Biological invasion and parasitism: invaders do not suffer from physiological alterations of the acanthocephalan *Pomphorhynchus laevis*

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SUMMARY

Biological invasions expose parasites to new invasive hosts in addition to their local hosts. However, local parasites are often less successful in infecting and exploiting their new hosts. This may have major consequences for the competitive ability of hosts, and finally on the fate of the parasite-host community. In Burgundy (Eastern France), the acanthocephalan parasite, *Pomphorhynchus laevis*, infects 2 amphipod species living in sympatry: the native *Gammarus pulex* and the invasive *Gammarus roeseli*. While *P. laevis* affects the behaviour and the immunity of *G. pulex*, *G. roeseli* seems unaffected by the infection. In this study, we examined in detail the ability of the parasite to affect the immune system and resource storage of both gammarid species. We found that the infection was associated with a general decrease of the prophenoloxidase activity, haemocyte density, resistance to an artificial bacterial infection and level of sugar reserves in *G. pulex*, but not in *G. roeseli*. These results demonstrate a differential ability of *P. laevis* to exploit its local and its invasive gammarid hosts. Potential mechanisms of these differential physiological alterations and their potential consequences on the coexistence of both gammarid species in sympatry are discussed.

Key words: acanthocephalan, disease resistance, *Gammarus pulex*, *Gammarus roeseli*, haemocytes, local adaptation, phenoloxidase, *Pomphorhynchus laevis*.

INTRODUCTION

Parasites are ubiquitous and they have recently been given a central position in the functioning of ecosystems (Hudson *et al*. 2006). Hence, studying the potential effects of parasites may help to understand host population dynamics and community structure, and to improve predictions on the emergence of infectious diseases (Grenfell and Dobson, 1995). Parasites have clear consequences in driving species interactions, as well as the spread and the establishment of non-indigenous species (Drake, 2003; Prenter *et al*. 2004; Hatcher *et al*. 2006). It is generally assumed that non-indigenous species suffer less from parasitism than native species (Dunn and Dick, 1998; Torchin *et al*. 2002; Genner *et al*. 2008) as they tend to loose their native range parasites (Torchin *et al*. 2003) and as the parasites from the local fauna are often less effective at infecting invading hosts (Ebert, 1994; Kaltz and Shykoff, 1998; Emblidge *et al*. 2006).

The pattern of local adaptation in infectivity may suggest that parasites are not adapted to face a novel immune system or might reflect differential investment to immune defences. However, studies that focused on immune activities in a biological invasion context are scarce (but see Lee and Klasing, 2004; Lee *et al*. 2005). Because immune defences are costly (Bonneaud *et al*. 2003; but see Schmid-Hempel, 2003 for a review), it has been suggested that introduced populations could evolve lower investment in resistance and could down-regulate their level of immunity as a plastic response to the absence of enemy. This would minimize the cost of immunity, and could allow the shifting of resources from defences towards growth and/or reproduction, providing further benefits to the invading populations (Kuo *et al*. 2008). However, such an evolutionary down-regulation of immune defences among invaders might nevertheless be limited because native and invasive populations sharing the same habitat suffer from similar risks of infection, and non-indigenous species often acquire parasites from the novel environment (Colautti *et al*. 2004). Although exposed to similar infection risk, native and non-indigenous hosts should be affected differently by the infection, and understanding the host-parasite interactions could shed light on the mechanisms underlying invasion success.

Obviously, parasites are not static entities and they have in turn evolved strategies to counteract, evade and/or exploit host immune defences (Damian, 1997; Maizels *et al*. 2004). Among such parasites, helminths modulate the immune system of their hosts in order to avoid the negative effects of the

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immune response (damage to parasite tissues or clearance) and to survive within the host, sometimes for years. Their effects on the host immune system have intensively been investigated in vertebrates (see Maizels *et al*. 2004). However, little is known on the modulatory effects of helminths in invertebrate hosts (Loker, 1994; Humbert and Coustau, 2001; Rigaud and Moret, 2003). In addition, modulating primary helminth infections, by reducing or suppressing the immune responsiveness of the hosts, may increase the probability of contracting other infections (Cox, 2001), particularly bacterial infections (Graham, 2008), that could challenge the immunosuppressing parasite and/or increase the death probability of the hosts. Hence, in the context of local adaptation, local parasites are expected to be more effective in immunosuppressing and exploiting resources of the local hosts, compared to non-indigenous hosts. Nevertheless, if the parasite infection only decreases the local host resistance to subsequent infections, then local hosts should be penalised when in competition with invasive hosts.

Biological invasions offer a context to study local adaptation. The acanthocephalan parasite – gammarid host association provides an interesting system to investigate the impact of shared parasites on their local and non-indigenous hosts, and on invasion success. In Burgundy (Eastern France), the acanthocephalan *Pomphorhynchus laevis* uses 2 gammarid species as intermediate hosts: the resident *Gammarus pulex* and the invasive *Gammarus roeseli*. *P. laevis* alters several phenotypic traits of the resident host, *G. pulex*, such as immune defences (Rigaud and Moret, 2003; Cornet *et al*. 2009*b*) and various behavioural traits (Cézilly *et al*. 2000; Kaldonski *et al*. 2007), which favour host exploitation and parasite transmission to the definitive fish hosts (Lagrue *et al*. 2007). For such a parasite, behavioural and physiological alterations of the intermediate host are strongly related to parasite success and have been shown to be more successful in the native species, *G. pulex*, than in the invasive species, *G. roeseli* (Rigaud and Moret, 2003; Tain *et al*. 2007).

