

Body size in the ant-associated isopod *Platyarthrus hoffmannseggii* is host-dependent

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Many symbionts live in association with different species. It can be expected that these distinct hosts might have a different effect on key life-history traits of the associated symbionts. Here, we compared the key trait body size of the obligatorily ant-associated isopod *Platyarthrus hoffmannseggii* collected in nests of two types of sympatric ant hosts. This isopod species showed surprisingly large differences in body size depending on type of host ant, with the head width of females and males associated with organic mound building red wood ants (RWAs) being, respectively, 1.30 and 1.17 times larger than isopods sympatrically living in earth nests of *Lasius flavus*. There was also a higher proportion of females in many RWA nests, but this pattern was not consistent across all nests. Genetic analyses and aggression trials did not reveal cryptic groups specialized to different hosts. Therefore, we argue that the isopods exhibit size plasticity because of different host nest conditions. Absence of host aggression and optimal abiotic conditions in RWA nests might promote a larger isopod body size. Overall, this study shows that the association of a symbiont with different hosts might induce phenotypic plasticity in a symbiont key trait.

ADDITIONAL KEYWORDS: ant guests – associates – ecotype – fitness – inquiline – isopoda – myrmecophile – symbiosis.

INTRODUCTION

Symbionts often engage in close associations with multiple hosts (Nuismer & Thompson, 2006). This complicates the study of symbioses as host–symbiont interactions should be studied as a dynamic network in which host species might differently affect the symbiont (Ivens *et al.*, 2016). Interestingly, symbionts can demonstrate local adaptation of distinct genetic populations to a preferred host (Carroll, Dingle, & Klassen, 1997; Mccoy *et al.*, 2001; Le Gac *et al.*, 2007). These distinct symbiont ecotypes or races might have different phenotypes (Carroll, Dingle, & Klassen, 1997) and are expected to have a higher fitness with their preferred host (Carroll, Dingle, & Klassen, 1997; Mccoy *et al.*, 2001). However, there are

also reports of phenotypic plasticity of symbionts without genetic structuring across host species. A nice example is the commensal bivalve *Neaeromya rugifera*, which has a host-specific shell morphology, but lacks host-specific genetic structuring (Li & O’Foighil, 2012).

A valuable system to test host–symbiont interactions is arthropods that are strictly associated with ants, so-called myrmecophiles (Kronauer & Pierce, 2011). Some very specialized parasitic myrmecophiles are restricted to a single host (e.g. Elmes *et al.*, 1999), but most European myrmecophiles associate with a group of related ants or can even live with most ants in their distribution range (Parmentier, Dekoninck, & Wenseleers, 2014). The nest conditions of sympatric ant species might be extremely different (Lach, Parr, & Abbott, 2010). Different types of nests such as nests in dead trunks, ground nests in the soil or litter, nests in acorns and nests with an above-ground mound of

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organic thatch can be found on a scale of a few square meters (Seifert, 2007). Apart from these different abiotic conditions, myrmecophiles might also be exposed to different levels of biotic stress as the tolerance or aggression of host ant species towards myrmecophiles can be highly variable (Parmentier, Dekoninck, & Wenseleers, 2016a). We hypothesize therefore that different abiotic and biotic nest conditions of sympatric ant species might strongly affect myrmecophile traits.

In this study, we analysed populations of the myrmecophilous isopod *Platyarthrus hoffmannseggii* Brandt, 1833 in different ant hosts. First, we looked whether the isopod has split into distinct groups specialized to different ant hosts by analysing host-specific genetic differentiation and by performing aggression experiments. Second, we examined the effect of living with different hosts on isopod body size and sex ratio. Body size is an environmentally plastic trait in isopods, which is easy to measure and is strongly positively correlated with fecundity (e.g. Lavy *et al.*, 2001; Hemmi & Jormalainen, 2004). Sex ratio has a strong effect on population dynamics and mating strategies and might also be affected by the environment in isopods (Rigaud *et al.*, 1997). Finally, we looked whether *P. hoffmannseggii* could benefit from host-specific plasticity in body size to avoid detection and aggression in distinct hosts.

