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ORIGINAL PAPER

# Examining the "evolution of increased competitive ability" hypothesis in response to parasites and pathogens in the invasive paper wasp *Polistes dominula*

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**Abstract** Successful invaders often become established in new ranges by outcompeting native species. The "evolution of increased competitive ability" hypothesis predicts that invasive species are subjected to less predation and parasitization than sympatric native species, and thus can allocate resources from defence and immunity to growth and fecundity, thereby achieving higher fitness. In this study, we examined whether American invasive *Polistes dominula* paper wasps have reduced immunocompetence. To explore this scenario, we tested their susceptibility towards parasites and pathogens at both the individual (immune defence) and colony levels, i.e. hygienic behaviour (removal of diseased individuals by nestmates). First, we examined the response to the specific coevolved parasite *Xenos vesparum* (lost after invasion) in terms of individual host susceptibility and hygienic behaviour. Second, we explored the response against general pathogens by quantifying the bacterial clearance in individual wasps after a challenge with *Escherichia coli* and hygienic behaviour after a challenge with the fungus *Beauveria bassiana*. Our results show that American invasive *P. dominula* have a higher response against *X. vesparum* at the colony level, but at the individual level their susceptibility is not significantly different from conspecifics of the native range. On the other hand, invasive *P. dominula* display lower response after a challenge with general pathogens at both the individual and colony levels. While supporting the hypothesis of a reduction of immunocompetence towards general pathogens in invasive species, these findings also suggest that the response against coevolved parasites might follow different evolutionary pathways which are not always easily predictable.

**Keywords** Bacterial clearance · Fungal infection · Hygienic behaviour · Invasion biology · Social insects · Strepsiptera

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## Introduction

Invasive species, i.e. non-native plants, animals and microbes, introduced to a new region and successfully established pose considerable ecological and economic risks (Sax et al. 2007; Paini et al. 2010). With increasing world trade, there has been a concomitant increase in the introduction of non-native species to new environments (Levine and D'Antonio 2003; Wilson et al. 2009). Invasive species can vector diseases, serve as agricultural pests and significantly reduce indigenous biodiversity. While many factors can play a role in the successful establishment of an invasive species, there has been a great deal of focus on the effects of interactions with predators, parasites and pathogens (Prenter et al. 2004). Two complementary models incorporating the effects of host–parasite interactions have been developed to explain the ability of introduced species to become invasive in the new habitats. The "enemy release hypothesis" (ERH) predicts that invasive species leave their predators, parasites and pathogens behind during the process of invasion, due to population bottlenecks or the lack of appropriate intermediate hosts in new environments (Keane and Crawley 2002; Torchin et al. 2003; Liu and Stiling 2006). According to the "evolution of increased competitive ability" (EICA) hypothesis, this reduction in natural enemies could result in selection for invasive populations that invest less in defence mechanisms (including immunocompetence) and shift resources to improving growth and fecundity, thereby achieving a competitive advantage over native species (Blossey and Notzold 1995; Lee and Klasing 2004). Despite their broad applicability, these theories have been examined mainly in plants and only in few animal models

(reviewed in Torchin and Mitchell 2004). Furthermore, these theories have not been examined in the context of coevolved host–parasite interactions or in social systems (Ugelvig and Cremer 2012), where behavioural adaptations may serve as immune defences.

Here, we examine the predictions of the EICA hypothesis in invasive *Polistes dominula* Christ paper wasps (Fig. 1). *P. dominula* is primitively eusocial and is organized in small annual colonies with one reproductive queen, non-reproductive cofoundresses and workers (Pardi 1948; Reeve 1991; Turillazzi and West-Eberhard 1996). These wasps have successfully invaded in North America, South Africa and Australia from the Mediterranean basin where they originated (Cervo et al. 2000). The introduction to North America likely took place several times over the last 30 years and *P. dominula* has rapidly spread over the continental USA (Liebert et al. 2006). In the USA, *P. dominula* is sympatric with other congeneric native paper wasps, and is outcompeting at least one native American paper wasp in Michigan, *Polistes fuscatus* (Gamboa et al. 2002; Gamboa et al. 2004). Multiple hypotheses have been formulated to explain the successful and rapid invasion of *P. dominula* in North America (Liebert et al. 2006) but the ERH and EICA have not been explicitly tested. However, there are signs that *P. dominula* has developed higher levels of colony productivity after invasion: for example, average colony size in Italy (native range) was 160 cells per nest in single-foundress colonies and 230 cells per nest in polygynous colonies (Queller et al. 2000), while in Michigan (invasive range) it was 200 and 300 cells per nest, respectively (Gamboa et al. 2002; Gamboa et al. 2004). Furthermore, a previous study by Wilson-Rich and Starks (2010) found that cellular and humoral immune responses—i.e. encapsulation and phenoloxidase activity—were significantly higher in *P. fuscatus* than *P. dominula* in Massachusetts, and that *P. fuscatus* displayed also higher grooming behaviour. However, this study did not compare invasive populations of *P. dominula* with populations from this species' native range.