The present study investigates the differential effects of the acanthocephalan, *P. laevis*, on immune defences and resource storage of both amphipod hosts, *G. pulex* and *G. roeseli*. First, we measured the level of activity of 2 major immune parameters: the activity of the phenoloxidase (PO) enzyme and the density of circulating haemocytes (Cerenius and Söderhäll, 2004), and the amount of energetic reserves (sugars and lipids) of *G. pulex* and *G. roeseli*, to estimate the impact of *P. laevis* infection on these host traits. The PO enzyme is involved in the melanization and encapsulation processes (Cerenius and Söderhäll, 2004). It is mainly stored in haemocytes as an inactive pro-enzyme (prophenoloxidase, ProPO), which is rapidly activated upon infection (Labbé and Little, 2009). Both PO and haemocytes are associated with disease resistance in crustaceans (Cerenius *et al*. 2003, 2008) and their impairment should enable acanthocephalan macroparasites to develop successfully in the host (Volkmann, 1991). Second, we investigated the ecological consequences of parasite-induced immune depression on the ability and efficiency of both host species to resist an infection mimicked by the inoculation of a bacterial suspension. Because many infectious organisms are highly host specific, the evolutionarily novel pathogen faced by invading host species might lack the mechanisms producing the disease symptoms. Due to differences in the coevolutionary history between *G. pulex* and *G. roeseli* with the acanthocephalan *P. laevis*, no significant physiological alteration induced by the parasite is expected in *G. roeseli*, this could confer a competitive advantage to the invasive species when invading native *G. pulex* populations.

MATERIALS AND METHODS

Study system

The acanthocephalan *P. laevis* is a macroparasite that uses an amphipod crustacean as intermediate host, and a fish as definitive host. In the study area, chubs *Leuciscus cephalus* are preferred definitive hosts of the parasite. Gammarids are orally infected when ingesting parasite eggs. The acanthor (mature egg) hatches and passes through the gut wall to the haemocoel where it undergoes successive growth events until it reaches the cystacanth stage (infectious for the next host). Transmission is achieved via predation of the gammarid by the definitive host. In the fish, the parasite attaches to the intestines by embedding its proboscis in the tissue. The parasite becomes mature and reproduces, with females releasing eggs into the digestive tract that are then released with the faeces (reviewed by Kennedy, 2006).

G. pulex is a resident amphipod species in France whereas *G. roeseli* has colonized Western European streams during the last century. The spread of *G. roeseli* from Central Europe or perhaps Minor Asia (Karaman and Pinkster, 1977; Jazdzewski and Roux, 1988) to Northern and Western Europe was facilitated by the development of a canal network between watersheds (Jazdzewski and Roux, 1988). Interestingly, *G. roeseli* infections by *P. laevis* have been recorded in several areas of the Danube area (Moret *et al*. 2007).

Sampling

Gammarids were collected using a kick sampling method in the River Ouche at Dijon in 2007. A total of 2303 gammarids were sampled to estimate the prevalence of infection by *P. laevis* in both species. As this sampling did not provide enough infected

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individuals for experimental comparisons, additional parasitized *G. pulex* and *G. roeseli* were actively sought. Infected gammarids could easily be identified as the parasite appeared as yellow-orange dots through the cuticle of the host. Animals were maintained in the laboratory under standard conditions (15 °C ± 1 °C, light : dark cycle 12 : 12h) in well-aerated tanks filled with dechlorinated UV-treated tap water and fed with elm leaves. At the end of the experiments (immune defence, energy reserves, bacterial clearance assays), individuals were measured by linear dimension (size of the fourth coxal plate) using a stereoscopic microscope (Nikon SMZ-10A) and a video-analysis system (VTO 232, Linkam Scientific Instruments). Gammarids were then dissected to check for infection and parasite intensity and bodies were frozen for later measurements of their lipid and sugar contents. All individual gammarids were measured for the level of immune defences, lipid and glycogen contents.

Haemolymph collection, haemocyte concentration and activities of the ProPO system

Haemolymph extracts were taken by wounding gammarids near the 7th dorsal segment with very fine forceps (Cornet *et al.* 2009*b*). Three μl of haemolymph were collected into a sterile, pre-chilled glass capillary and flushed into 20 μl of cold phosphate-buffered saline (PBS: 8·74 g NaCl, 1·78 g $Na_2HPO_4 \cdot 2H_2O$, 1 L dH_2O, pH 6·5). Ten μl were immediately used for the determination of haemocyte concentration and the remainder was then frozen in liquid nitrogen and stored at −80 °C for later phenoloxidase assays. Haemocyte concentration was determined using a Neubauer counting chamber as the number of cells counted in 0·1 μl and reported relative to 1 μl of pure haemolymph.

