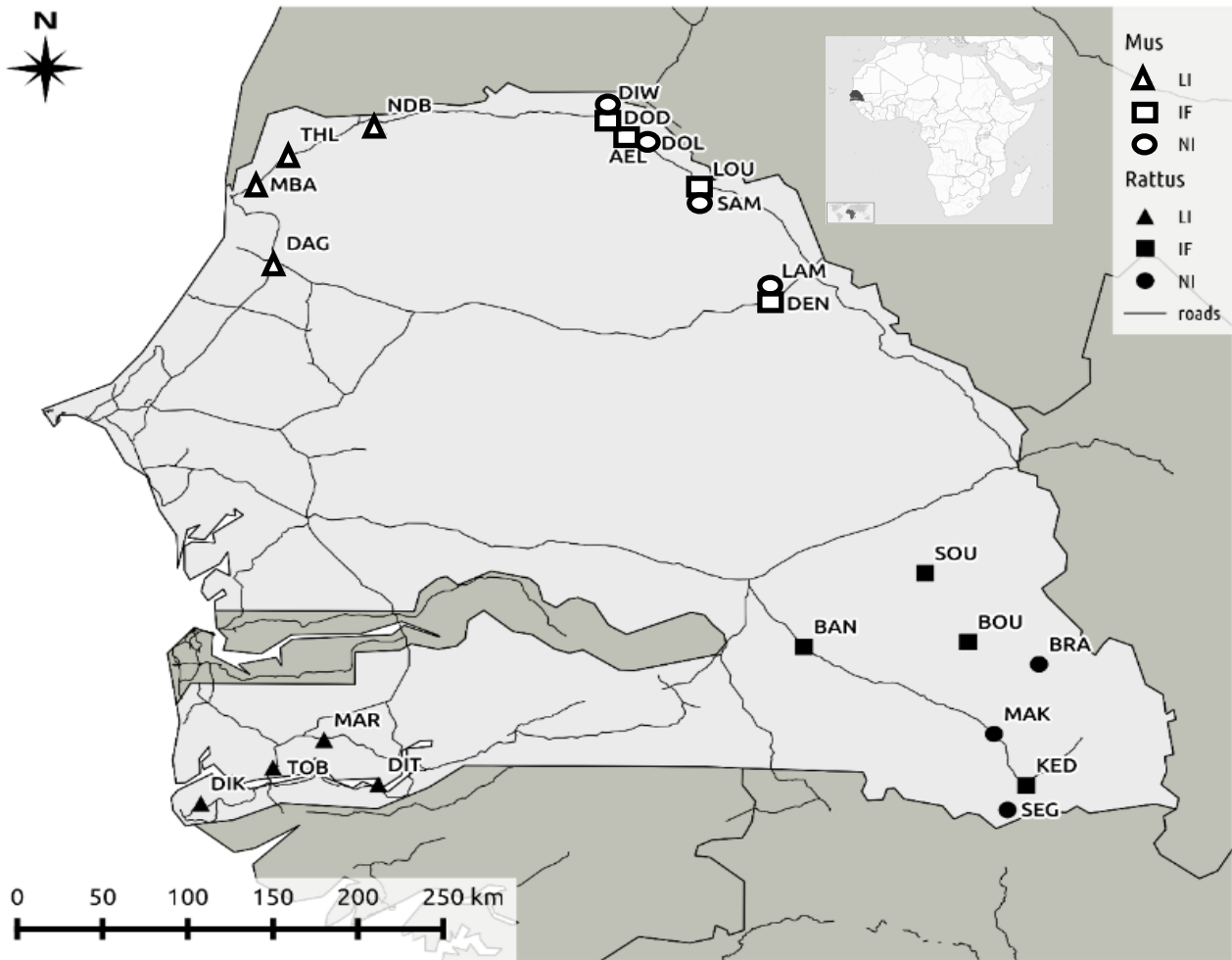
705

706 b)

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)

