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# Bumble bee parasite prevalence but not genetic diversity impacted by the invasive plant *Impatiens glandulifera*

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**Citation:** Vanderplanck, M., N. Roger, R. Moerman, G. Ghisbain, M. Gérard, D. Popowski, S. Granica, D. Fournier, I. Meeus, N. Piot, G. Smagghe, L. Terrana, and D. Michez. 2019. Bumble bee parasite prevalence but not genetic diversity impacted by the invasive plant *Impatiens glandulifera*. Ecosphere 10(7):e02804. 10.1002/ecs2.2804

**Abstract.** While many bee species are experiencing population declines, some host plant generalist bees remain common in Europe, partly because they seem able to shift to new resources. However, foraging on a new alternative plant, such as an invasive species, can modify diet quality and have a potentially detrimental effect on bee health. Herein, we investigated whether the spread of the invasive plant *Impatiens glandulifera* affects *Bombus pascuorum* population regarding parasite prevalence, genetic structure, and nest density in Belgium. While no difference in bumble bee genetic structure was detected between invaded and uninvaded sites, we show that *I. glandulifera* occurrence was significantly correlated with a decrease in the prevalence of *Apicystis bombi* but not the prevalence of three other parasite species (i.e., *Crithidia bombi*, *Nosema bombi*, *Nosema ceranae*, and *Nosema* sp.). Regarding our investigations, this effect was likely not due to variation in local bumble bee population fitness before *I. glandulifera* flowering, nor to the relative abundance of other pollinators such as *Apis mellifera*, but the unique chemical composition (i.e., polyphenol rich) of the pollen of *I. glandulifera* remained as an interesting hypothesis. Whereas *B. pascuorum* queens probably colonize all the potential nesting sites in an area, invaded by *I. glandulifera* or not, the abundance of polyphenol ampelopsin in pollen from *I. glandulifera* pollen might reduce local parasite prevalence. Our field study confirms that bumble bee parasite prevalence is potentially related to the particular chemical composition of collected pollen. Plant traits such as secondary metabolite occurrence could play a key role in the health and conservation of bumble bees.

**Key words:** bumble bee; conservation; insect pollinator; invasive plants; parasites; population genetic diversity.

**Received** 6 November 2018; revised 28 May 2019; accepted 5 June 2019. Corresponding Editor: T'ai Roulston.

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## Introduction

Bees play a key role in natural and agricultural ecosystem maintenance through the pollination of flowering plants (Ollerton et al. 2011). It is now well established that several wild and domesticated bee species are experiencing significant population declines, which raises concerns about the services they provide (Nieto et al. 2014). The key drivers of these declines are the

loss and fragmentation of habitats, pesticide spread, climate change, changes in land management practices, and alien plant invasion (Scheper et al. 2014, Goulson et al. 2015, Kerr et al. 2015, Woodcock et al. 2016). However, some bumble bee species remain common in Europe, as it is the case for the so-called big-six: *Bombus hortorum*, *B. lapidarius*, *B. lucorum*, *B. pratorum*, *B. pascuorum*, and *B. terrestris* (Goulson et al. 2008). Some species traits or trait combinations common to this group are assumed to partly explain such population stability: (1) large climatic ranges (i.e., being more resilient to extreme climatic events; Williams and Osborne 2009); (2) early emergence (i.e., having access to early floral resources with low competition; Williams and Osborne 2009); (3) flexibility in choice of nesting substrate (i.e., occupying a high diversity of sites; Darvill et al. 2004); and (4) generalist foraging behavior (i.e., foraging on a large range of phylogenetically distant host plant species; Goulson and Darvill 2004). In particular, generalist foraging behavior could allow bumble bees to deal with a change in habitat quality such as the spread of invasive plants by integrating these plants into their diets as new floral resources (Chittka and Schurkens 2001).

However, new host plant occurrence as well as decrease in plant diversity that may result from plant invasion can comprise an alternative diet that may contain low concentration of primary nutrients (e.g., protein content [Roulston and Cane 2000] or amino acid content [Roger et al. 2017]) or detrimental secondary metabolites (Bennet and Wallsgrove 1994). Many studies have already highlighted that pollen diets with low protein concentration may negatively affect colony development (i.e., total brood mass; Vanderplanck et al. 2014, 2018), individual size (Moerman et al. 2016), and the immune system of bumble bees (Brunner et al. 2014). In addition, some plants also contain secondary metabolites in their pollen and nectar, which evolved to deter herbivores or plant parasites (Hadacek 2002). These secondary compounds can have a wide range of negative biological effects such as poisoning bees (e.g., alkaloids, saponins, cardiac glycosides, and cyanogenic glycosides; Detzel and Wink 1993) or reducing colony fitness when consumed (e.g., lupanine; Arnold et al. 2014). Alternatively, some secondary metabolites can display positive effects on bees by improving memory and foraging efficiency (e.g., caffeine [Wright et al. 2013]; iridoid glycosides [Richardson et al. 2016]), and also decreasing parasite infection (e.g., gelsemine [Manson et al. 2010]; anabasine, catalpol, nicotine, and thymol [Richardson et al. 2015]). Moreover, some studies have shown that infected bees could actively self-medicate by consuming plant secondary metabolites (Gherman et al. 2014, Baracchi et al. 2015). Finally, the spread of new plant species can modify pollinator–flower interaction networks through different plant traits, including floral resource quantity and quality, spatial and temporal availability, and accessibility (Ghazoul 2002, Nienhuis et al. 2009, Stout and Tiedeken 2017). The impacts of invasive plants can occur at the individual and population levels, including decreasing interspecific competition among pollinators (Fontaine et al. 2008) and pathogen transmission (Tylianakis et al. 2008). Moreover, these impacts are species-dependent as different plant species can impact pollinators in different ways and pollinators do not all respond uniformly to new plant species (Tiedeken et al. 2016, Stout and Tiedeken 2017). Invasive plants could therefore impact pollinators at individual (i.e., nutrition, health, and fitness) and population levels in terms of size, density, and growth rates, but also population genetic diversity and structure (e.g., heterozygosity; Lenda et al. 2013).

