Is the host or the parasite the most locally adapted in an amphipod–acanthocephalan relationship? A case study in a biological invasion context

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Abstract

Manipulative endoparasites with complex life cycles can alter their intermediate host immunity and behaviour in ways that increase survival probability within the host body cavity and enhance successful transmission to the definitive host. These parasitic manipulations are variable among and within parasite species and may result from co-evolutionary processes, in which the parasite is constrained for adaptation to the local intermediate host. Hence, arrival of a new host species in a local host population may promote local parasite maladaptation. This study tested the occurrence of local adaptation in two distantly located populations of the acanthocephalan parasite Pomphorhynchus laevis and its effect on the immunity and behaviour of its gammarid intermediate host Gammarus roeseli. This was done in France (an area for which G. roeseli is a recent invader) and Hungary (an area from which G. roeseli was believed to be native). As expected, we found no alteration in G. roeseli’s immune defence and behaviour associated with infection by P. laevis in localities, where the gammarid is invasive. Unexpectedly, we found similar results in Hungarian populations, where the parasite was even more exposed to the host immune response. Whilst these results suggest maladaptation of the parasite to the gammarid in both countries, they also suggest that the gammarid host might be locally adapted to the parasite. Genetic analyses were performed on both the parasite and the host and the results suggest that the two subsets of populations we studied harbour rather isolated host–parasite systems, both probably deriving from a common ancestral population. We propose that G. roeseli is also of recent acquisition in Hungary, and that a recent co-evolutionary history between P. laevis and G. roeseli in association with a long generation time in the parasite has constrained parasite adaptations in Europe or even favoured host adaptation to the parasite.

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1. Introduction

Some parasites with complex life-cycles involving more than one host have the capacity to affect the physiology and the behaviour of their intermediate host in ways that increase their transmission rate to the final host, where they reproduce (Moore, 2002; Thomas et al., 2005). First and foremost, these parasites have to ensure their survival within the host body cavity by evading the host immune response (Locker, 1994; Damian, 1997). This parasitic manipulation is variable among populations (Thomas et al., 2005; Moret, unpublished results) and, as in many other host–parasite relationships, may result from co-evolutionary processes, in which the parasite is constrained for adaptation to the local intermediate host (reviews in Kaltz and Shykoff, 1998; Kawecki and Ebert, 2004).

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Biological invasions provide an interesting context to study the evolution of such host–parasite relationships (Lee and Klasing, 2004). Differential migration rates between parasites and their hosts can cause local parasite maladaptation. For instance, high host gene flow compared with that of the parasite may prevent local adaptation of the parasite (e.g., Gandon et al., 1996). Such a hypothesis is supported when introduced species and their parasites have different migration rates (Torchin et al., 2003). However, invasive species may face new, local manipulative parasites, and the infection of this novel host might be compromised, as the parasite may not succeed in evading the new host's immune system and/or manipulating its behaviour. More specifically, Rigaud and Moret (2003), studying the infection effect of two species of acanthocephalan parasites, Pomphorhynchus laevis and Polydora minutus, on immune defence of native and invasive species of gammarids (Gammarus pulex and Gammarus roeseli, respectively) in Burgundy (eastern France), found that local parasites seem to be able to immunosuppress their local host but not the invasive one, suggesting local maladaptation of the parasites to evade the new host's immune system. Local maladaptation may also apply at the level of host behaviour manipulation, since P. laevis typically manipulates the behaviour of its local intermediate host, G. pulex, but is unable to affect the behaviour of the invasive gammarid species, G. roeseli (Bauer et al., 2000). Hence, there are reasonable indications that the capacity of P. laevis to affect both the physiology and the behaviour of the host results from long co-evolutionary processes between the host and the parasite.

**Gammarus roeseli** is thought to be native to Asia Minor and south-eastern Europe (Balkan and Hungarian plains of the Danube), and has colonised rivers of the north-eastern part of France about one century ago (Karaman and Pinkster, 1977; Jazdzewski and Roux, 1988). As mentioned earlier, in Burgundy, G. roeseli co-occurs in sympathy with the native gammarid species, G. pulex, which is the usual intermediate host of P. laevis.