MATERIAL AND METHODS

STUDY ORGANISMS AND STUDY SITES

Platyarthrus hoffmannseggii is a small, white and blind isopod which is strictly associated with ants. It scavenges on ant brood and ant prey but probably feeds on organic material as well (Parmentier *et al.*, 2016a). This generalist myrmecophile can be found in nests of multiple ant species (Parmentier, Dekoninck & Wenseleers, 2014).

We sampled *P. hoffmannseggii* in northwestern Belgium and northern France (Fig. 1) in nests of two host types: *Lasius flavus* and red wood ants (RWAs) (*Formica rufa* and *F. polyctena*). *Lasius flavus* constructs ground nests in meadows, lawns and along the periphery of woodlands (Seifert, 2007). *Formica rufa* and *F. polyctena* are closely related RWA species that might hybridize (Seifert, 2007). They build similar above-ground mounds of organic thatch along forest edges in our study region.

Sampling was conducted in five sites during July and August 2016. In four sites (sites 1, 3, 4, 5), isopods were collected both in *L. flavus* and RWA nests (Table 1), in site 2 only isopods associated with *L. flavus* were sampled. Site and nest details (soil, coordinates, habitat and nest distances) are included in Supporting Information, Appendix 1.

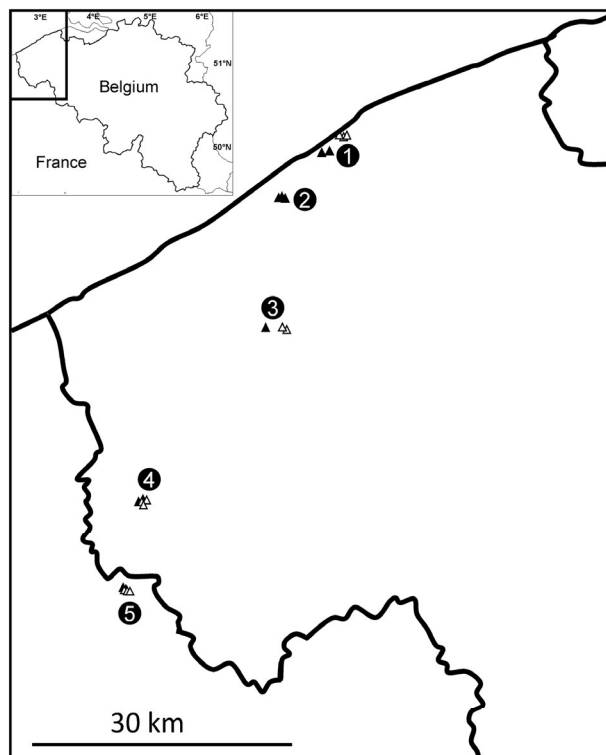


Figure 1. Study sites in Belgium (site 1: De Haan, site 2: Oostende, site 3: Vladslo, site 4: West-Vleteren) and France (site 5: Boeschepe). Black triangles refer to *Lasius flavus* nests, white triangles are red wood ant nests.

HOST SPECIALIZATION OF *P. HOFFMANNSEGGII* GROUPS

cytochrome c oxidase I (COI) Gene

The mitochondrial COI was examined to identify the presence of distinct host races adapted to *L. flavus* and RWAs. The DNA of one specimen of each nest of the study site (Table 1) was extracted and sequenced (protocol see Supporting Information, Appendix 2).

Behavioural Specialization

Myrmecophiles can employ host-specific and even colony-specific behavioural and chemical adaptations to optimize their integration in the host colony (Kronauer & Pierce, 2011). Here, we tested whether ants discriminate isopods found in their own nest from individuals transferred from a nest of the other host type (*L. flavus* vs. RWAs). In case of behavioural specialization to the host colony or host type, workers are expected to display elevated aggression towards individuals transferred from a colony of the other host type compared to individuals found in the same nest. Trials were conducted in a rectangular plastic arena (8 × 5.5 cm) filled with 1 cm plaster and coated with

Table 1. Maximum head width and sex ratio in *Platyarthrus hoffmannseggii* populations associated with *Lasius flavus* and RWAs