To evaluate positive, neutral, or negative impacts of a new floral resource on the health and genetic diversity of common bumble bees, we need additional field studies comparing invaded and uninvaded sites. Here, we investigated whether the spread of the alien species *Impatiens glandulifera* impacted a common bumble bee species in Belgium, *Bombus pascuorum*. We estimated the impact of this plant on bumble bee health by assessing the prevalence of common parasite species (i.e., *Apicystis bombi*, *Crithidia bombi*, *Nosema* species) in bumble bee populations at invaded and uninvaded sites during two consecutive years. Moreover, we used DNA sampling of workers to determine the population genetic diversity and infer the number of nests at each site. Our hypothesis is that even a common and generalist bumble bee species like *Bombus pascuorum* will display a higher parasite prevalence or a decrease in genetic diversity and

nest density, following the invasion of *Impatiens glandulifera*.

## Materials and Methods

### *Selected species*

*Bombus pascuorum* is a ubiquitous, generalist, and medium-tongued bumble bee species (Goulson et al. 2008), which is easy to identify in Belgium thanks to its typical ginger-colored thorax. Like most bumble bees, *B. pascuorum* is primitively eusocial with an annual life cycle, with queens emerging at the beginning of the spring and workers active until October (Goulson and Darvill 2004). It has a flexible diet (Goulson and Darvill 2004), which can include *Impatiens glandulifera* in Belgium, The Netherlands, and the United Kingdom (Kleijn and Raemakers 2008). This alien invasive plant, known as Himalayan balsam, is spreading rapidly across Europe and quickly integrating into pollination networks because of its highly rewarding flowers (Chittka and Schurkens 2001, Lopezaraiza-Mikel et al. 2007). Its flowering period starts at the beginning of July until the end of August. Many bee species have been recorded foraging on this plant in Europe, including bumble bees, honey bees, and solitary bees (Nienhuis et al. 2009).

Three taxa of potential bumble bee parasites were investigated: the intestinal trypanosomatid *Crithidia bombi*, the neogregarine *Apicystis bombi* which mainly infects the fat body, and the obligate intracellular microsporidian *Nosema* sp. (including *Nosema bombi, Nosema ceranae*, and *Nosema apis*; Meeus et al. 2011). *Crithidia bombi* is primarily transmitted among individuals from the same colony through contact with contaminated materials within the nest (e.g., feces), but parasite cells may also pass from infected members to healthy ones, and eventually to future queens (Imhoof and Schmid-Hempel 1999, Folly et al. 2017). Transmission may also occur via flowers (Durrer and Schmid-Hempel 1994) among individuals from the same species but also across bumble bee species (Ruiz-González et al. 2012) or vectored by non-susceptible honey bees (Ruiz-González and Brown 2006). Little is known about *Apicystis bombi* transmission or epidemiology (Meeus et al. 2011), but interspecific transmission has been detected between honey bees and bumble bees (Graystock et al. 2015). The principal transmission mode for *Nosema bombi* is horizontal and occurs when larvae feed on food contaminated by long-lived spores (Rutrecht and Brown 2008). We additionally screened for the presence of other *Nosema* species since spillover from *Apis* to *Bombus* has been described (e.g., *Nosema apis* and *N. ceranae*; Plischuk et al. 2009, Li et al. 2012, Graystock et al. 2013, 2014) with detrimental effects partly demonstrated (Graystock et al. 2013).

### *Study sites and sampling*

This study was conducted along various watercourses near Mons (Belgium) where *I. glandulifera* was patchily distributed. Ten sites of approximately 30 × 5 m were selected in six localities (Dour, Havré, La Louvière, Mons, Quévy, Tertre; Appendix S5: Table S1), including five sites where density of *I. glandulifera* did not significantly differ (85 ± 38 flowers/m², $F_{4,15}$ = 2.51, $P$ = 0.086) and five other sites where the plant was absent (Appendix S5: Table S1). As *B. pascuorum* workers have relatively short foraging ranges for bumble bees (maximum foraging range of 363 m; Wood et al. 2015), we considered sites at least 600 m apart to ensure that sampled workers from different sites came from different colonies (Schmid-Hempel 2001).

As bumble bees and honey bees share pathogens (Graystock et al. 2014), we conducted a survey of the beekeepers with the local association (CEIAM, *Centre d'étude et d'information apicole de Mons*) to avoid sites with potential high density of hives. It was impossible to locate all hives in the area, but we had confirmation of a regular density of hives around the sites (i.e., all apiaries included less than ten hives and maximum five beekeepers per locality, which are average values for Belgium; Chauzat et al. 2013). Moreover, we assessed the abundance of flower visitors to *I. glandulifera*, including *Apis mellifera*, at the different study sites (Appendix S1). In the ten sites, flower visitors were clearly dominated by *B. pascuorum* (64.5%), followed by *B. terrestris* (11%), *A. mellifera* (6.6%), and *B. lapidarius* (6%). Floral visitor communities (i.e., proportions of visitors recorded in the ten sites) significantly differed between the site types ($F_{1,9}$ = 5.64, $P$ = 0.014) with *Bombus pascuorum* being more abundant in invaded sites (IC = 0.64, $P$ = 0.013) and *Bombus lapidarius* in uninvaded sites (IC = 0.95,

$P = 0.020$; Appendix S5: Table S2; see Appendix S1 for statistical details). As no significant difference was detected for *A. mellifera* and *B. terrestris*, we can argue that any potential difference in parasite prevalence between the site types was likely not due to managed pollinators.

We collected foragers of *B. pascuorum* from all sites for both 2014 and 2015 at the peak flowering of *Impatiens glandulifera* (sampling on one single day in mid-July), for a total number of 536 specimens ($n = 22$–38 per site). We put individuals directly into 70% ethanol and stored them at −20°C. We used all specimens for genetic structure analyses and 12 randomly selected specimens from each site and each year for parasite detection. At each site, we recorded all plants in bloom as well as *Bombus* host plant species (i.e., plants visited by bumble bees; Appendix S5: Table S1). At invaded sites, all collected workers were foraging for pollen on *I. glandulifera,* the dominant species. At uninvaded sites, workers were caught on different plant species (Appendix S5: Table S1).