**Fig. 1** American invasive *P. dominula* collected in State College, PA. *Left*: two foundresses on the nest. *Right*: late larvae (fourth and fifth instars) and pupae (*capped cells*) within a paper wasp nest. *Scale bars*= 1 cm. *Photos by Fabio Manfredini*

As predicted by the scenario described by the ERH, the parasites and parasitoids that commonly affect *P. dominula* colonies in the native range are missing in the USA (Cervo et al. 2000): among these, the highly specific and coevolved Strepsipteran *Xenos vesparum* Rossi. Strepsiptera are parasitic insects that infect hosts from seven orders of insects, usually following species-specific associations (Kathirithamby 2009) and they are widespread at a global scale, including North America where they infect several paper wasp species (Kathirithamby and Taylor 2005). Two studies, one in New York State and the other one in Michigan, showed that *P. dominula* colonies were not affected by *X. vesparum* while colonies of the sympatric *P. fuscatus* were often heavily affected by their own specific Strepsipteran *Xenos peckii* (Pickett and Wenzel 2000; Gamboa et al. 2004). This seems to be the case in Pennsylvania as well, where about 45 % of *P. fuscatus* colonies collected over the course of 2 years were parasitized by Strepsiptera while none of *P. dominula* were affected (personal observation). *X. vesparum* coexists with *P. dominula* in the host's native range, where the parasite prevalence among wasp colonies can be high: 58 % of the nests and about one third of the brood were parasitized in Tuscany (Italy) according to Hughes et al. (2003). The host–parasite association starts when the infective stage of the parasite (first instar larva, the so-called triungulin) penetrates the wasp larva in its nest cell. Thereafter, the parasite develops within the wasp's hemocoel in perfect synchronization with the wasp's developmental maturation, until the wasp reaches adulthood (Manfredini et al. 2007). Unlike healthy workers, parasitized wasps do not contribute to the growth and welfare of the colony but instead abandon the nest early in their adult life and aggregate in groups where mating of the parasites may occur (Hughes et al. 2004; Beani et al. 2005). Wasps with female parasites overwinter in large aggregations with healthy future queens (Beani et al. 2011). In the spring, parasitized wasps function as active vectors of the parasite: they start releasing triungulins on flowers or directly on wasp nests (Hughes et al. 2003; Beani and Massolo 2007) where they will randomly infect larval hosts (Manfredini et al. 2010c). Less is known about the host–parasite interaction at the physiological and molecular levels. *X. vesparum* does not kill its host, nor does it compromise nutrient uptake of the adult host (Giusti et al. 2007) or immunocompetence towards other pathogens (Manfredini et al. 2010a, b). There is apparently no cost of parasitism for larval wasps in terms of mortality and weight loss (Hughes and Kathirithamby 2005). However, *X. vesparum* induces permanent sterility in host female wasps (Strambi and Strambi 1973; Strambi et al. 1982) and alters their morphology, behaviour and fate (Beani 2006; Beani et al. 2011): this suggests that parasitization might have remarkable costs for the host at the colony level, though the fitness of infected and uninfected colonies has yet to be characterized.

Here, in the framework of the EICA hypothesis, we examine individual and colony-level responses to *X. vesparum* parasitization and immune challenge with bacteria and fungi. We examined the responses to *X. vesparum* in order to determine how immune responses towards a coevolved parasite that is widespread in the native range but absent in the invasive range evolve, while infections with general pathogenic bacteria and fungi allowed us to examine the differences in core, conserved innate immune pathways between the two populations (for more details on this approach see Govind 2008; Boughton et al. 2011). We tested these responses in American invasive *P. dominula* (from Pennsylvania, USdom), and in *P. dominula* from their native range (from Italy, ITdom). At the level of the individual, wasps can attack parasites and pathogens through cellular and humoral immune pathways. At the level of the colony, social insects can adopt behavioural changes, including hygienic behaviour (Cremer et al. 2007; Wilson-Rich et al. 2009); however, hygienic behaviour in terms of removal of larvae from their cell by adult nestmates has not previously been demonstrated for a social wasp. According to the EICA hypothesis, USdom should have lost the ability to mount a strong defense response to *Xenos* parasites and other pathogens, both at the level of the individual and the colony, if maintaining high levels of immunocompetence is costly. Therefore, we predict higher levels of parasitization and higher pathogen loads in USdom than ITdom following laboratory infections.