The activity of naturally activated phenoloxidase (PO) enzymes only (therein after called PO activity) and the activity of the pro-enzymes (ProPO) in addition to that of the PO (therein after called total activity) were measured for each individual haemolymph extract using a spectrophotometric assay (see the detailed protocol in Cornet *et al.* 2009*b*). The assay was performed using 5 μl of haemolymph extract added to a microplate well containing 20 μl of PBS buffer and either 140 μl of dH_2O to measure PO activity only or 140 μl of chymotrypsin solution (Sigma C-7762, 0·07 μg ml^{-1} of dH_2O) to measure total activity. After an incubation period of 4 min, 20 μl of L-dopa solution (Sigma D-9628, 4 mg ml^{-1} of dH_2O) was added and the reaction was followed in a microplate reader (Versamax, Molecular Devices) for 40 min at 490 nm. Enzyme activity was analysed using the software SOFT-Max®Pro 4·0 (Molecular Devices) and measured as the slope (V_{max} value) of the reaction curve and reported relative to the activity of 1 μl of pure haemolymph.

Immune challenge and bacterial clearance

Gammarids were exposed to a bacterial pathogen to assess the efficiency of the immune system to clear an infection (Cornet *et al.* 2009*b*). We used a bacterial strain of *E. coli* (strain CIP 103410, Pasteur Institute, Paris, France) resistant to tetracycline. Crustacean exposure to bacterial infection was done by injection. Gammarids were briefly immobilized on sticky gum. A small hole was made laterally on the animal third dorsal segment using a fine needle. Then 0·5 μl of a bacterial suspension at a concentration of 8×10^4 bacteria μl^{-1} (for details see Cornet *et al.* 2009*b*) was injected into the animal haemocoel using a Hamilton syringe equipped with a fine needle (gauge 33). After injection, gammarids were kept at controlled temperature (15 °C ± 1 °C) for 8 h before haemolymph extraction. Each individual provided 2 μl of haemolymph that were flushed into 198 μl of PBS buffer. After homogenization, 100 μl of the mixture were spread on agar Petri dishes containing 20 μg ml^{-1} tetracycline. Petri dishes were incubated overnight at 37 °C and colonies (colony-forming unit, CFU) were counted. The number of colonies is expected to be inversely proportional to the immune defence level.

Lipid and sugar contents

Both lipid and sugar (glucose) contents were quantified using a colorimetric assay following the protocol of Lemaître *et al.* (2009) modified from Rivero and Fergusson (2003). Gammarids were crushed using a micro-centrifuge tube pestle into 100 μl of a solution of sodium sulphate 2% and 750 μl of a chloroform/methanol (1/2) solution. Samples were centrifuged (4 °C, 1500 ***g***, 4 min) and 2 fractions of 350 μl each were extracted and separated into clean culture tubes for lipid and sugar analyses respectively. Samples were placed into a water bath at 95 °C to enable solvent to evaporate.

For lipid determination, 40 μl of sulphuric acid were added and samples were reheated for 2 min at 95 °C and then 960 μl of vanillin-phosphoric acid reagent were added. Samples were mixed and 150 μl were transferred into a 96-microplate well and optical density was read at 525 nm in a microplate reader (Versamax, Molecular Devices) and analysed using the software SOFT-Max®Pro 4·0. Lipid concentration was obtained from a standard curve ranging from 1 to 64 μg ml^{-1} of a commercial vegetable oil.

For sugar determination, residues after evaporation were heated for 15 min at 95 °C with 1 ml of anthrone reagent. After cooling, 150 μl were transferred into a 96-microplate well and optical density was read at 625 nm. Sugar concentration was obtained from a standard curve ranging from 3 to 192 μg ml^{-1} of a glucose solution.

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Statistics

Data on haemocyte concentration were square-root transformed and data on phenoloxidase activities (PO and total activity), animal body size, colony counts, lipids and sugars were natural-log transformed to meet assumptions for parametric tests.

The prevalence of infection between species was compared using a Chi-square test. Overall, variation in immune and energy parameters was investigated using a multivariate analysis of covariance (MANCOVA, Pillai's trace) with respect to 'host species', 'status of infection', their interaction and 'size' as covariate. Then, variation within host species was analysed with respect to 'status of infection', 'sex' and 'size' and their interactions. Gender did not explain any variation in the dependent variables either as main effect or interactions with other factors and was therefore removed from the statistical models. The best models were searched by a stepwise procedure that removed non-significant higher interaction terms. Data from the experiment of resistance to a bacterial infection were analysed with the same method.

All tests were performed using JMP v5.0 for Windows (SAS Institute, Cary, USA) and referred to two-tailed tests with significant differences considered at the level of $P \leqslant 0{\cdot}05$.