### *Floral resources and bumble bee population fitness*

To assess how the pollen of *I. glandulifera* was integrated into the diet of *B. pascuorum* at invaded sites, we performed palynological analysis of pollen loads from 101 workers (Appendix S2) that were randomly collected at the studied sites. Regarding the chemical composition of the floral resources of *I. glandulifera*, the composition of pollen and nectar has already been determined for some compounds including sugar and protein, but secondary metabolites have only been analyzed for the whole flower (Vieira et al. 2016, Roger et al. 2017). We analyzed the polyphenols from pollen and nectar of this alien invasive species (Appendix S3).

To check whether the local bumble bee population fitness was similar between invaded and uninvaded sites before the flowering period of *I. glandulifera,* we assessed wing size of foraging workers as a body size proxy measurement (Appendix S4). Based on previous studies, we made the assumption that body size may contribute to colony-level fitness. Indeed, worker size positively correlates with the numbers of egg cells and emerging workers produced (Cnaani and Hefetz 1994). Larger workers are also known to bring back more resources to the colony than smaller ones, and they are more resistant to starvation, fly at cooler temperatures, and could be less prone to predation (Goulson et al. 2002, Spaethe and Weidenmuller 2002, Couvillon and Dornhaus 2010, De Luca et al. 2013). Moreover, larger workers forage at longer distances, which is an advantage in fragmented landscape displaying unsuitable habitats and disconnection between nesting places and foraging places (Tscharntke and Brandl 2004, Greenleaf et al. 2007). Besides, body size can be affected by resource availability and quality in a landscape or other environmental stresses and landscape simplification can decrease adult size (Chown and Gaston 2010, Persson and Smith 2011, Renauld et al. 2016, Gérard et al. 2018). It can then be viewed as a valuable proxy of local bumble bee population fitness on both uninvaded and invaded sites and is related to the conditions before the flowering period of *I. glandulifera*. If resource availability or suitability was, for instance, lower in invaded sites than in uninvaded ones prior to the flowering of *I. glandulifera*, foraging workers fed as larvae before *I. glandulifera* flowering should be smaller (see Appendix S4 for statistical details).

### *Genetic structure and sibship reconstruction*

Total genomic DNA was extracted from the last third of the median leg using 5% Chelex 100 resin (Walsh et al. 1991). Genotypes of individuals were determined at ten statistically independent microsatellite loci (Estoup et al. 1995, 1996) divided into two multiplex sets (set M1: B10, B11, B96, B121, and B131; set M2: B100, B118, B124, B126, and B132). Polymerase chain reaction (PCR) amplifications were carried out in a 10 μL volume containing 1 μL of genomic DNA, 5 μL of 2× Qiagen Multiplex PCR Master Mix, 1 μL of 5× Q-solution, and 0.5 μL of 10× primer mix (2 μM of each primer). Amplifications were performed in a TProfessional thermocycler. The PCR conditions were as follows: An initial denaturing step of 15 min at 95°C was followed by 35 cycles of 30 s at 94°C, 90 s at 49°C, and 90 s at 72°C and terminated with a final extension step of 30 min at 72°C. Analysis of the PCR products was performed by capillary electrophoresis on an ABI 3100 automated DNA sequencer (Applied Biosystems, Foster City, California, USA). Data were

visualized and allele sizes were scored using GeneMapper software (version 4.0; Applied Biosystems). In total, all 536 sampled workers were successfully genotyped. The presence of null alleles, errors due to microsatellite stuttering, and large-allele dropout were checked using MicroChecker version 2.2.3 (Van Osterhout et al. 2006). Tests of deviations from Hardy–Weinberg equilibrium and linkage disequilibrium were conducted in Fstat 2.9.3 (Goudet 1995).

Genetic diversity parameters (i.e., the number of alleles, allelic richness, and expected and observed heterozygosities) and fixation indices were estimated using Fstat 2.9.3 and averaged across loci. Genetic differentiation among sites was calculated using $F_{ST}$ and pairwise exact tests of genetic differentiation with GenAlEx 6.3 (Peakall and Smouse 2012). Measures of gene flow (*Nm*) among sites were calculated from pairwise estimates of $F_{ST}$ (Wright 1949). Mean allelic richness, expected and observed heterozygosities, and genetic differentiation were compared between site types (i.e., invaded versus uninvaded sites) using a two-sided permutation test implemented in Fstat 2.9.3.

The computer program Colony version 2.0 (Jones and Wang 2010) was used to detect sister relationships among workers. Three runs with different random number seeds were conducted for each replicate. Each Colony run was a short run, with both sexes selected as monogamous (Schmid-Hempel and Schmid-Hempel 2000), without inbreeding, with the full likelihood method (Wang 2012) and medium precision, and assuming an error rate of 0.05 for both allelic dropout and genotyping error. Allele frequencies were set as unknown and were not updated. The best configuration (as indicated by the highest likelihood score) was used to determine the number of nests within a site. The complete database of genetic data is available from the Dryad Digital Repository: http://datadryad.org/review?doi=doi:10.5061/dryad.n8g87.

### *Parasite prevalence*

The abdomen of sampled workers was cut off with a sterile scalpel, rinsed with distilled water, and immediately put into a 1.5-mL reaction tube. Abdomens were crushed, and genomic DNA was extracted with the Invisorb® Spin Tissue Mini Kit (Stratec, Berlin, Germany). DNA extraction was performed according to the manufacturer's instructions. DNA extracts were stored at −20°C and used as a template for PCR.

Parasite diagnostics were conducted using Ready-To-Go PCR Beads (GE Healthcare, Amersham, Buckinghamshire, UK). For each PCR, negative controls (i.e., water instead of the DNA extract) were run together with DNA extracts. To detect *C. bombi* and *A. bombi* (i.e., presence/absence data), we amplified fragments of the small subunit 18S ribosomal DNA using the primers SEF/SER and NeoF/NeoR, respectively (see Appendix S5: Table S3 for the sequences; Meeus et al. 2010). Bumble bees' 18S rDNA was amplified with the primers ApidaeF/ApidaeR as positive control (see Appendix S5: Table S3 for the sequences; Meeus et al. 2010). Each reaction was performed in a total volume of 25 μL with 1 μL of genomic DNA. Multiplex PCR conditions were as follows: 2 min at 94°C, 35 amplification cycles of 30 s at 94°C, 30 s at 57°C, 45 s at 72°C, and a final elongation step of 3 min at 72°C. To confirm that amplicons belonged to hosts and parasites, some PCR products were randomly chosen and sent for Sanger sequencing (LGC Genomics, Berlin, Germany).