**Pomphorhynchus laevis** is a non-specific parasite with a larval stage (cystacanth) infecting various gammarid amphipods, whereas adults develop in several freshwater fish. (Hine and Kennedy, 1974). While P. laevis systematics is still under debate with probably more than one species under this generic name (Perrot-Minnot, 2004), this parasite species shows a Palaearctic distribution (Kennedy, 2006) with a lot of genetic and/or morphological variants recognized in its whole range (e.g., Kral’ova-Hromadova et al., 2003; O’Mahony et al., 2004; Perrot-Minnot, 2004). This wide distribution applies for P. laevis sensu stricto (Perrot-Minnot, 2004). The gammarids are orally infected when ingesting parasite eggs released in the faeces of the definitive host. After hatching, the acanthor passes through the gut wall of the crustacean host to occupy its haemocoel. From there, the parasite alters the behaviour of most of its common intermediate hosts, alteration that favours predation by the definitive host (review in Kennedy, 2006). Behaviour alteration typically translates into modification of the gammarid host’s reaction to light by changing its photophobia into photophilia (Bauer et al., 2000). Prior to this, however, the cystacanth needs to ensure its own survival within the host haemocoel whilst exposed to the host immune response (Damian, 1997). Gamarid crustaceans possess an innate immunity, which relies on both cellular (Ratcliffe et al., 1985) and humoral (Ratcliffe and Rowley, 1979) components. Pathogens entering the host haemocoel are usually phagocytosed or encapsulated by haemocytes (Ratcliffe et al., 1985). These reactions are often accompanied by the proteolytic activation of the phenoloxidase (PO) enzyme (Söderhäll and Cerienius, 1998). This enzyme synthesises melanin and is also used to signal non-self, as well as kill and isolate internal parasites (Söderhäll, 1982) such as acanthocephalans.

Whilst in France G. roeseli is invasive and represents a new host species for P. laevis, it should be the common intermediate host of this acanthocephalan parasite in its native range. Consequently, the co-evolutionary history between P. laevis and G. roeseli should be relatively short in France and long in south eastern Europe. We may then expect that P. laevis should be able to manipulate both the immune system and behaviour of G. roeseli in native populations as a result of local adaptation of the parasite to its host. The main problem is that the biogeography of both the host and the parasite has largely been overlooked, and acquisition of such data is necessary before exploring local adaptation by transplant or common garden experiments, as suggested by Kawecki and Ebert (2004). Therefore, using a correlative approach, we studied geographical patterns of adaptation (Kaltz and Shykoff, 1998).

This study aims to test the hypothesis that P. laevis is locally adapted to G. roeseli in Hungarian populations (western part of G. roeseli’s native range, Jazdzewski and Roux, 1988), but not in French populations (invasive range). We compared the degree of adaptation of parasites to their local hosts by measuring behavioural scores and levels of immune defence. In addition, using a genetic analysis from each population, we estimated the genetic differentiation of both P. laevis and G. roeseli between Hungary and France, with an additional sampling in another putative invasive population in the Czech Republic for comparison.

2. Materials and methods

2.1. Gammarid sample

Gammarids used in this study were sampled in September and October, 2004, in the rivers Ouche and Tille at Dijon and Les Maillys, respectively (Burgundy, eastern France), in the river Tapolca at Tapolca and Raposka in Hungary near Lake Balaton, and in the river Rokytna, south of the Czech Republic. This last sample was only used for the genetic analyses. They were sampled using
the kick-sampling method (Hynes, 1954). At each site, a first random sample of 160–900 G. roeseli was collected and immediately stored in absolute alcohol for later estimation of parasite prevalence. Sampling effort was the same for all the populations i.e., five landing net samples (opening: 20 × 25 cm) in each representative micro-habitat (weed, sand and gravel). In a second sample G. roeseli parasitized and non-parasitized by acanthocephalan parasites were actively sought. Parasitized gammarids could be distinguished from non-parasitized ones because acanthocephalan parasites could be seen through the transparent cuticle of the host as orange-yellow dots. The animals were kept in pots filled with water from the river in which they were caught, were provided with oxygen and kept at a low temperature during return to the laboratory. In the laboratory, the animals were kept in aquaria under standard conditions (15 °C) and were fed with dead leaves before being used to perform behavioural and immune measurements. For this study, the gammarids were used within 24 h following sampling.

2.2. Behavioural measures

All the gammarids of the second sample were individually tested for their reaction to light (phototaxis) and gravity (geotaxis).