	Host	N	Maximum head width female	Maximum head width male	Sex ratio
Site 1: De Haan					
RWA_1	<i>F. polycytena</i>	41	0.93 ± 0.10	0.77 ± 0.05	0.85
RWA_2	<i>F. polycytena</i>	54	0.96 ± 0.11	0.81 ± 0.10	0.83
RWA_3	<i>F. rufa</i>	59	0.90 ± 0.08	0.70 ± 0.06	0.85
Flavus_1	<i>L. flavus</i>	28	0.68 ± 0.11	0.62 ± 0.05	0.50
Flavus_2	<i>L. flavus</i>	35	0.71 ± 0.08	0.63 ± 0.05	0.71
Site 2: Oostende					
Flavus_3	<i>L. flavus</i>	37	0.75 ± 0.06	0.64 ± 0.06	0.51
Flavus_4	<i>L. flavus</i>	43	0.74 ± 0.09	0.63 ± 0.04	0.53
Flavus_5	<i>L. flavus</i>	41	0.67 ± 0.08	0.57 ± 0.08	0.44
Site 3: Vladslo					
RWA_4	<i>F. rufa</i>	50	0.89 ± 0.08	0.69 ± 0.07	0.80
RWA_5	<i>F. rufa</i>	89	0.90 ± 0.09	0.67 ± 0.05	0.91
Flavus_6	<i>L. flavus</i>	44	0.67 ± 0.10	0.65 ± 0.06	0.45
Site 4: West-Vleteren					
RWA_6	<i>F. rufa</i>	39	0.94 ± 0.16	0.69 ± 0.09	0.53
RWA_7	<i>F. rufa</i>	57	0.97 ± 0.11	0.77 ± 0.08	0.61
RWA_8	<i>F. rufa</i>	62	0.93 ± 0.08	0.73 ± 0.07	0.76
Flavus_7	<i>L. flavus</i>	51	0.74 ± 0.09	0.66 ± 0.06	0.59
Flavus_8	<i>L. flavus</i>	39	0.69 ± 0.07	0.65 ± 0.05	0.49
Site 5: Boeschepe					
RWA_9	<i>F. rufa</i>	31	0.91 ± 0.06	0.76 ± 0.05	0.45
RWA_10	<i>F. rufa</i>	67	0.86 ± 0.11	0.73 ± 0.06	0.49
RWA_11	<i>F. rufa</i>	63	0.87 ± 0.08	0.77 ± 0.06	0.75
Flavus_9	<i>L. flavus</i>	77	0.69 ± 0.06	0.63 ± 0.06	0.57
Flavus_10	<i>L. flavus</i>	20	0.72 ± 0.07	0.64 ± 0.04	0.55

Sex ratio is expressed as the proportion of female individuals.

flouon. Thirty *L. flavus* and *F. rufa* workers were acclimatized in two different arenas for 1 h. Ants were calm and showed typical grooming and inspection behaviour. Then a *P. hoffmannseggii* individual was added to one arena and the first 20 interactions (i.e. when the antennae of a worker passed over an isopod) with the ants were recorded after a settling period of 30 s. Ant aggression was scored by the proportion of aggressive interactions (biting, opening mandibles) out of the first 20 interactions. Every isopod was subsequently introduced in the arena of the other ant species and aggression was similarly scored. During the introductions of the isopod, we were unaware (“blind observations”) of its origin (same nest as ant workers or from a hetero-specific nest). These aggression tests were first done with workers and isopods from RWA_9 (19 isopods) and Flavus_3 (17 isopods) nests, and subsequently repeated with workers and isopods from RWA_6 (17 isopods) and Flavus_1 (15 isopods) nests. The order of aggression trials in either an arena with *L. flavus* or RWAs was randomly chosen for each isopod. Workers were re-used for several trials.

HOST-DEPENDENT SIZE AND SEX RATIO VARIATION OF *P. HOFFMANNSEGGII*

We measured the head of every isopod at its widest point with an Olympus-M3 binocular (×70). This parameter has been reported as a reliable body size proxy (Williams & Franks, 1988). Isopod individuals were grouped by host type (*L. flavus* vs. RWAs) and by sex. Immature males with undeveloped genitalia could be wrongly classified as females. However, the smallest individuals measured in this study were clearly male, so the number of females was not overestimated. Previous studies reported a higher proportion of females than males in *P. hoffmannseggii* (Vandel, 1962; Williams & Franks, 1988). The proportion of females was calculated in each colony and compared between the two hosts.