A second PCR was performed on the same DNA extracts to detect the presence of *Nosema* parasites. The universal forward primer UF was used with the three specific reverse primers Ra, Rb, and Rc amplifying *N. apis, N. bombi*, and *N. ceranae*, respectively (see Appendix S5: Table S3 for the sequences; Menail et al. 2016). PCR conditions were as follows: 2 min at 95°C, 35 amplification cycles of 30 s at 95°C, 30 s at 60°C, 1 min at 72°C, and a final elongation step of 2 min at 72°C. Electrophoreses were run on a 1% agarose gel containing 0.05 μL/mL of GelRed. This did not allow species differentiation. All *Nosema*-positive samples were PCR-amplified with general *Nosema* screening primers as described by Ravoet et al. (2013) and sent for Sanger sequencing (LGC Genomics, Berlin, Germany) to identify the species level. We report both *N. bombi* and *N. ceranae* up to species level as both species were reported in bumble bees. While the microsporidian *N. bombi* is described to cause real infection in bumble bees, the *N. ceranae* virulence in bumble bees remains unclear (Brown 2017). *N. apis* has not been reported in bumble bees. The resulting *Nosema* sp. group also contains sequences which do not match with

*N. apis* nor *N. ceranae*, indicating that the initially used multiplex primer set has cross-reactivity with other *Nosema* species. Species identification of these and whether they cause real infections are beyond the scope of this manuscript. The complete database of parasite prevalence is available from the Dryad Digital Repository: http://datadryad.org/review?doi=doi:10.5061/dryad.n8g87.

### *Statistical analyses*

To test for the effects of *Impatiens glandulifera* and parasite species (i.e., independent variables) on the prevalence of parasites in *Bombus pascuorum* (i.e., dependent variable), we performed a generalized linear mixed model (GLMM; lmer function, R package lmerTest; Kuznetsova et al. 2014) with the categorical variables Site type (two levels), Parasite species (five levels), and their interaction term as fixed-effect terms, as well as the categorical variables Year (two levels) and Site nested within Site type (five levels per site type) as random-effect terms. Parasite prevalence (which was a proportion variable) was analyzed using binomial model with the number of infected bees (successes) and the number of non-infected bees (failures) as a bivariate response. After checking for overdispersion, a type III ANOVA was performed to determine the effect of plant invasion and parasite species on the prevalence of parasites (ANOVA function, R package car; Fox and Weisberg 2011). When a significant difference was found, multiple pairwise comparison tests were performed using Tukey contrasts (glht function, R package multcomp; Hothorn et al. 2008). Besides, we tested whether the prevalence of the different parasites was correlated across sites using Pearson's r correlation coefficients on log-transformed data (rcorr function, R package Hmisc; Harrell 2015).

We used a second Gaussian GLMM to evaluate the effect of the year on the prevalence of parasites in *Bombus pascuorum.* We included the categorical variables Year (two levels), Parasite species (five levels), and their interaction term as fixed-effect terms, as well as the categorical variable Site (ten levels) as a random-effect term. The dependent variable (i.e., parasite prevalence expressed as proportions) was log-transformed to achieve normality of the residuals. Assumptions (i.e., normality of residuals and overdispersion) were checked, and the effects of fixed and random factors were assessed using the step function.

Finally, the impact of *I. glandulifera* on the parasite community (i.e., number of parasite species per specimen as dependent variable) was assessed using a third GLMM with a Poisson probability distribution for modeling count data (glmer function, R package lme4; Bates et al. 2015). We included the categorical variable Site type (two levels) as a fixed-effect term as well as the categorical variables Year (two levels) and Site nested within Site type (five levels per site type) as random-effect terms. After checking for overdispersion, a type III ANOVA was performed to determine the effect of plant invasion on bumble bee infection. All analyses were performed in R version 3.0.2 (R Development Core Team 2013).

## Results

### *Floral resources and local bumble bee population fitness*

Among the 101 pollen loads collected at invaded sites, a total of 54 loads (53.5%) contained pollen of *I. glandulifera* represented by more than 10% of the counted grains, a threshold which prevents biases due to contamination (Müller and Kuhlmann 2008). For twenty of these loads, the percentage of *Impatiens* pollen was over 50%, eight of them being pure loads (more than 95% of the counted grains). Whereas no compounds were detected in nectar, five polyphenols were identified and quantified in pollen, ampelopsin being the major one ($13.81 \pm 1.26$ mg/g; Appendix S5: Table S4).

The two-way nested ANOVA conducted on the wing size of foraging workers (i.e., body size proxy related to population fitness components) detected a significant effect of the site type ($F_{1,\ 334} = 36.72$, $P < 0.001$) but also a significant interaction between site type and sites (interaction term; $F_{8,\ 334} = 4.37$, $P < 0.001$). This interaction effect indicated that the difference in wing size of foraging workers between site types (main effect) depended on the site itself and could not be meaningful. Biological interpretation had then to rely on a careful analysis of the interaction effect: Multiple pairwise comparisons revealed that the uninvaded sites were not more similar to one another than to invaded sites (Appendix S5: Table S5). This indicated that the

local bumble bee population fitness was overall quite similar between invaded and uninvaded sites before the flowering of *I. glandulifera*, but was variable among sites.

### *Population genetic and worker sibship*

Populations of 2014 and 2015 were in Hardy–Weinberg equilibrium, and no linkage disequilibrium was detected (data not shown). No evidence of stuttering or large-allele dropout was detected. Null alleles seem to be present at loci B10, B118, and B121. These three loci showed similar values of heterozygosity than loci for which null alleles were not detected.