RESULTS

Prevalence of Pomphorhynchus laevis

In total, 2303 gammarids were analysed. The native species *G. pulex* was predominant (70·13%), and *G. roeseli* individuals represented one third of the animals (29·87%). The prevalence of *P. laevis* in the river Ouche did not differ between *G. pulex* (2·17%, $n=1615$) and *G. roeseli* (2·99%, $n=688$, $\chi^2_1=1{\cdot}13$, $P=0{\cdot}287$). We found *G. pulex* harbouring 1 ($n=29$) and 2 ($n=10$) cystacanths of *P. laevis* whereas all *G. roeseli* ($n=21$) were infected by a single parasite only. There was no gender effect upon the prevalence of infection, neither in *G. pulex* ($\chi^2_1=0{\cdot}85$, $P=0{\cdot}3575$) nor in *G. roeseli* ($\chi^2_1=0{\cdot}001$, $P=0{\cdot}9852$).

Levels of immune defences

No effect of parasite load on *G. pulex* immunological parameters was detected (PO activity $F_{1,37}=0{\cdot}06$, $P=0{\cdot}8042$; total activity $F_{1,37}=1{\cdot}44$, $P=0{\cdot}2385$; haemocyte concentration $F_{1,37}=1{\cdot}46$, $P=0{\cdot}2342$). Data from all infected *G. pulex* were therefore pooled and compared to data from uninfected animals for further analyses.

The variation of immune functions was related to animal body size, especially for PO activity and haemocyte concentration (Table 1). This global size effect was mostly due to the impact of size on immune parameters in *G. pulex* (Table 2). Overall, immune activities were influenced by the host species, the parasite infection status and the interaction (Table 1). Whereas PO activity and haemocyte concentration were found to be similar between native and invasive species (non-significant effect of 'host species' on its own, Table 1), *G. roeseli* had a greater total activity (Table 1; Fig. 1A, B, C). However, the host species effect was the product of the interaction with the status of infection. Indeed, uninfected amphipods of the two species did not differ in their total activity (for equal animal size, *G. pulex* $1{\cdot}07 \pm 0{\cdot}11$, *G. roeseli* $1{\cdot}21 \pm 0{\cdot}17$; $F_{1,60}=0{\cdot}26$, $P=0{\cdot}6112$), whereas they did when infected (*G. pulex* $0{\cdot}18 \pm 0{\cdot}10$, *G. roeseli* $0{\cdot}95 \pm 0{\cdot}14$; $F_{1,57}=19{\cdot}06$, $P<0{\cdot}0001$). Parasite infection had a strong effect on the level of the 3 immunological variables (Table 1), but this depended on the gammarid species as shown by the significant interaction between 'host species' and 'status of infection' (at least for PO and total activity, Table 1). The infection by *P. laevis* was associated with a strong decrease of all of the immune variables in parasitized *G. pulex* (Table 2; Fig. 1A, B, C) (differences for equally-sized individuals, uninfected *vs P. laevis*-infected; PO activity: $-0{\cdot}75 \pm 0{\cdot}19$ *vs* $-1{\cdot}86 \pm 0{\cdot}20$; total activity: $1{\cdot}07 \pm 0{\cdot}11$ *vs* $0{\cdot}18 \pm 0{\cdot}12$; haemocyte concentration: $37{\cdot}56 \pm 2{\cdot}13$ *vs* $24{\cdot}56 \pm 2{\cdot}23$). In contrast, immune effectors of the invasive host *G. roeseli* were not affected by the parasite (Table 2; Fig. 1A, B, C).

Sugar and lipid reserves

Measures of energetic reserves were correlated with animal body size, likely as a consequence of the method we used. Body size was therefore included in the models as a covariate. Gammarid species differed in their sugar contents (Table 1; Fig. 2A). Equally-sized *G. roeseli* had slightly more sugar than *G. pulex* (uninfected only, *G. roeseli* $3{\cdot}76 \pm 0{\cdot}15$, *G. pulex* $3{\cdot}32 \pm 0{\cdot}10$; $F_{1,60}=5{\cdot}64$, $P=0{\cdot}0208$) but no difference was detected for lipid contents (Fig. 2B). Neither the status of infection nor the interaction explained a significant fraction of variation in the overall model. However, within species, we found a significant effect of infection by *P. laevis* on the local hosts only; parasitized *G. pulex* had less sugar than uninfected ones ($2{\cdot}98 \pm 0{\cdot}09$ and $3{\cdot}30 \pm 0{\cdot}09$, respectively; Fig. 2A). Lipid content remained unaffected in *G. pulex* and none of the parasite effects were found in *G. roeseli* (Table 2; Fig. 2A, B).

Bacterial clearance efficiency

Non-significant effects ('sex' and 'size') were dropped from the model by a backward stepwise procedure. The number of bacterial colonies (inversely proportional to disease resistance) was explained by both 'host species' (global model, $F_{3,73}=10{\cdot}64$, $P<0{\cdot}0001$; $F_{1,73}=20{\cdot}39$, $P<0{\cdot}0001$) and 'status of

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Table 1. Multivariate (Pillai's trace) and univariate analyses of covariance investigating variation in immune parameters and energetic contents as a function of gammarid host species, infection by *Pomphorhynchus laevis* and body size