## Materials and methods

### Insect collection and maintenance

Mature colonies of the European invasive paper wasp *P. dominula* Christ (USdom) were collected in the surrounding areas of State College, PA, between June and August in 2010 and 2011. Native *P. dominula* (ITdom) and *X. vesparum* Rossi were collected in Italy in two localities, Trespiano and Impruneta (Florence, Tuscany) where a high prevalence of the parasite has been reported since 2003 (Hughes et al. 2003). Since these locations are only ~20 km apart, these wasps are likely from the same breeding population and not genetically distinct. Wasp colonies were sampled at the beginning of June, while overwintered wasps harbouring one or two females of the parasite were collected in March under roof tiles in the same locations as above (12 wasps in 2010 and 16 in 2011). Both colonies and parasitized wasps were shipped from Italy to USA (APHIS-USDA permit number P526P-10-02052). All colonies were placed in 40×20×20-cm Plexiglas cages at room temperature and provided ad libitum with water, powdered sugar and wax moth larvae as a food source. All wasps were housed in the Penn State Department of Entomology Quarantine Facility (12 L/12D, 25±2 °C and 50 % humidity). In total, we used 37 colonies of USdom and 20 of ITdom in 2010, and 25 and 22, respectively, in 2011 (see Table 1 for more details). While testing the response to *X. vesparum*, some colonies were used at multiple timepoints due to the limitations associated with sample size in ITdom: we allowed these colonies to recover from the previous manipulation (2 weeks at least) before infecting them again and we treated some of the USdom colonies in the same way for consistency. Before starting the experiments, colonies were observed daily for a minimum of 2 weeks to monitor for the emergence of parasites and parasitoids naturally occurring in the field. Diseased colonies were excluded from our assays. In 2011, we excluded two USdom and four ITdom colonies and in 2010 only one USdom colony.

### Individual and colony-level responses to the parasite *X. vesparum*

For the individual-level response, infected and control larvae were incubated outside of the nest. Wasp larvae (third and fourth instars collected during the colony cycle in which workers, not reproductives, are produced) were gently removed from their nest cell with forceps and placed inside plastic tubes where the main opening was closed by means of a wet cotton ball, while a small secondary opening was created on the opposite side, where the larva was located. From this tiny opening, 5–10 triungulins (for harvesting protocol see Manfredini et al. 2007) were introduced inside the tube using a thin needle and they were placed directly on the larval body; other samples from the same colonies were reared in analogous conditions but not infected to be used as controls. Specimens (see Table 1 for sampling size) were housed in an incubator for 3 days at 30 °C and 50 % humidity and checked daily for mortality. After 3 days, wasp larvae were dissected under a microscope and examined for (1) the number of triungulins that managed to enter the host and (2) the number of parasites that were able to undergo the first moult. Based on our previous work, in standard lab conditions triungulins need approximately 24 h to cross host cuticle and epidermis and 2 days to perform the first moult, therefore 3 days at the above conditions is a reasonable time window for both processes to take place. Empty exuvia of the triungulins where counted as successfully moulted parasites (this procedure is easier than detecting recently moulted second instar larvae, see Manfredini et al. 2007) while non-moulted or encapsulated triungulins were considered to be unsuccessfully moulted parasites.

To measure colony-level responses, we examined hygienic behaviour (the percentage of infected wasp larvae removed from their nest cell) by adult nestmates. We placed five to ten triungulins directly on the cuticle of each larval

**Table 1** Timeline and sampling size for the performed assays

| Challenge | Year | Treated | | Untreated, handled control | | Saline-injected control | |
|---|---|---|---|---|---|---|---|
| | | USdom | ITdom | USdom | ITdom | USdom | ITdom |
| *Xenos*—individual | 2011 | 33[a] (9)[b] | 25 (11) | 9 (9) | 9 (9) | | |
| *Xenos*—colony | 2010 | 231 (13) | 220 (14) | 83 (3) | 21 (5) | | |
| Bacteria—immatures | 2010 | 25 (7) | 26 (5) | 5 (3) | 5 (4) | 4 (2) | 6 (5) |
| Bacteria—adults | 2011 | 33 (5) | 32 (7) | 6 (5) | 7 (7) | 6 (5) | 6 (2) |
| Fungi—colony | 2010 | 65 (6) | 44 (6) | 25 (6) | 16 (6) | | |