Across all the study sites, the number of alleles per locus varied from 7.25 to 10.13 and the allelic richness (*Ar*) from 6.95 to 9.58 (Table 1). Observed and expected heterozygosities ranged from 0.68 to 0.80 and from 0.71 to 0.77, respectively (Table 1). Mean $F_{IS}$ values over all sites were low and were not significantly different from zero, as indicated by their 95% CI (Table 1). Low mean $F_{ST}$ values indicate that there is little divergence among the populations (Table 1). Thirteen and 16 different private alleles have appeared in 2014 and 2015, respectively (these variations between sites and years may be due to small numbers of samples taken). Deduced from the $F_{ST}$ values, the levels of gene flow (*Nm*) were estimated at 7.90 (range, 5.82–10.33) and 9.03 (range, 5.35–11.5) for 2014 and 2015, respectively. There was no difference in genetic diversity

Table 1. Comparison of genetic parameters, nest size, and number of colonies among sites.

| | $A_r$ | $H_o$ | $H_S$ | $F$ | Nest size | Nbr colonies |
|---|---|---|---|---|---|---|
| 2014 | | | | | | |
| Invaded | | | | | | |
| Dour 1 ($n$ = 31) | 8.553 | 0.714 | 0.782 | 0.056 | 1.4 | 23 |
| Mons 1 ($n$ = 25) | 8.141 | 0.719 | 0.736 | 0.041 | 1.5 | 17 |
| Mons 2 ($n$ = 25) | 7.849 | 0.695 | 0.724 | 0.039 | 1.7 | 15 |
| Havré 1 ($n$ = 27) | 8.435 | 0.694 | 0.737 | 0.034 | 2.5 | 18 |
| Quévy ($n$ = 25) | 8.104 | 0.795 | 0.767 | 0.048 | 1.9 | 13 |
| Mean (SD) | 8.216 (0.280) | 0.723 (0.042) | 0.749 (0.024) | 0.044 (0.009) | 1.8 (0.4) | 17 (4) |
| Uninvaded | | | | | | |
| Dour 2 ($n$ = 22) | 7.215 | 0.766 | 0.770 | 0.054 | 1.8 | 12 |
| La Louvière ($n$ = 29) | 6.945 | 0.754 | 0.720 | 0.036 | 1.5 | 14 |
| Havré 2 ($n$ = 29) | 9.150 | 0.758 | 0.778 | 0.032 | 1.2 | 24 |
| Tertre 1 ($n$ = 30) | 8.469 | 0.787 | 0.775 | 0.035 | 1.6 | 19 |
| Tertre 2 ($n$ = 38) | 8.178 | 0.799 | 0.768 | 0.052 | 1.8 | 21 |
| Mean (SD) | 7.991 (0.909) | 0.773 (0.019) | 0.762 (0.024) | 0.042 (0.010) | 1.6 (0.2) | 18 (5) |
| Between-site-type comparison | $P$ = 0.633 | $P$ = 0.042* | $P$ = 0.462 | $P$ = 0.084 | $P$ = 0.528 | $P$ = 0.781 |
| 2015 | | | | | | |
| Invaded | | | | | | |
| Dour 1 ($n$ = 25) | 8.558 | 0.710 | 0.771 | 0.035 | 1.6 | 16 |
| Mons 1 ($n$ = 29) | 8.396 | 0.707 | 0.734 | 0.007 | 1.5 | 19 |
| Mons 2 ($n$ = 27) | 9.335 | 0.773 | 0.749 | −0.056 | 1.4 | 19 |
| Havré 1 ($n$ = 23) | 8.000 | 0.690 | 0.76 | 0.050 | 1.3 | 18 |
| Quévy ($n$ = 25) | 8.564 | 0.735 | 0.758 | −0.011 | 1.6 | 16 |
| Mean (SD) | 8.571 (0.485) | 0.723 (0.032) | 0.754 (0.014) | 0.005 (0.042) | 1.5 (0.1) | 18 (2) |
| Uninvaded | | | | | | |
| Dour 2 ($n$ = 25) | 9.041 | 0.740 | 0.760 | −0.027 | 1.4 | 18 |
| La Louvière ($n$ = 26) | 8.630 | 0.739 | 0.766 | −0.005 | 1.5 | 17 |
| Havré 2 ($n$ = 27) | 9.584 | 0.745 | 0.768 | −0.007 | 1.3 | 20 |
| Tertre 1 ($n$ = 25) | 8.468 | 0.735 | 0.745 | −0.029 | 1.4 | 18 |
| Tertre 2 ($n$ = 23) | 9.125 | 0.679 | 0.764 | 0.055 | 1.3 | 17 |
| Mean (SD) | 8.970 (0.440) | 0.728 (0.027) | 0.761 (0.009) | −0.003 (0.034) | 1.4 (0.1) | 18 (1) |
| Between-site-type comparison | $P$ = 0.226 | $P$ = 0.817 | $P$ = 0.402 | $P$ = 0.922 | $P$ = 0.196 | $P$ = 0.659 |

*Notes:* $A_r$: allelic richness; $H_o$: observed heterozygosity; $H_s$: gene diversity; $F$: fixation index. $P$-values are obtained based on 2000 permutations (*$P < 0.05$). Nbr colonies: number of colonies per site; Nest size: mean of the number of workers per nest within site.

parameters between uninvaded and invaded sites (Table 1, Fig. 1).

Twelve to 24 full-sibships per site were reconstructed by Colony from the genotypes of workers (Table 1). Worker sibships are of sizes 1–7 for a probability of inference of 0.17 to 1.00. 65% of sibships were reconstructed from only one worker, and for more 85% of full-sib families, configurations inferred by Colony exhibited high probabilities (>0.8) of including all full-sib individuals in a given family. Regardless of the effect of site, the number of nests varied from 12 to 24 with no significant differences between invaded and uninvaded sites (Table 1). No difference in the mean number of workers within sibships was observed between types of sites (i.e., with or without *I. glandulifera*; Mann–Whitney *U*-tests; 2014: $U = 4271$, $P = 0.179$; 2015: $U = 4080$, $P = 0.679$; Table 1).

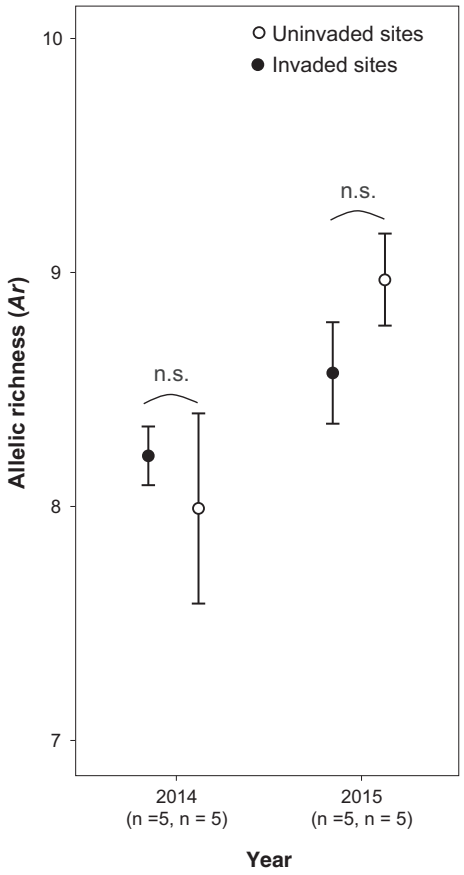


Fig. 1. Comparison of the allelic richness (*Ar*) in invaded and uninvaded sites for the two years of sample collections (2014 and 2015). Independently of the year, no significant difference was observed between site types (n.s., non-significant difference). Error bars represent standard error of the mean.