BENEFITS OF HOST-SPECIFIC SIZE DIFFERENCES

We found in a previous study that myrmecophiles much smaller than their host were mostly ignored, whereas myrmecophiles that match the size of their host were attacked (Parmentier, Dekoninck, & Wenseleers 2016a).

Therefore, host workers might be more aggressive towards larger *P. hoffmannseggii* individuals, especially in *L. flavus* of which the workers equal the size of the largest isopods. Smaller isopods could better mask their presence in *L. flavus* and consequently the phenotypic plasticity could be beneficial for *P. hoffmannseggii*. As we used isopods over a large size range in the aforementioned aggression trials, we could also examine the effect of isopod size on ant aggression with these trials.

STATISTICAL ANALYSIS

We ran for *L. flavus* and RWA-associated isopods two different generalized linear mixed models (GLMMs) with a binomial distribution to compare the proportion of aggressive interactions across different treatments (isopod found in the same nest or transferred from a heterospecific nest) and controlled for isopod size. “Treatment”, “isopod size” and their interaction were modelled as fixed factors, “nest combinations” (RWA_9-Flavus_3 and RWA_6-Flavus_1) as a random factor. We also added an observation level random factor to deal with overdispersion (Browne et al., 2005).

The maximum head width (hereafter head width) of isopods was compared with a general linear mixed model (LMM), including the fixed factors “ant host” and “isopod sex” and its interaction term and the random factor “host nest” nested in the factor “site”. Host-dependent differences in proportion of female *P. hoffmannseggii* were analysed using a GLMM with a binomial distribution. “Ant host” was implemented as a fixed factor and “site” and an observation level factor as random factors.

Significance of the fixed factors in all aforementioned models were tested with a likelihood ratio test using the ANOVA function of package car in R version-3.2.1.

RESULTS

HOST SPECIALIZATION OF *P. HOFFMANNSEGGII* GROUPS

COI Gene

After alignment and trimming to equal length, a 537-bp fragment of the COI gene was obtained. This fragment was identical in specimens collected in 20 out of the 21 sampled nests (GenBank accession KY038165). The fragment of the specimen found in nest Flavus_1 differed in one base pair with those fragments (GenBank accession KY020404). Three other COI haplotypes of *P. hoffmannseggii* are hitherto deposited on GenBank (KR424592-KR424594). These haplotypes come from Italian *P. hoffmannseggii* populations (Javidkar et al., 2015), but are clearly different from the two *P. hoffmannseggii* haplotypes of this study (similarity between 78 and 79%).

Behavioural Specialization

Lasius flavus regularly showed aggression towards *P. hoffmannseggii* [mean proportion aggressive interactions (95% CI) = 0.12 (0.03–0.37)]. Conversely, RWAs mostly ignored *P. hoffmannseggii* [mean proportion aggressive interactions (95% CI) = 0.01 (0.00–0.05)]. Both *L. flavus* and RWAs did not discriminate isopods living in their own nest from individuals transferred from a nest of the other ant type (GLMM_{flavus}: $P = 0.184$, GLMM_{RWAs}: $P = 0.735$, detailed output of statistical tests in Supporting Information, Appendix 3).