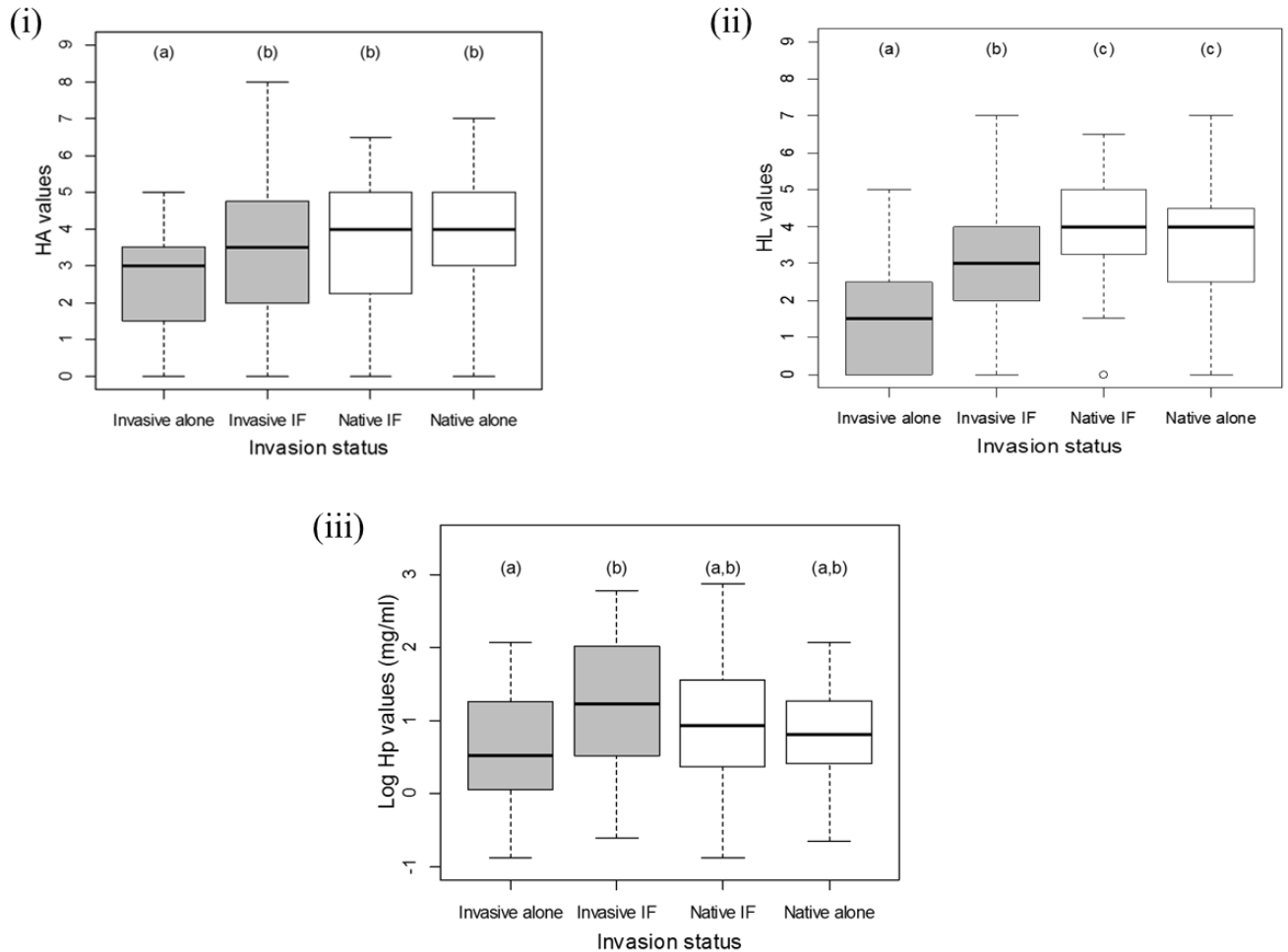
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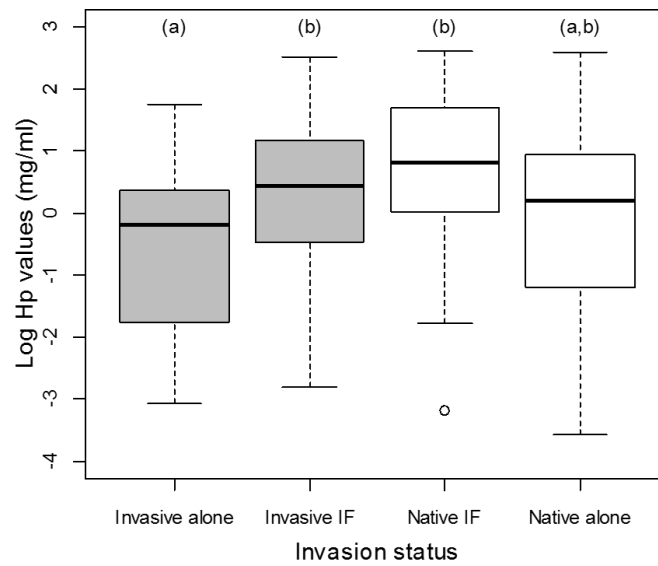
Figure 1. Sampling sites. Triangles, squares and circles correspond respectively to sites of long-established invasion (> 100 years), recently invaded sites (< 30 years) and non-invaded sites. Mouse invasion route (symbols in white): Dagathie (DAG), Mbakhana (MBA), Thilene (THL), Ndombo (NDB), Dodel (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. Δ : difference between the model chosen and the model with the lowest AICc. N: total number of models selected as $\Delta\text{AICc} < 2$ with respect to the model with the lowest AICc. S: number of models selected in which the factor was significant.