### *Parasite prevalence and parasite community*

Out of 240 workers, 112 were infected with *Apicystis bombi*, 50 with *Crithidia bombi*, 46 with *Nosema bombi*, five with *N. ceranae*-infected, and 10 with *Nosema* sp. (Appendix S5: Table S6). We found a significant difference in prevalence among the different parasite species (Wald $\chi^2 = 47.87$, $P < 0.001$). Overall, the prevalence of *A. bombi* was significantly higher than for all other parasites ($P < 0.05$; Fig. 2). Except for *N. bombi* ($P = 0.708$), the prevalence of *C. bombi* was significantly higher than for *Nosema* species ($P < 0.01$; Fig. 2). *Nosema* sp. prevalence did not significantly differ from *N. bombi* prevalence ($P = 0.100$; Fig. 2) and *N. ceranae* prevalence ($P = 0.534$; Fig. 2). The effect of year was significant for the prevalence of *A. bombi* ($P = 0.048$), *N. bombi* ($P = 0.026$), and *Nosema* sp. ($P = 0.036$), with there being lower prevalence of each parasite in 2015 than in 2014 (Table 2).

Half of specimens had just one parasite species (50%) while a few specimens were jointly infected with 2 or 3 parasites (7% and 3%, respectively). Using a Pearson correlation test, there was no correlation between the prevalence of the different parasites ($P > 0.05$) at site level regardless of study site, sampling year, and the occurrence of *I. glandulifera*.

### *Impact of invasive plant on parasite prevalence and parasite community*

At uninvaded sites, out of 120 workers, 69 were infected with *A. bombi*, 27 with *C. bombi*, 30 with *N. bombi*, four with *N. ceranae*, and five with *Nosema* sp. (Fig. 2; Appendix S5: Table S6). Only 21% of the specimens did not show any infection, while 50% had a single parasite species, 25% had two parasites, and 4% had three parasites. At invaded sites, out of 120 workers, 43 were infected with *A. bombi*, 23 with *C. bombi*, 16 with *N. bombi*, one with *N. ceranae*, and five with *Nosema* sp. (Fig. 2; Appendix S5: Table S6). Forty percent of the specimens were not infected with any of the investigated parasites. Regardless of parasite species, the proportion of infected bumble bee workers was significantly lower in sites invaded by

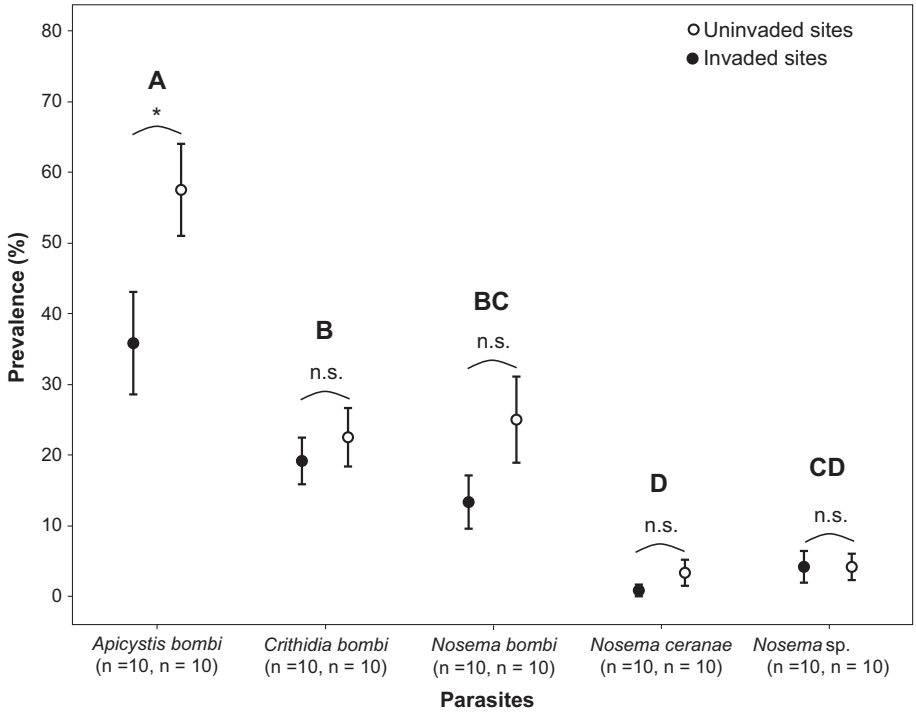


Fig. 2. Prevalence of *Apicystis bombi*, *Crithidia bombi*, *Nosema bombi*, *Nosema ceranae*, and *Nosema* sp. for the 2 yr of study for the *Impatiens glandulifera*-invaded sites and the uninvaded ones. Parasite prevalences differing significantly from each other, regardless of invasive plant occurrence, are marked with different letters. For each parasite, comparisons between invaded and uninvaded sites were also performed (n.s., non-significant difference; $^{**}P \leq 0.01$). Error bars represent standard error of the mean.