Reaction to light was quantified following the method of Perrot-Minnot (2004). Gammarids were individually placed into a horizontal plastic tube (length 23 cm and diameter 3 cm) filled with oxygenated water. Half of the tube was completely opaque and the other half was transparent. A hole placed in the middle tube allowed the introduction of the gammarid to be tested. After an acclimatization of 5 min, the position of the gammarid was recorded every 30 s for 5 min. A score of 0 was given when the tested animal was found in the opaque half of the tube and a score of 1 was given when the gammarid was found in the transparent side. Thus, at the end of the trial, the final score could range from 0 (strongly photophobic) to 10 (highly photophilic).

Quantification of gammarid reaction to gravity was as in Bauer et al. (2005). Gammarids were placed individually in a translucent plastic 500 ml test-tube (diameter 5.5 cm) filled with oxygenated water. Half lines. The test-tube rested on a black plastic sheet and the top was covered with a hard black top to avoid potential confounding phototactic reaction resulting from room lighting. After 5 min of acclimatization, the position of the gammarid was recorded every 30 s for 5 min. A score was given according to the position of the tested animal in the water column (1 at the bottom, up to 5 at the top of the column). At the end of the trial, the final score given to a gammarid could range from 10 to 50.

After being tested for both behavioural assays, gammarids were placed individually into 5 ml plastic vials on ice waiting for haemolymph collection.

2.3. Haemolymph collection and phenoloxidase activity

The method used to collect haemolymph was as described in Rigaud and Moret (2003). Haemolymph extracts were taken by perfusing the haemocoel of chilled gammarids with 250 µl of ice-cold sodium cacodylate buffer (0.01 M Na-cacodylate, 0.005 M CaCl2, pH 6.5). For this the telson of the gammarids was removed with dissecting scissors to create a hole, out of which haemolymph was collected. Gammarids were injected with sodium cacodylate buffer through the second tergite behind the head, using a 1 ml disposable syringe (Clinipack U-40 Insulin, Pharma–Plast) and the perfused liquid (buffer plus haemolymph) was collected through the posterior hole into 1.5 ml micro-centrifuge tubes. Samples were immediately frozen in liquid nitrogen and then stored in a freezer (−80 °C). For the PO assay, samples were thawed on ice and 20 µl were placed into a micro-titre plate well containing 140 µl of cold distilled water, 20 µl of cold saline phosphate buffer (PBS: 8.74 g NaCl; 1.78 g Na2HPO4·2H2O; 1000 ml distilled water, 20 µl of cold L-dopa solution (4 mg/ml of distilled water) were added into each well and the reaction allowed to proceed for 40 min at 30 °C in a micro-titre plate reader (Versamax, Molecular Devices). Readings were taken at 490 nm and analysed using SOFT-max®PRO 4.0 software (Molecular Devices). Enzyme activity was measured as the slope (V max value) of the reaction curve during the linear phase of the reaction (Barnes and Siva-Jothy, 2000) within a time scale between 5 and 30 min after the reaction mix was made.

2.4. Size, sex and dissection

Immediately after haemolymph collection, the gammarids were sexed and measured by linear dimensions (distance from fourth coxal plate basis to individual dorsal limit) using a stereoscopic microscope Nikon SMZ-10A and a video – analysis system VTO 232 from Linkam scientific instruments (Bollache et al., 2000). Animals were then dissected in Ringer’s solution under a stereoscopic microscope to identify the acanthocephalan species in their body cavity, according to Perrot-Minnot (2004). A piece of gammarid muscle and the parasite were then isolated into 0.5 ml micro-centrifuge tubes filled with absolute alcohol for later genetic analysis.

2.5. Genetic analysis

Genetic differentiation among populations and between countries for both P. laevis and G. roeseli was measured on the basis of a 714 bp partial sequence of the Cytochrome Oxidase I (COI) mtDNA gene and a 400 bp partial sequence of the 16S rDNA mitochondrial gene, respectively. Total
DNA from both \textit{P. laevis} and \textit{G. roeseli} was extracted following a standard phenol/chloroform method described in \textit{Perrot-Minnot} (2004).