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

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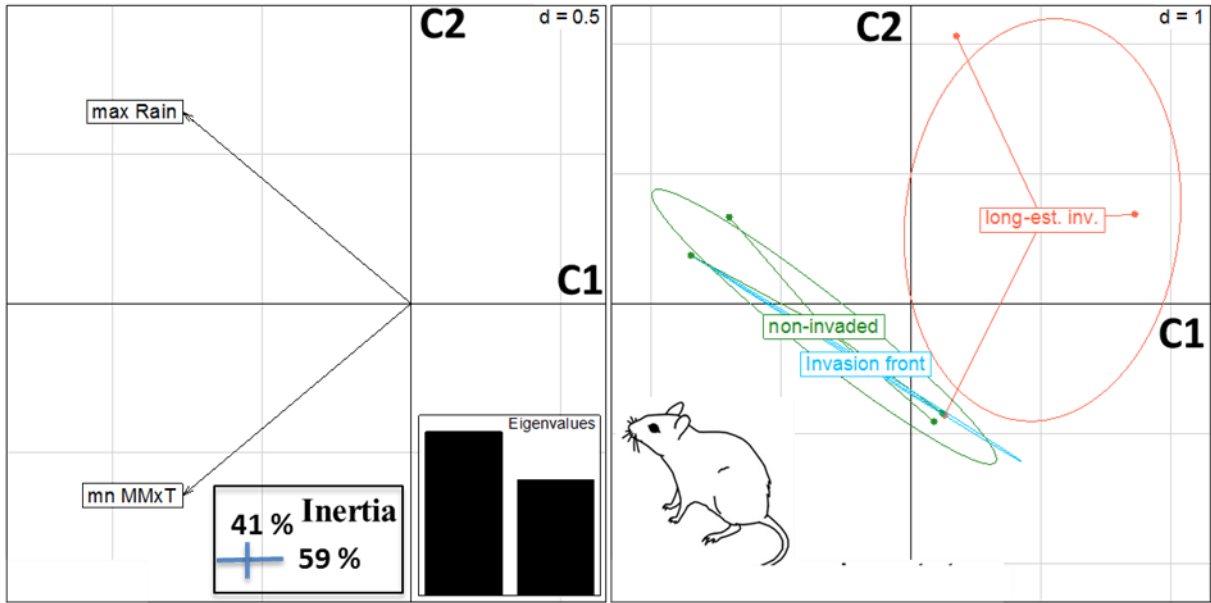
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Table S2: Results of the linear mixed-effect models for hemagglutination (HA), hemolysis (HL) and haptoglobin (Hp) variations along the *Rattus. rattus* invasion route. ‘i;j’ indicates the interaction between the factors ‘i’ and ‘j’. Significant effects are highlighted in bold. AICc: Akaike’s information criterion with correction for finite sample size. Δ: 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.