Table 2. *Bombus pascuorum* parasite prevalence according to the year (n.s., non-significant difference; $^{*}P < 0.05$).

| | 2014 | Significance | 2015 |
|---|---|---|---|
| *Apicystis bombi* | 55.82 ± 26.95% | * | 37.49 ± 17.25% |
| *Crithidia bombi* | 22.49 ± 11.83% | n.s. | 19.16 ± 11.83% |
| *Nosema bombi* | 26.66 ± 19.57% | * | 11.66 ± 8.97% |
| *Nosema ceranae* | 2.50 ± 5.63% | n.s. | 1.66 ± 3.50% |
| *Nosema* sp. | 7.50 ± 7.31% | * | 0.83 ± 2.62% |

*Impatiens glandulifera* (Wald $\chi^2 = 11.45$, $P < 0.001$). However, separate analyses performed on each parasite species revealed that this difference between invaded and uninvaded sites was more or less pronounced according to the parasite species (Fig. 2; Appendix S5: Table S6). Whereas *C. bombi* (Wald $\chi^2 = 0.40$, $P = 0.525$), *N. bombi* (Wald $\chi^2 = 3.30$, $P = 0.069$), *N. ceranae* (Wald $\chi^2 = 1.04$, $P = 0.308$), and *Nosema* sp. (Wald $\chi^2 = 0$, $P = 1$) prevalence did not differ between site types, the proportion of *A. bombi*-infected workers was significantly lower in invaded sites than in uninvaded ones (Wald $\chi^2 = 4.45$, $P = 0.035$; Fig. 2; Appendix S5: Table S6).

Regarding the parasite community, the presence of *I. glandulifera* was associated with a significant decrease in the amount of co-infecting parasite species per bumble bee (Wald $\chi^2 = 9.79$, $P = 0.002$), with 0.73 ± 0.73 parasites for bumble bees in invaded sites and 1.13 ± 0.78 in uninvaded ones.

## Discussion

### *Genetic structure and nest densities*

We detected an average of 18 nests per site, with this total unaffected by the presence of the invasive plant *I. glandulifera*. It must be noted that

the number of detected nests was probably underestimated (Darvill et al. 2004), but since sampling effort was similar between invaded and uninvaded sites, data comparisons on genetic diversity (i.e., number of alleles and heterozygosities) remain reliable. Interestingly, the most available resource (pollen and nectar) in the invaded sites during our sampling period was nearly exclusively *I. glandulifera* (see Appendix S5: Table S1), while nutritive sources at the uninvaded sites were mostly Fabaceae species (*Trifolium pratense, T. repens, Lotus corniculatus, Vicia cracca*; see Appendix S5: Table S1). These results seem to confirm that *I. glandulifera*-invaded areas can support populations of *B. pascuorum* which are at least similar to those of uninvaded Fabaceae-rich sites (Appendix S5: Table S2).

We did not detect any difference in bumble bee genetic structure between invaded and uninvaded sites. This confirms that bumble bee populations exhibit low levels of genetic structuring at a fine spatial scale (Dreier et al. 2014) and that geographical distance is a poor predictor of genetic divergence among populations (Widmer and Schmid-Hempel 1999). Our results reveal that new queens of *B. pascuorum,* able to disperse by at least 3 km (Lepais et al. 2010) near the flowering site, do not preferentially establish in *I. glandulifera*-invaded areas. These results are consistent with inferences drawn from studies of population structuring in common bumble bee species and suggest that regular gene flow over several kilometers due to queen dispersal is likely to be sufficient to maintain genetic cohesion of common species over large spatial scales (Lepais et al. 2010). In the same way, except for the observed heterozygosity estimated for 2014, the populations of *B. pascuorum* collected at invaded sites did not have significantly different genetic diversity (allelic richness, gene diversity) than the populations from uninvaded sites.

### *Parasite prevalence and impact of* Impatiens glandulifera

Regardless of the occurrence of the invasive plant and the sampling year, *Apicystis bombi* prevalence was significantly higher than the prevalence of *Crithidia bombi* and *Nosema* species for foragers of *Bombus pascuorum* at all sites (in the region of Mons, West of Belgium). Given that the bell-shape morphology of the flower of *Impatiens glandulifera* is likely to maximize the contact between the visitor's body and flowers, parasite transmission via flowers, and then parasite prevalence, were expected to be higher at invaded sites (Graystock et al. 2015). In spite of an expectation that parasite prevalence would be similar in populations with similar genetic diversity (Whitehorn et al. 2010), there was a detectable difference in parasite prevalence between invaded and uninvaded sites.

This unexpected negative effect of *I. glandulifera* on parasite prevalence for *B. pascuorum* could be explained by three alternative scenarios based on the characteristics of the plant: (1) its impact on pollinator network (e.g., lower abundance of parasites-sharing bees; Thijs et al. 2012), (2) its resource availability and suitability (i.e., host nutrition status directly impacts population of the parasite; Logan et al. 2005), and/or (3) the presence of secondary metabolites in its floral resources (i.e., medicinal effects on bumble bee health; Richardson et al. 2015).

The presence of *Impatiens glandulifera* can have a strong impact on plant–pollinator networks by increasing or decreasing the relative abundance of pollinator species (Thijs et al. 2012). Different studies showed that bumble bee abundance (including *Bombus pascuorum*) is positively correlated with the invasion of *I. glandulifera* while many bee species are negatively impacted (Lopezaraiza-Mikel et al. 2007, Nienhuis et al. 2009). The lower prevalence of bees infected with parasites observed in our study could therefore be explained by a lower abundance of foraging vectors at the invaded sites. Although the occurrence of managed pollinators did not differ between the site types (i.e., *Apis mellifera* and *Bombus terrestris;* Appendix S5: Table S2), unmanaged bumble bee species could have also played an important part in disease prevalence. Actually, cross-species transmission experiments have shown that *B. lapidarius* is likely to act as an important vector of *Crithidia bombi,* a true multihost parasite across *Bombus* spp. (Ruiz-González et al. 2012). The higher density of *B. lapidarius* in the uninvaded sites compared to the invaded ones could then play a role on parasite prevalence. However, as we detected no significant difference in the prevalence of *C. bombi* between sites uninvaded or invaded by *I. glandulifera*, it is unlikely that the lower occurrence of *B. lapidarius*

fully explains the lower parasite prevalence in the invaded sites. Nevertheless, caution has to be paid since our data did not allow for plainly ruling out this hypothesis.