Models	Source of variation	DF	F	P[a]
MANCOVA	Global model	20, 468	5·53	**<0·0001**
	Host species	5, 114	5·27	**0·0002**
	Status of infection	5, 114	4·28	**0·0013**
	Host species × status of infection	5, 114	2·23	**0·0461**
	Size	5, 114	10·47	**<0·0001**
ANCOVAs				
PO activity	Global model	4, 118	6·95	**<0·0001**
	Host species	1, 118	1·81	0·1807
	Status of infection	1, 118	4·02	**0·0471**
	Host species × status of infection	1, 118	6·94	**0·0095**
	Size	1, 118	10·52	**0·0015**
Total activity	Global model	4, 118	10·97	**<0·0001**
	Host species	1, 118	11·43	**0·0010**
	Status of infection	1, 118	17·98	**<0·0001**
	Host species × status of infection	1, 118	5·31	**0·0229**
	Size	1, 118	0·07	0·7804
Haemocyte concentration	Global model	4, 118	5·24	**0·0006**
	Host species	1, 118	1·62	0·2051
	Status of infection	1, 118	9·97	**0·0020**
	Host species × status of infection	1, 118	1·46	0·2291
	Size	1, 118	4·80	**0·0303**
Sugar content	Global model	4, 118	12·62	**<0·0001**
	Host species	1, 118	20·89	**<0·0001**
	Status of infection	1, 118	1·74	0·1898
	Host species × status of infection	1, 118	1·61	0·2065
	Size	1, 118	17·89	**<0·0001**
Lipid content	Global model	4, 118	2·05	0·0919
	Host species	1, 118	2·72	0·1014
	Status of infection	1, 118	0·002	0·9617
	Host species × status of infection	1, 118	0·71	0·4024
	Size	1, 118	5·85	**0·0171**

[a] Significant values shown in bold.

infection' ($F_{1,73}=5·71$, $P=0·0194$). The clearance of *E. coli* was higher in uninfected *G. roeseli* than in uninfected *G. pulex* ($F_{1,42}=4·89$, $P=0·0324$; Fig. 3). Moreover, *P. laevis* had a different influence on gammarid species as shown by the 'host species × status of infection' interaction ($F_{1,73}=4·20$, $P=0·0439$). Infected *G. pulex* were less able to clear the bacteria as shown by the greater number of developing colonies compared to uninfected animals ($F_{1,40}=9·13$, $P=0·0044$; Fig. 3). No difference in the ability to clear the bacterial infection was detected in *G. roeseli* ($F_{1,33}=0·07$, $P=0·7903$; Fig. 3).

DISCUSSION

Because of their major impact on the ecosystems, biological invasions have been intensively studied in the last years. Much emphasis has been put on the intrinsic characteristics of invading species to explain invasion success (Sakai *et al*. 2001). Nevertheless, the importance of parasites should not be underestimated as they also play a great role in species interactions and in the outcome of animal invasion (Drake, 2003; Prenter *et al*. 2004; Hatcher *et al*. 2006). In this study, we showed that despite a similar prevalence of infection between native and invasive gammarid hosts, the detrimental effects of the acanthocephalan *P. laevis* occurred only in the local host *G. pulex*. *P. laevis* infection was associated with a reduced immunocompetence and an energetic cost for its local host *G. pulex*, supporting the idea of a maladaptation of the parasite to the invasive host *G. roeseli*. On the contrary, *G. roeseli* is likely to be more tolerant, and suffers less *P. laevis*-induced alterations.

The ProPO system and haemocytes are general effectors of the crustacean immune system (Cerenius and Söderhäll, 2004) and immune depression induced by the acanthocephalan worm is likely to be a strategy developed to exploit and survive inside their gammarid intermediate hosts until transmission (Loker, 1994). The infection in *G. pulex* was associated with a decrease of the activity of the ProPO system and the concentration of circulating haemocytes (Cornet *et al*. 2009*b*). Since phenoloxidase enzymes are mainly synthesized and stored in

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Table 2. Multivariate (Pillai's trace) and univariate analyses of covariance investigating variation in immune parameters and energetic contents in *Gammarus pulex and Gammarus roeseli* as a function of infection by *Pomphorhynchus laevis* and body size

Host species	Immune parameter	Source of variation	DF	F	P^a
MANCOVAs					
Gammarus pulex		Global model	10, 152	9·05	**<0·0001**
		Status of infection	5, 75	9·16	**<0·0001**
		Size	5, 75	8·93	**<0·0001**
Gammarus roeseli		Global model	10, 70	1·52	0·1502
		Status of infection	5, 34	0·72	0·6130
		Size	5, 34	2·48	0·0506
ANCOVAs					
Gammarus pulex	PO activity	Global model	2, 79	14·94	**<0·0001**
		Status of infection	1, 79	15·61	**0·0002**
		Size	1, 79	14·04	**0·0003**
	Total activity	Global model	2, 79	15·04	**<0·0001**
		Status of infection	1, 79	29·21	**<0·0001**
		Size	1, 79	0·78	0·3782
	Haemocyte concentration	Global model	2, 79	12·57	**<0·0001**
		Status of infection	1, 79	17·59	**<0·0001**
		Size	1, 79	7·38	**0·0081**
	Sugar content	Global model	2, 79	12·34	**<0·0001**
		Status of infection	1, 79	5·76	**0·0187**
		Size	1, 79	19·07	**<0·0001**
	Lipid content	Global model	2, 79	1·15	0·3214
		Status of infection	1, 79	0·64	0·4270
		Size	1, 79	1·68	0·1985
Gammarus roeseli	PO activity	Global model	2, 38	0·25	0·7816
		Status of infection	1, 38	0·48	0·4890
		Size	1, 38	0·04	0·8486
	Total activity	Global model	2, 38	1·68	0·2003
		Status of infection	1, 38	1·28	0·2649
		Size	1, 38	1·57	0·2170
	Haemocyte concentration	Global model	2, 38	0·39	0·6789
		Status of infection	1, 38	0·78	0·3825
		Size	1, 38	0·007	0·9297
	Sugar content	Global model	2, 38	0·76	0·4718
		Status of infection	1, 38	0·01	0·9056
		Size	1, 38	1·44	0·2374
	Lipid content	Global model	2, 38	3·42	**0·0432**
		Status of infection	1, 38	0·46	0·5027
		Size	1, 38	6·75	**0·0133**