DNA sequences were amplified by PCR in a total volume of 20 $\mu$l including 5 ng DNA template, 200 $\mu$M deoxyribonucleotide triphosphate (dNTP), 200 nM each primer, 1$\times$ buffer and 1 U HotMaster$^\text{R}$ DNA polymerase (Eppendorf). The primers used for \textit{P. laevis} COI mtDNA sequence amplification were LCO1490 5'-GGTCAACAAATCATCAAAA GATATTGG-3' and HCO1298 5'-TAAACTTCAGGG TGTGACCAAAAAATCA-3' (Gomez et al., 2002), and those for the \textit{G. roeseli} 16S rDNA sequence were LR-J-Gam 5'-ATTTAAATTCAACATCGAGGTTGC-3' and LR-N-Gam 5'-TTTAACGGXTGCGGTATTTTGAC-3' (Muller et al., 2000). A T3 thermocycler$^\text{R}$ (Biometra) was used with an initial denaturation at 94 $^\circ$C for 2 min, followed by 35 cycles consisting of 20 s at 94 $^\circ$C, 30 s at the annealing temperature (50 $^\circ$C for \textit{P. laevis} and 40 $^\circ$C for \textit{G. roeseli}) and 20 s at 65 $^\circ$C. The final extension step was done at 65 $^\circ$C for 3 min. Fifteen microlitres of PCR product was cleaned from residual oligonucleotides and dNTPs with Exonucleases I and Shrimp Alkaline Phosphatase, both from MBI Fermentas, according to the manufacturer's recommendations. Direct sequencing of the PCR products, using the PCR forward primer for cycle sequencing, was performed by Macrogen Inc. (Seoul, South Korea).

Sequences were edited and aligned using Bioedit 5.0.9 software (Hall, 1999). At least two independent PCR products were sequenced for each polymorphism observed. Genetic differentiation was investigated using F-statistics (Weir and Cockerham, 1984) using the program Arlequin 3.01 (Excoffier et al., 2005).

2.6. Statistics

Variation among populations and between countries for the prevalence of infection of \textit{G. roeseli} by \textit{P. laevis} was analysed using a logistic regression. Geotaxis and phototaxis scores were not normally distributed and could not be satisfactorily transformed. These data were therefore analysed using Wilcoxon two-sample tests for differences between countries of origin and status of infection within each population. Data on PO activity were first analysed using Wilcoxon two-sample tests for differences within each population. Data on PO activity were then analysed with a mixed model of analysis of variance (ANOVA) including country of origin, infection status and sex as factors, and their interaction. A population factor was nested within the country factor to take into account potential between-population variations within each country studied. The data were analysed using SPSS 11 for Macintosh and JMP 5.0 for Windows. For each statistical test, the null hypothesis ($H_0$) was rejected for $P \leq 0.05$.

3. Results

3.1. Variable parasite prevalence among populations but similar prevalence between countries

Among the investigated populations, infected gammarids were mostly infected by \textit{P. laevis}. Only one individual was found infected with a different species of acanthocephalan parasite, \textit{Pomphorhynchus terreticolis} (Perrot-Minnot, 2004), in the river Ouche at Dijon. Considering the rare occurrence of infection by this particular parasite we restricted our analysis to the prevalence of \textit{P. laevis}. Prevalence of infection by \textit{P. laevis} was variable among populations (Fig. 1, logistic regression, Wald statistic = 58.57, df = 3, $P < 0.001$) but not between countries since this later factor was withdrawn from the statistical model by the stepwise procedure. The highest prevalence was found in Dijon (France), which was about 4.7 times higher than the overall prevalence of the four populations together (Wald statistic = 50.63, df = 1, Odd ratio = 4.68, $P < 0.001$), and the lowest was found in Tapolca (Hungary), which was about 11 times lower than the overall prevalence of the four populations (Wald statistic = 23.52, df = 1, Odd ratio = 0.113, $P < 0.001$). The prevalences of \textit{P. laevis} in Les Maillys (France) and Raposka (Hungary) were similar and did not differ significantly from the overall prevalence of the four populations (Les Maillys Wald statistic = 0.88, df = 1, Odd ratio = 1.39, NS; Raposka Wald statistic = 1.87, df = 1, Odd ratio = 1.36, NS).

3.2. No behavioural alteration associated with \textit{P. laevis} infection

Within each population, the infection status did not affect \textit{G. roeseli} behaviour for either phototropism (Dijon...
z = −0.31, NS; Maillys z = 1.00, NS; Raposka z = 1.35, NS; Tapolca z = 1.15, NS) (Fig. 2).

3.3. Changes of phenoloxidase activity associated with P. laevis infection

Gammarid sex, either alone or in interaction with other factors, never had a significant effect on PO activity of G. roeseli. This factor was therefore removed from the analysis. PO activity differs between countries and between populations within each country (Table 1), a result explained mainly by variation between Hungarian populations (Fig. 3). Despite this variation, a significant effect of the interaction between country and infection was found (Table 1). Infection by P. laevis affects G. roeseli PO activity in France and in Hungary differently (Fig. 3). No significant difference in PO activity was found between infected and uninfected animals in France (t_{166} = 1.52, P = 0.13) while the infection was associated with higher levels of PO activity in Hungary (t_{204} = 2.46, P = 0.01), a phenomenon due mainly to the large difference observed in the Raposka population (Fig. 3).