HOST-DEPENDENT SIZE AND SEX RATIO VARIATION OF *P. HOFFMANNSEGGII*

The size of *P. hoffmannseggii* was strongly host-dependent (LMM, Chisq = 173.45, $P < 0.001$, Supporting Information, Appendix 3). Females were significantly larger than males in both host ant types (LMM, Chisq = 435.10, $P < 0.001$). There was also a significant interaction effect between host and isopod sex as the size difference between males and females was much more prominent in RWAs than in *L. flavus* (LMM, Chisq = 74.99, $P < 0.001$) (Fig. 2). The head width of females associated with RWAs (mean \pm SE = 0.91 mm \pm 0.01) was 1.30 times larger than head width of females associated with *L. flavus* (mean \pm SE = 0.70 mm \pm 0.01). The head width of males

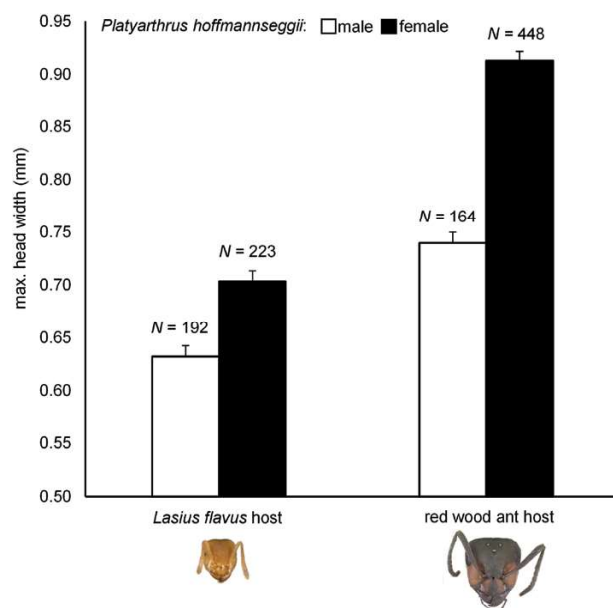


Figure 2. Maximum head width \pm SE (mm) of males and females of *Platyarthus hoffmannseggii* associated with *Lasius flavus* and red wood ants. Total number of individuals sampled for each category is given above the bars. Ant heads adapted from figures at www.antweb.org.

associated with RWAs (mean \pm SE = 0.74 mm \pm 0.01) was 1.17 times larger than head width of males living in nests of *L. flavus* (mean \pm SE = 0.63 mm \pm 0.01). The differences were consistent across all sampled nests and sites (Table 1).

A significantly larger proportion of females occurred in RWA nests [mean (95% CI) = 0.74 (0.63–0.82)] than in *L. flavus* nests [mean (95% CI) = 0.55 (0.43–0.66)] (GLMM, Chisq = 11.30, $P < 0.001$, Supporting Information Appendix 3), but this pattern was not consistent for all nests (Table 1).

BENEFITS OF HOST-SPECIFIC SIZE VARIATION

Isopod size did not affect host aggression (GLMM_{flavus}: $P = 0.400$, GLMM_{RWAs}: $P = 0.218$, Supporting Information Appendix 3), nor did the interaction between size and isopod origin affect ant aggression (GLMM_{flavus}: $P = 0.179$, GLMM_{RWAs}: $P = 0.726$).

DISCUSSION

Our study shows that living with different hosts can have a strong impact on a key trait of a symbiont. Particularly, we showed that the association with different ant hosts induce size plasticity in the myrmecophilous isopod *P. hoffmannseggii*.

Symbionts might split in distinct genetic groups specialized to different hosts (e.g. McCoy *et al.*, 2001). Similarly, *P. hoffmannseggii* might have diverged in different host ant-specific cryptic races with specialized morphologies. The COI gene was used as a successful marker to uncover genetic population structuring in isopods (Markow & Pfeiler, 2010). However, we found no COI-gene differentiation in *P. hoffmannseggii* across the hosts or study sites, whereas this species displays high variation in this gene (cf. with Italian *P. hoffmannseggii* Javidkar *et al.*, 2015). Fine-scale genetic structuring and gene flow could be detected with microsatellites or RAD sequencing. Nevertheless, *P. hoffmannseggii* is expected to have a much larger gene flow between nests of different host species within a site than between nests of the same host located in different sites. The isopod also lacked behavioural specialization to their host colony or host species as isopods transferred from a nest of the other host type did not provoke more aggression. The absence of host specialization is further underlined by the rapid association of *P. hoffmannseggii* with invasive ants (Dekoninck, Lock, & Janssens, 2007) and the lack of matching host recognition cues (Parmentier, 2016). Therefore, we argue that the isopods found in both host types form no distinct races. Some studies relied on very subtle morphological variation to split the taxonomic status of a myrmecophile in different lines

each specializing on related ants (Zerche, 2009; Zagaja, Staniec, & Pietrykowska-Tudruj, 2014). However, in the case of unspecialized myrmecophiles, which poorly interact with their host, easily switch between related hosts and do not match the nestmate recognition cues (Parmentier, 2016), divergent selection is expected to be low. Phenotypic plasticity depending on abiotic and biotic conditions of the host nests might therefore be a more plausible explanation.