[a] Significant values shown in bold.

haemocytes, their reduction and/or impairment may have led to a decreased concentration of proteins and enzymes involved in the melanization response. However, the mechanisms by which acanthocephalans induce depression of humoral and cellular immune defences are still unknown. In invertebrates, immune evasion often involves excretory-secretory products that affect both the number and functions of haemocytes (de Jong-Brink, 1995; Humbert and Coustau, 2001) and interfere with plasma melanization by inhibiting the ProPO activating cascade (Shelby *et al*. 2000; Gomes *et al*. 2003). Parasites also express lectin- and mucin-like molecules that are thought to be involved in the avoidance of the host immune response and the invasion of host tissues (Loukas and Maizels, 2000; Theodoropoulos *et al*. 2001). The reduced phenoloxidase activity and the fewer number of circulating haemocytes in the haemolymph of *P. laevis*-infected *G. pulex* may then prevent the parasite from suffering the harmful effects of the host immune response and/or melanization and encapsulation.

We showed that the impairment of immune defences observed in the local host *G. pulex* was absent in the invasive host *G. roeseli* supporting the idea that the ability to infect and induce pathogenic effects is likely to be host specific, and the result of an adaptation of the parasite to the local hosts, as previously suggested (Rigaud and Moret, 2003). Even if hosts are closely related, pathogens are adapted to their local hosts and will often lack the mechanisms to produce disease symptoms in the invading host species. However, it is worth mentioning that *P. laevis* parasites infecting the invasive host were

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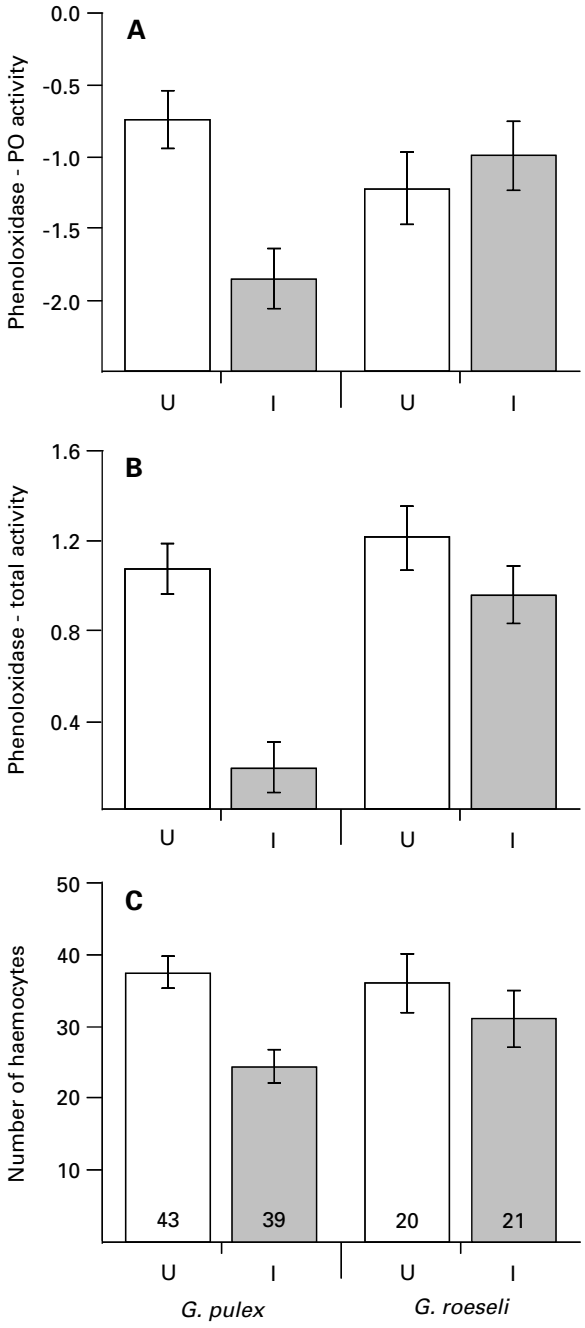

Fig. 1. Comparison of the level of immune defences according to the status of infection by *Pomphorhynchus laevis* (U, uninfected; I, infected) in *Gammarus pulex* and *Gammarus roeseli*: (A) PO and (B) total phenoloxidase activity (natural-log transformation) and (C) number of haemocytes (square-root transformation) per μl of haemolymph, mean $\pm$ S.E. Sample sizes are given within bars.