3.4. Populations of G. roeseli and P. laevis are genetically isolated but share a common origin

Sequencing of 55 G. roeseli for rRNA 16S (312 nucleotides) identified five nucleotide variants, allowing the definition of five haplotypes (R1–R5, Fig. 4a) (GenBank Accession Nos. EF044314–EF044318). Similarly, sequencing of 48 P. laevis for the COI gene (610 nucleotides) identified 12 nucleotide variants and distinguished 10 haplotypes (L1–L10, Fig. 4b) (GenBank Accession Nos. EF051062–EF051071). Despite the high levels of differentiation sometimes observed (see below), molecular divergence between haplotypes for both host and parasite was extremely low (Fig. 4), strongly advocating that we did not analyse two different species of hosts or parasites according to their geographic location. The analysis of the spatial distribution of this polymorphism based on haplotype frequencies revealed contrasting results between the host and the parasite. For the host, a strong differentiation was observed between French and Hungarian populations, but not between populations within both France and Hungary. No significant differentiation was observed between

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**Table 1**

Analysis of variance for phenoloxidase activity (PO) in *Gammarus roeseli*, as a function of country of origin, population of origin (nested within country of origin as a random factor) and infection by *Pomphorhynchus laevis*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country</td>
<td>263.52</td>
<td>1</td>
<td>4.92</td>
<td>0.03</td>
</tr>
<tr>
<td>Population (country)</td>
<td>485.88</td>
<td>2</td>
<td>4.54</td>
<td>0.011</td>
</tr>
<tr>
<td>Infection</td>
<td>0.95</td>
<td>1</td>
<td>0.02</td>
<td>0.894</td>
</tr>
<tr>
<td>Country*Infection</td>
<td>404.62</td>
<td>1</td>
<td>7.56</td>
<td>0.006</td>
</tr>
<tr>
<td>Error</td>
<td>19702.48</td>
<td>368</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Prior to analysis, PO activity was corrected for gammarid size, and values were transformed using Box–Cox procedure to meet normality and homogeneity of variances.

Global model: F_{5,373} = 4.177, P = 0.001.
the Czech population and the two Hungarian populations (Table 2a, Fig. 4a). For parasites, differentiation was observed for all comparisons between populations except between populations within both France and Hungary (Table 2b, Fig. 4b).

4. Discussion

This study revealed high variation in prevalence of infection of G. roeseli by P. laevis among populations, without an obvious geographic pattern: a strong prevalence was found in one French population, a very low prevalence in one Hungarian population, and medium prevalences in the two countries. However, no significant difference was found between countries for the prevalence of infection of G. roeseli by P. laevis.

Gammarus roeseli PO activity was also variable among populations. However, PO activity was higher in infected gammarids than in uninfected ones in Hungarian populations, whilst an opposite, but not significant, tendency was observed in French populations. This suggests that in Hungary, gammarids are developing an immune response against the parasite by enhancing the PO activity of their haemolymph. In contrast, in France, the immune response is mostly unaffected by the parasite. This contradicts results from a previous study (Rigaud and Moret, 2003) where, in one of the French populations studied here (Les Maillys), the infection by P. laevis was found to be associated with higher levels of PO activity than in uninfected hosts. This difference may be due to a between-year variation (the present study was made in 2004 while results of Rigaud and Moret (2003) were obtained in 2002).