We found that *P. hoffmannseggii* was characterized by a larger body size in RWA nests than in *L. flavus* nests. It was hard to keep the isopods in lab nests for a long period. Consequently, we could not measure other key traits, such as growth rate and offspring number, but it is likely that they show plasticity across hosts as well. Life-history traits of isopods are strongly affected by abiotic conditions such as diet (Lavy *et al.*, 2001), temperature and moisture (Dixie, White, & Hassall, 2015). For example the isopods *Armadillidium vulgare* and *Idotea baltica* have, respectively, a faster growth rate and a larger size in patches with high-quality food (Hassall, Helden & Benton, 2003; Hemmi & Jormalainen, 2004). Similarly, conditions in RWA nests might be better than in *L. flavus* nests and could lead to larger body sizes in *P. hoffmannseggii*. RWA mounds might provide more optimal abiotic conditions than *L. flavus* nests for two reasons. First, RWAs maintain a high (25–30 °C) and stable temperature in their nest from early spring to late autumn (Rosengren *et al.*, 1987). This nest heating is caused by efficient solar absorption, microbial activity and metabolic heat production of the workers (Frouz, 2000). The soil nest of *L. flavus* also heat by solar absorption, but its temperature mainly fluctuates with the ambient temperature (Bierbaß, Gutknecht, & Michalzik, 2015). Second, RWA nests provide ample food sources for *P. hoffmannseggii* which scavenges on organic material, prey and brood (Parmentier *et al.*, 2016b). Nests of *L. flavus* contain little detritus and the number of dead prey is also lower, as *L. flavus* mainly feeds on honeydew (Seifert, 2007). Besides abiotic conditions, biotic stressors might also affect isopods (Sparkes, 1996; Kight & Nevo, 2004; Castillo & Kight, 2005). *Lasius flavus* showed some aggression towards *P. hoffmannseggii*, whereas it was mostly ignored by RWAs. Possibly, the higher biotic stress in *L. flavus* nests might negatively affect body size of *P. hoffmannseggii*. Lower size asymmetries between host and myrmecophile are likely to increase the detectability of the myrmecophile (Parmentier, Dekoninck, & Wenseleers, 2016a). Consequently, larger *P. hoffmannseggii* individuals might be more easily detected by *L. flavus* and therefore it would be beneficial to be smaller in *L. flavus* nests. However, *P. hoffmannseggii* might not benefit from the observed host-specific phenotypic plasticity as aggression and corresponding biotic stress

induced by *L. flavus* was size-independent. Therefore, *P. hoffmannseggii* is expected to have a lower fitness in *L. flavus* nests as body size is strongly positively correlated with reproductive success in isopods (Hemmi & Jormalainen, 2004). Still, the small isopod size in *L. flavus* nests might be adaptive as it could permit easier access to the relatively small tunnels of *L. flavus* nests.

Isopods associated with RWAs had on average a much stronger female-biased sex ratio than isopods living with *L. flavus*, but the sex ratio of some RWA-associated populations deviated from this pattern. Interestingly, populations of the ant-associated congeneric species *P. aiasensis* ranged from 63% females to complete parthenogenesis (Montesanto, Caruso, & Lombardo, 2008). The female-inducing bacteria *Wolbachia* is widespread in isopods (Bouchon, Rigaud, & Juchault, 1998) and might cause the observed variation in sex ratio. It would be interesting to test whether different ant hosts promote, either directly or indirectly, the infection of *P. hoffmannseggii* by *Wolbachia*.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Table S1. Details on the different nests of the five sites.

Appendix S2. COI protocol.

Appendix S3. Detailed results statistical analyses.