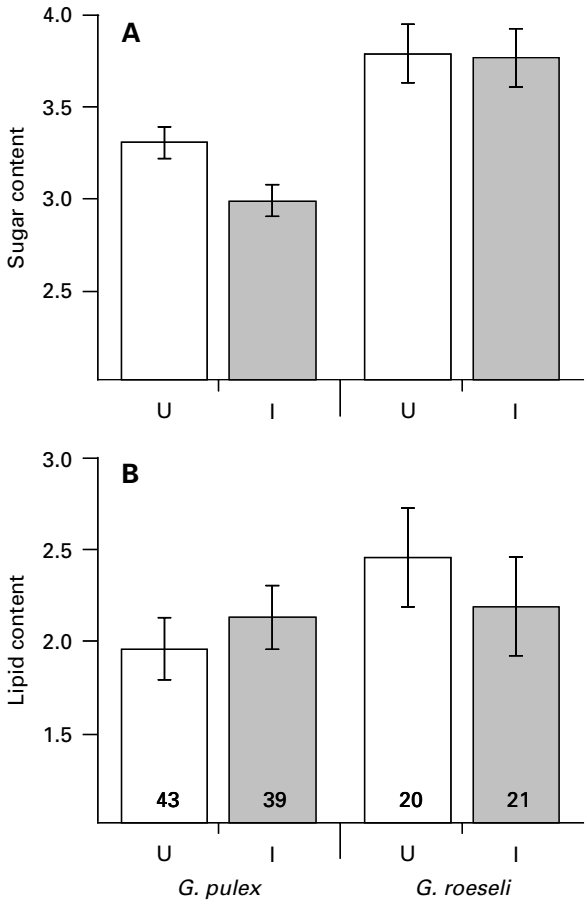

Fig. 2. Sugar (A) and lipid (B) contents (natural log-transformation) according to the status of infection by *Pomphorhynchus laevis* (U, uninfected; I, infected) in *Gammarus pulex* and *Gammarus roeseli*, mean $\pm$ S.E. Sample sizes are given within bars.

never found melanized (as they should be having elicited an immune response) and are able to devaginate when stimulated by fish bile extracts (a test for parasite survival; SC, personal observation). In addition, cystacanths of acanthocephalans (and especially for *P. laevis*) are surrounded by a membrane layer formed by microvilli, which acts as a protective barrier (Taraschewski, 2000). In *Echinogammarus stammeri* infected by *P. laevis*, haemocytes in the vicinity of the cystacanths are rare and often partially or completely disintegrated, attesting to the unsuccessful *E. stammeri* cellular response (Dezfuli *et al.* 2008). Even if the *P. laevis* parasite does not induce significant changes in the immune system of its invasive host (a sign of non-optimal exploitation), it might nevertheless be able to survive through other protective mechanisms.

Parasites also modify the energetic storage of their host by diverting resources to their own benefits and, as such, impose an energetic cost. For instance, mosquitoes *Aedes aegypti* infected with microsporidia have lower sugar, glycogen and lipid contents than uninfected conspecifics (Rivero *et al.* 2007). Here, following the same pattern observed for immune defences, sugar contents were depleted in infected local *G. pulex* whereas sugar contents were the same in uninfected and infected *G. roeseli*. Both gammarid species had similar lipid contents and *P. laevis* infection did not change the level in either the local nor the invasive hosts. Several studies have reported similar observations, suggesting that lipid reserves are rarely affected by parasite infection (Plaistow *et al.*

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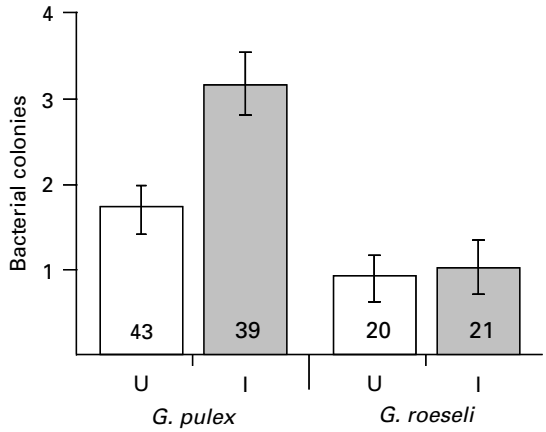

Fig. 3. Comparison of live bacteria (number of colonies per μl of haemolymph, natural-log transformation) after infection by *Escherichia coli* according to the status of infection by *Pomphorhynchus laevis* (U, uninfected; I, infected) in *Gammarus pulex* and *Gammarus roeseli*, mean $\pm$ S.E. Sample sizes are given within bars.