Gammarids infected by P. laevis in Hungarian and French populations had similar behavioural scores to uninfected ones, for both phototaxis and geotaxis. Such a result was expected for geotaxis scores since infection by P. laevis has not been observed to affect the geotropism of other gammarid species (e.g., Bauer et al., 2000). However, we were expecting to observe enhanced phototaxis in infected G. roeseli from Hungarian populations if, according to our hypothesis, P. laevis was locally adapted to this gammarid host in Hungary. The parasite seems unable to perform any behavioural manipulation on G. roeseli, or evade the gammarid resistance to behavioural manipulation, both in Hungary and in France. Either way, this suggests a local maladaptation of the acanthocephalan to G. roeseli in all the studied populations. Another possibility is that an unknown behavioural modification by P. laevis exists in G. roeseli. This remains to be tested, but in other gammarid species infected by P. laevis, phototropism behaviour is always modified by the acanthocephalan (e.g., Maynard et al., 1998). Some acanthocephalans are also known to modify the appearance of some of their crustacean intermediate hosts (reviewed in Kennedy, 2006). In G. roeseli, the orange-yellow spot in the host cuticle produced by cystacanths is much weaker than in G. pulex, and no increase of cuticle melanization is observed in infected animals (unpublished observations). In P. laevis/G. roeseli interaction, it is therefore unlikely that an enhanced probability of transmission to the definitive host is driven by a change in colour or appearance, as observed in other systems (Kennedy, 2006).
In summary, the data about immune defence and behavioural measurements do not support our hypothesis about the respective local maladaptation and local adaptation of the parasite to its host in Hungary and France. This hypothesis was based on two assumptions.

The first assumption was that the French invasive populations of *G. roeseli* were coming from an area of origin including the Hungarian part of the Danube plain (Jazdzewski and Roux, 1988). The genetic analyses of both *P. laevis* and *G. roeseli* provide an explanation as to why our hypothesis was probably not valid. The molecular divergence between haplotypes for both *P. laevis* and *G. roeseli* in Hungarian and French populations is extremely low, suggesting that individuals from both countries are originating from the same original population. However, the observed differentiation based on haplotype frequencies might reflect founding effects during a recolonization process and a subsequent absence of gene flow. At least, it is difficult to consider that *G. roeseli* found in French rivers is a subset of Central European populations as originally believed. Conversely, the Czech population of *G. roeseli* is genetically closely related to the Hungarian ones. All this suggests that *G. roeseli* from both Central Europe and France probably come from the same original population or area and then independently diverged from each other after establishment in their present respective areas. A similar conclusion held for *P. laevis* whilst belonging to the same species (*P. laevis* sensu stricto, see Perrot-Minnot, 2004 for comparison), all the populations studied here were rather isolated between countries. It means that the two subsets of populations we studied harbour two isolated host–parasite systems. Consequently, co-evolutionary history of *P. laevis* and *G. roeseli* in both countries is probably relatively recent and may explain why *P. laevis* is not more adapted to *G. roeseli* in Hungary than in France. In fact, our results suggest that the host is probably more adapted to the parasite than the reverse. Indeed, *G. roeseli* seems able to mount a significant immune response against *P. laevis* (Rigaud and Moret, 2003; this study), suggesting an adaptation of the host to fight the parasite. Such a potential adaptation of the host to the parasite may result from differential migration rates between the partners as proposed by Gandon et al. (1996), since populations of *G. roeseli* are less structured than those of *P. laevis*.

The second assumption of our initial hypothesis was based on an evolutionary advantage of the parasite in host–parasite co-evolutions. Parasites, with relatively shorter generation times, could evolve faster than hosts and should therefore adapt rapidly to the most common host genotypes and their resistance mechanisms (Kaltz and Shykoff, 1998 and references therein). Freshwater-living acanthocephalans have a life-cycle (from egg to egg) of about 3–12 months, considering their minimal development time at all stages and their maximum survival time in their different hosts (Kennedy, 2004; Rigaud and Bollache, unpublished observations). Generation time of *G. roeseli* is similar to that of the acanthocephalan parasite (Pockl, 1993). Consequently, the parasite may not have an obvious evolutionary advantage in this host–parasite system and parasite adaptation to the host might be slower. As stated earlier, our data even suggest an advantage to the host in Hungarian populations. Our interpretation would be that the host, rather than the parasite, is currently winning the co-evolutionary race in Hungary, but not in France. Nevertheless, it is difficult to make firm conclusions on this point, because the slow dynamics of selection may enhance the probability of confounding effects caused by environmental variations (e.g., Blanford et al., 2003; Mitchell et al., 2005).

In conclusion, local adaptation and maladaptation of *P. laevis* to *G. roeseli* could only be tested in the real area of origin of this gammarid species, which still needs to be found. The presence of *P. laevis* should be investigated in these original populations of *G. roeseli*. If *P. laevis* is present in this original area, infection prevalence and the effects of the parasite on *G. roeseli* immunity and behaviour should be examined. Such a study will help in understanding why the central and western-European populations of *P. laevis* are not adapted to this host. Alternatively, the absence of *P. laevis* in native populations of *G. roeseli* would mean that this gammarid species is a recently acquired host and the parasite has not adapted to the total invasive range.

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