Although *I. glandulifera* was the only major food source at invaded sites, it is a highly rewarding plant owing to its massive flower display (Showler 1989, Bjerknes et al. 2007). Moreover, *I. glandulifera* could be an even more important foraging source for *Bombus pascuorum* as food competition is reduced by the lower occurrence of honey bees on this plant species (Lopezaraiza-Mikel et al. 2007, Nienhuis et al. 2009). Based on one of our previous studies, *I. glandulifera* pollen shows a relatively low concentration of total amino acids (~25% vs. 37–44%) and a different amino acid profile (i.e., a lower relative abundance of proline, glycine, and threonine) compared to the dominant host plants recorded in uninvaded sites like *E. vulgare*, *L. corniculatus*, *T. repens*, or *T. pratense* (Roger et al. 2017). A previous research indicates that the food environment can influence the parasite loads in the bumble bee–*Crithidia* system with the pollen-starved bees displaying lower *Crithidia* counts (i.e., lack of nutrition disrupting the parasite development; Logan et al. 2005). In the same way, Sadd (2011) found that *Crithidia* counts were low in bees fed with a low-quality diet (i.e., sugar concentration in nectar) but that counts were higher in bees fed with a medium-quality diet compared to those fed with a high-quality diet. Thus, for *Crithidia* and perhaps other parasites as well, we might expect that resources of lower but not terrible quality such as *I. glandulifera* might be associated with a greater incidence of parasite infections compared to higher quality diet offered by native plants. However, such pattern was not observed herein. Another possibility is that a poorer diet for the bee host is also a poorer diet for the parasite, in which case parasite prevalence might be lower where *I. glandulifera* is dominant. However, the fact that only particular parasite species are impacted by the presence of the plant is still puzzling, and it is unlikely that suitability of *I. glandulifera* resources affected the local bumble bee population fitness in such a way that it reduced parasite prevalence. Nevertheless, once again, caution has to be paid since our data did not allow for unambiguously ruling out this hypothesis. Our understanding of how diet and nutrients interact with bee disease is still lacking, and patterns may differ depending on the parasite and its biology (e.g., intracellular versus gut parasite).

Although we cannot provide conclusive evidence for the third hypothesis, the specific medicative effect of ampelopsin remains a very strong hypothesis for explaining the lower prevalence of *Apicystis bombi* in bumble bee foragers from invaded sites. Ampelopsin, also known as dihydromyricetin, is a dihydroflavonol that occurs in the leaves of some plant species, including *Hovenia dulcis* that has been used in traditional Japanese, Chinese, and Korean medicines to treat parasitic infection (Hyun et al. 2010). It has been shown that occurrence of such secondary metabolites in floral resources (i.e., nectar or pollen) can reduce pathogen activity in bees (Cory and Hoover 2006, Richardson et al. 2015). Although generalist bee species have only poor acuity for the detection of nectar toxins (Tiedeken et al. 2014), a series of toxicological, microbiological, and behavioral experiments have shown that bumble bees infected with *C. bombi* preferred sugar syrup with nicotine to sugar solutions without this substance (Baracchi et al. 2015). Despite relatively weak effect of this compound on the parasite (i.e., infection delayed but not cleared), such behavior could provide subtle benefits for the host fitness and raises the question about self-medication in bumble bees (Baracchi et al. 2015). In this study, the multidirectional biological activity of *I. glandulifera* (especially antimicrobial properties due to flavonoids occurrence in pollen) coupled with the active foraging of bumble bee workers on the invasive plant could explain the lower *Apicystis bombi* prevalence in invaded sites (Appendix S5: Table S4). The growth-inhibiting effect of flavonoids has already been demonstrated for *Plasmodium falciparum,* belonging to the same phylum as *A. bombi* (i.e., Apicomplexa; Slavic et al. 2009). Although the presence of secondary metabolites is a very strong hypothesis for explaining the lower prevalence of a subset of parasite species detected in *Bombus pascuorum* population from invaded sites, additional experimental assays (e.g., test on the biological activities of the secondary metabolites) are needed to fully validate this hypothesis and to understand the associated mechanisms.

### *Bumble bee conservation*

We confirmed in this study that parasite prevalence is context-dependent for bumble bees (Brown et al. 2003). We provided the first evidence that an alien invasive plant could benefit some bee species by decreasing their pathogen loads. Even if our study is limited in time, space, and diversity, we show that the composition of the plant community may significantly influence the pathogen community of their pollinators. This is in line with previous research showing that variation among plant species, through their influence on pathogen transmission, may shape bee disease dynamics (Adler et al. 2018). In addition, among floral traits, the chemical composition of floral resources and especially secondary compounds has been shown to impact interactions between pollinators and their parasites by reducing parasite transmission (Richardson et al. 2015) and parasite load (Manson et al. 2010, Spear et al. 2016, Giacomini et al. 2018, LoCascio et al. 2019). Such potential for floral resources to provide natural resistance to pathogens has been partly studied in bumble bees (Manson et al. 2010, Giacomini et al. 2018, LoCascio et al. 2019) and honey bees (Giacomini et al. 2018) but also in some solitary bee species (Spear et al. 2016). Understanding how plant species affect parasite prevalence is important for the selection of plant species for mitigating strategy (e.g., composition of floral strips to support pollinators and optimize pollinator health) (Vaudo et al. 2015, Adler et al. 2018). As we also underlined that the presence of *Impatiens glandulifera* has no impact on the population of a common and polylectic native bumble bee, it seems that some alien exotic species could be considered a positive alternative resource in poor habitat where natural native resources are missing before plant invasion. Additional quantitative and qualitative empirical data, including plant traits (e.g., pollen proteins, secondary metabolites) and pollination networks, are still needed (e.g., IPBES 2016, Carvell et al. 2017), as well as new experimental studies to evaluate the potential efficiency of the selected plant for bee conservation.

## Acknowledgments

We thank E. Curtis, M. Dehon, and B. Contino for helping in fieldwork, BOMB Lab and Agrozoology Lab for technical help with genetic analyses, D. Evrard for technical support, and T.J. Wood for proofreading and English improvement. This work was partly supported by the FRFC project 2.4.613.12 (F.R.S.-FNRS—Fonds National de la Recherche Scientifique) and the Belspo project Belbees (BR/132/A1). NR and LT are PhD students of the FRIA. MV was F.R.S.-FNRS postdoctoral researcher (grant fellowship "Chargé de recherches"). DM and MV conceived the ideas and designed methodology; NR, GG, RM, DP, SG, NP, and LT collected the data; MV, NR, GG, MG, RM, SG, GS, IM, and DF analyzed the data; and MV, DM, NR, and RM led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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## Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2804/full