2001; Franz and Kurtz, 2002; Rivero and Fergusson, 2003). Mounting an immune response is energetically costly (Schmid-Hempel, 2003). In mosquitoes, *Plasmodium* infection induces a reduction in longevity and fecundity (Ferguson and Read, 2002). However, these costs are reduced when mosquitoes are reared with *ad libitum* access to glucose. It has been hypothesized that parasite-mediated energy depletion might be related to virulence (Rivero and Ferguson, 2003). Highly virulent parasite strains are expected to draw a larger fraction of host resources for growth and replication than less virulent ones. However, evidence for this is scarce. It was found recently that among populations of *G. pulex*, total phenoloxidase activity was positively correlated with sugar content (Cornet *et al.* 2009*a*), suggesting that sugars may fuel the activity of the ProPO system. Here, we found that the local host *G. pulex* infected by the parasite had depleted sugar content and impaired immune defences. We may therefore wonder whether *P. laevis* immunodepresses its local host by depleting sugar reserves. The comparison of the cystacanth volume, a surrogate of parasite growth rate, could reflect differential depletion of energy reserves. Since energy reserves are not depleted in *G. roeseli*, parasites might be less efficient in acquiring resources and this could be reflected in a reduced growth rate.

Although local adaptation has favoured parasites able to circumvent the immune defence of its local host, the reduction of the level of immunity may have some underlying costs, as suggested by the bacterial clearance experiment. Co-infections occur at a high frequency in the wild and immunodepressed hosts primarily infected by a helminth parasite are more likely to contract microparasite secondary infections (Cox, 2001; Graham, 2008). In the local hosts, the *P. laevis* primary infection and the suppression of the immune system negatively affected the ability of *G. pulex* to clear a secondary infection when experimentally infected by the bacteria *E. coli*. Both the host and the parasite may pay a cost for these opportunistic infections since it could challenge the already established immunosuppressing parasite, as well as increasing the death probability of the host by higher pathogen exploitation. However, infected *G. roeseli* did not suffer from a reduced resistance and the bacterial clearance was as effective in uninfected as in parasitized individuals. Such a potential consequence of the differential immunodepression may therefore provide an advantage to the invader, *G. roeseli*, especially when the parasitic pressure imposed by the acanthocephalan parasite is high. Other acanthocephalan species also immunodepress *G. pulex* (Cornet *et al.* 2009*b*). If these parasites have no effect on *G. roeseli* immune defences, then the increased parasite-mediated disadvantage from which *G. pulex* suffers, compared to *G. roeseli*, could even be larger.

Invaders were slightly more immune competent than natives, they were found to have a higher level of total phenoloxidase activity (higher investment into the ProPO system). They were also more efficient in their response against bacteria. Nevertheless, it is hard to draw a conclusion from this result as these differences might result from fundamental differences in the physiology between the two species. Based on the differential effects of infection on immune defences, energetic reserves and bacterial resistance between native and invasive gammarids, it may be assumed that the local parasite *P. laevis* is not efficient in manipulating the non-indigenous species. However, *G. roeseli* can be infected by the local parasite in Burgundy. This might be explained by the fact that *P. laevis* has a wide geographical distribution (Kennedy, 2006) and that the amphipod had already experienced infection with this parasite species in its area of origin or during the invasion process (Moret *et al.* 2007). Although infected by *P. laevis*, *G. roeseli* suffers lower levels of damage caused by parasitism (immune depression, lower bacterial resistance, energetic budget modification) relative to the local species *G. pulex*, and can be maintained in the population and act as a reservoir (Kuo *et al.* 2008). Hence, it is not surprising that infection is as prevalent in *G. roeseli* as in *G. pulex*. The acanthocephalan *P. laevis* also relies on parasite-induced behavioural alterations (Cézilly *et al.* 2000; Kaldonski *et al.* 2007) to increase the trophic transmission to fish definitive hosts (Lagrue *et al.* 2007), but these only occur in the native hosts (Tain *et al.* 2007). The absence of behavioural manipulation, as well as morphological anti-predator defences (Bollache *et al.* 2006) and habitat segregation (Kaldonski *et al.* 2008) are likely to lead to lower predation rate of *G. roeseli* by fish (Lagrue *et al.* 2007). As they suffer less parasite-induced mortality

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(Lagrue *et al*. 2007), infected *G. roeseli* persist in the population.

To conclude, this study underlines that the local acanthocephalan *P. laevis* alters differentially the physiology of its native and non-indigenous hosts. Note that a primary acanthocephalan infection and the depression of the immune system will influence the host response to secondary infection. Hence, the higher exploitation of the native *G. pulex* is associated with costs. Recent studies suggested that immunity of introduced hosts added to the maladaptation of local parasites to face a novel host immune system provides a strong advantage to the invaders over the indigenous species (Emblidge *et al*. 2006; Genner *et al*. 2008). However, in rivers of Burgundy (especially in the river Ouche) where *G. pulex* and *G. roeseli* live in sympatry, the proportion of *G. roeseli* does not exceed 30% of the crustacean population (also reported by Lagrue *et al*. 2007). More work focussing on the interactions between the two species (e.g. studying the functional response to each species according to the status of infection, Bollache *et al*. 2008) should help to understand the ecological consequences of the differential effects induced by local parasites on their local and invasive host, and the outcome of the invasion.

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