[a]Total number of specimens
[b]Number of colonies

wasp, and infected all the larvae (third, fourth and fifth instars, worker phase) present in the nest. The cell walls of the target larvae were paint marked so removal could be tracked. We scored the hygienic behaviour in controls (non-infected larvae) and infected wasps. Since an excessive disturbance inflicted by detailed inspections of the brood cells altered the behaviour of adult nestmates (personal observation) we used different sets of colonies for each timepoint. We also used a different set of colonies for controls, since the triungulins are quite mobile and can easily move between larvae in a nest (personal observation). Hygienic behaviour in control colonies was scored after 3 days only, while infected colonies were scored after 24 h, 3 days and 1 week. Reduced observations in the control colonies and reduced numbers of replicates in the 24-h treatment timepoints were necessary due to the limited numbers of colonies available. Total sampling size is reported in Table 1: in particular, for the 24-h infections, four USdom (44 larvae) and four ITdom (43 larvae) colonies were used; for the 3-day infections, seven USdom (85 larvae) and eight ITdom (80 larvae) colonies were used; for 1 week infections, ten USdom (102 larvae) and ten ITdom (97 larvae) colonies were used.

## Individual-level response to the pathogen *Escherichia coli*

To test for the hypothesized reduction of individual immunocompetence in USdom, we injected wasps from both populations with the gram-negative bacterium *E. coli*. *E. coli* has been used to characterize and quantify immune responses in a wide variety of insects (Hillyer et al. 2004; Lavine et al. 2005; Yang and Cox-Foster 2005; Haine et al. 2008; Kaneko et al. 2008), including *P. dominula* (Manfredini et al. 2010a, b). This is an excellent immunostumulant to use because it activates the immune deficiency (IMD) pathway, which is one of four conserved immune response pathways in insects (reviewed in Hoffmann 2003 and in Hultmark 2003). Since developmental stage may affect immune function (Evans et al. 2006; Wilson-Rich et al. 2008; Laughton et al. 2011), we performed the same experiment with immatures (fourth and fifth instar, worker phase) and adults (putative workers), see Table 1. We used live *E. coli* of the strain NRRL B-2422, mutant, streptomycin resistant. Bacterial cultures were grown overnight at 37 °C in Luria-Bertani (LB) Broth containing streptomycin at a concentration of 50 μg/mL. After centrifugation, bacteria were washed twice, resuspended in 1× phosphate-buffered saline (PBS) and diluted to the desired concentration with PBS. The approximate amount of bacterial cells in the solution was determined using a Neubauer Hemocytometer and confirmed by plating the bacterial solution on LB agar and counting the colony forming units (CFU) that grew overnight at 37 °C.

Larval wasps were challenged outside the nest. They were gently removed from their nest cell with forceps and kept on ice. One microlitre of sterile PBS containing $1\times10^4$ bacterial cells was injected with a microsyringe after cleaning the injection site with 90 % ethanol. Thereafter, specimens were placed in sterile plastic tubes and incubated for 24 h at 28 °C and 50 % humidity. For adult wasps, we injected 2 μl of a bacterial solution (total $2\times10^5$ cells) since, based on our preliminary tests, they had higher bacterial clearance. The microsyringe was inserted ventrally between fourth and fifth abdominal sternites and treated individuals were housed in sterile plastic boxes for 24 h at room temperature and provided with water and powdered sugar. Six USdom died before the end of the experiment, probably due to handling or infection: since it would have been impossible to quantify the antibacterial activity of these individuals for the total duration of the experiment, they were not included in the analysis. For each group, we also included a set of PBS-injected wasps and non-injected controls. Abdomens from adult wasps and whole larvae were surface sterilized with 75 % ethanol, then washed in sterile distilled water and finally placed in sterile plastic tubes containing ten Zirconia beads and 200 μl sterile PBS. Samples were homogenized using a Fast-Prep machine (MP Biomedicals, Santa Ana, CA) set at 45 s and speed 6 for 1 cycle. We serially diluted 10 μl of the homogenate and plated 100 μl of the 1× and 3× dilutions on a Petri Dish containing LB agar and streptomycin (50 μg/mL). Plates were incubated at 37 °C for 24 h and the number of colony-forming units was recorded from each plate.