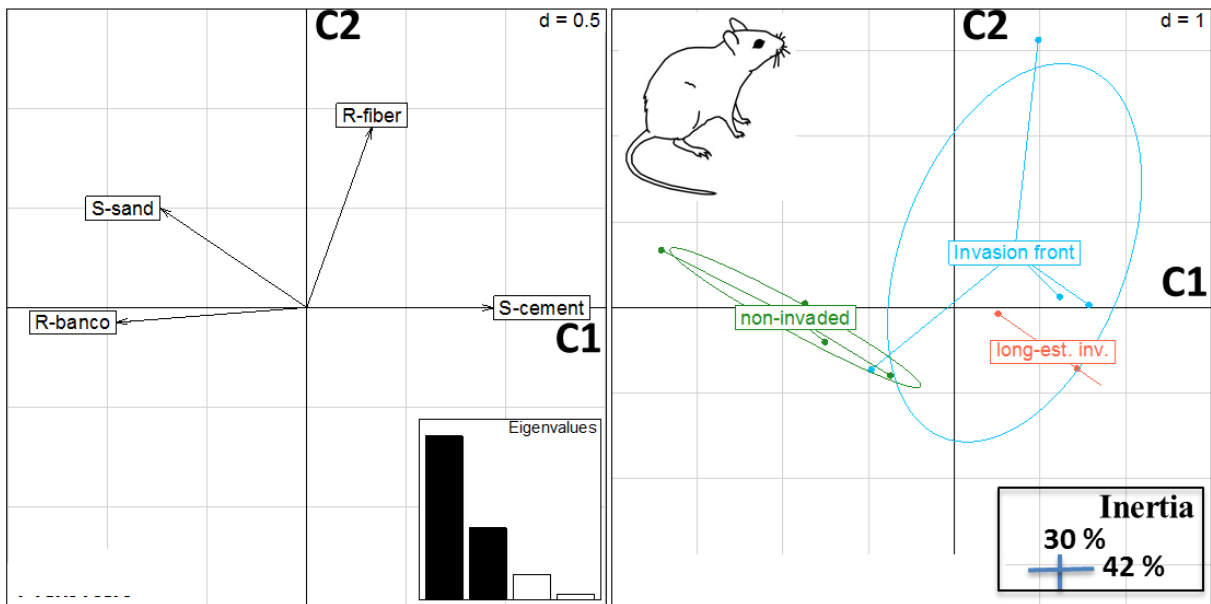
Assay	AICc (Δ)	Factors in the model chosen	F-value	p-value	S (N)
Hemagglutination (HA)	1210.3 (0.84)	Serum hemolysis	12.74	0.0004	8 (8)
		Age	9.40	0.0024	8 (8)
		Body mass	4.93	0.0270	8 (8)
		Climate (component 1)	12.91	0.0004	8 (8)
Hemolysis (HL)	765.6 (1.33)	Serum hemolysis	57.76	< 0.0001	16 (16)
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

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(a)



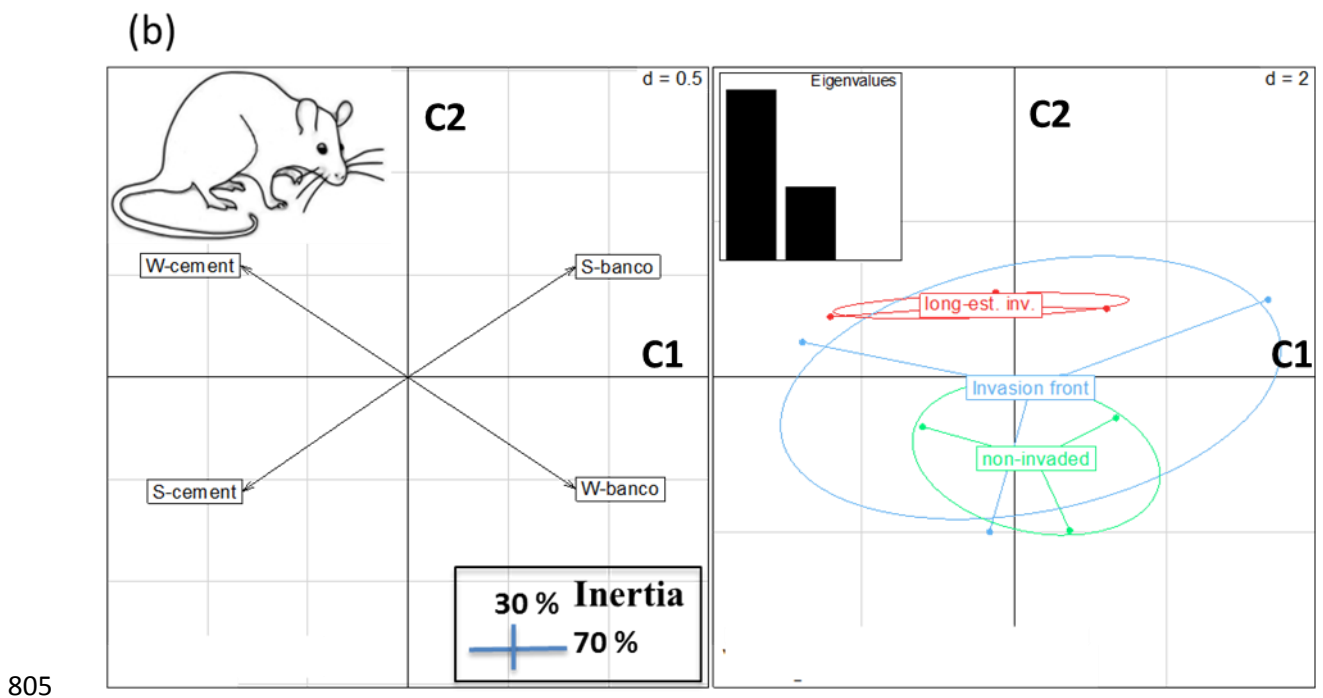
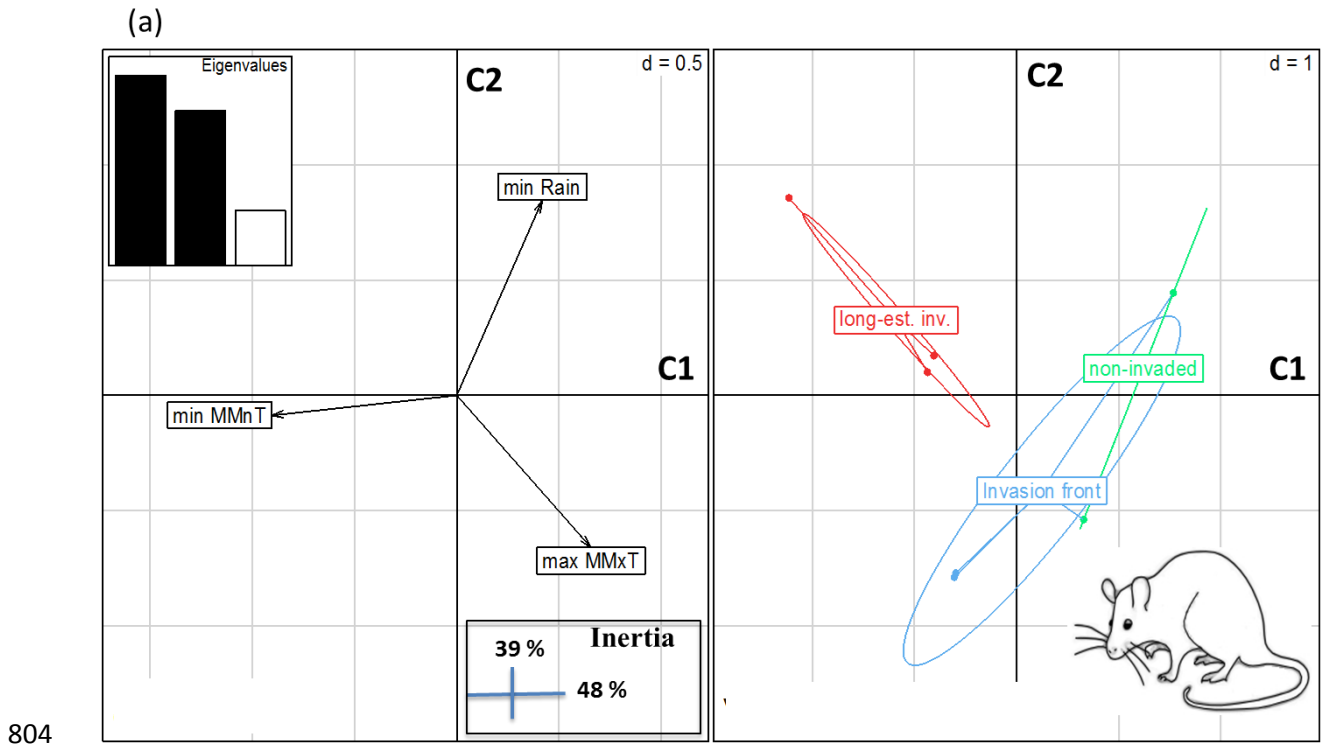
(b)



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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