### Colony-level response to the pathogen *Beauveria bassiana*

Immune responses at the colony level were quantified by monitoring the hygienic behaviour of adult nestmates, i.e. the percentage of larvae removed after a challenge with *B. bassiana*. *B. bassiana* is a common soil-borne entomopathogenic fungus, infecting a broad range of insects. Analogously to *E. coli*, this pathogen has been extensively used to test the innate immune response in insects (Kraaijeveld and Godfray 2008; Toledo et al. 2010; Kikankie et al. 2010; Migiro et al. 2010) since it triggers another conserved immune pathway, the Toll-like Receptors (TLRs) pathway (Broderick et al. 2009). This fungus was used rather than *E. coli* for the colony level behavioural responses because larvae could be infected without wounding, and wounding likely would have resulted in hygienic behaviour by adults. Wasp colonies were briefly cooled at 4 °C and adults were temporarily removed from the nest. Wasp larvae were infected without being removed from their nest cell; after challenge, the original number of adult nestmates was placed back into the colony (on average, nine for USdom and ten for ITdom) and the colony was reared in standard lab conditions (see above). We used a dose of $1 \times 10^5$ spores of the fungus per larva: this dose was chosen based on preliminary trials. The fungal solution was obtained by resuspending fungal spores in a 0.05 % Tween solution to the desired concentration as determined with the hemocytometer. Ten microlitres of the final solution were applied directly on the larval cuticle using a micro-pipette (third, fourth and fifth instars, worker phase). A set of larvae from the same nest were kept as controls and treated with 10 μl Tween solution alone. We screened for the presence/absence of the targeted specimens at 2 and 7 days (see Table 1 for sampling size).

### Statistical analysis

Normality was tested with the Shapiro–Wilcoxon test and equality of variances was assessed with Levene's test. Non-parametric Kruskal–Wallis test was used to analyze the response to *Xenos* parasites at the individual level. We pooled wasp larvae from the same colony and we compared the ratios of the parasites that were able to enter the host and perform the first moult in USdom and ITdom. For the same assay, significant differences in wasp survival after *Xenos* infection were assessed using a Fisher's exact test. Individual-level response to *E. coli* was tested after transforming bacterial counts by cube root transformation. We used a generalized linear model (GLM) with the colony of origin as a nested factor. Finally, for the colony-level responses, hygienic behaviour after infection with *Xenos* was assessed for focal contrasts using Pearson's Chi-squared test with Oddsratios (since we used different batches of colonies for each time point) while data of hygienic behaviour after fungal challenge were analyzed by means of a GLM that adopted a multiple logistic regression to investigate the association of the predictor variables treatment, time after challenge and population of origin with the response variable hygienic behaviour (Logan 2010). We used the following softwares: JMP 9.0.2 (SAS Statistical Institute, Cary, NC) and R (version 2.11.1, 2009; R Development Core Team 2009).

## Results

### Individual and colony-level responses to the parasite *X. vesparum*

At the individual level, larval mortality was slightly higher in USdom than ITdom (36 and 30 %, respectively) but not significantly different at 3 days post-infection (Fisher's exact: $P=0.47$, data not shown). Notably, a comparable level of mortality was observed simply due to the handling in control wasps, with the loss of three larvae out of ten in USdom and four out of nine in ITdom. Infection success (the ability of the triungulins to enter wasp larvae and moult) of the USdom larvae was slightly lower, though not significantly different (Fig. 2). Fewer triungulins were able to enter into USdom larvae (22 out of 87 vs. 26 out of 72 in ITdom) but this difference was not significant (Kruskal–Wallis chi-squared = 2.88, $df = 1$, $P=0.09$). The fact that the result for this test was close to statistical significance prompted us to perform an analysis of the sampling size for this experiment. Aiming for a power of analysis at least equal to 0.8 (80 % probability of detecting an effect if one exists) and with a Cohen's $d$ (=effect size) of 0.45, the minimum number of replicates should be 64 (Kenny 1987): we used 87 parasites for USdom and 72 parasites

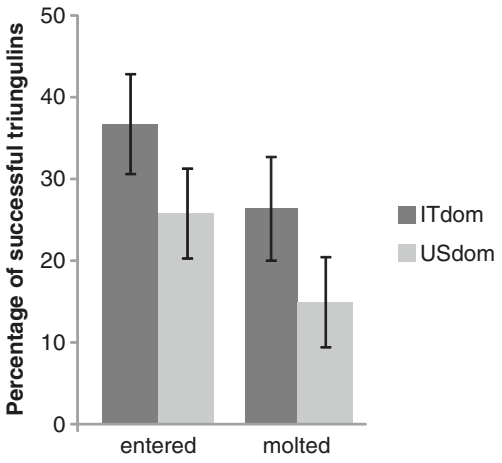


**Fig. 2** Individual-level response to *X. vesparum*. *Bars* represent the mean percentage of parasitic larvae ± S.E. that managed to enter and to perform the first moult after being placed on wasp larvae from ITdom and USdom colonies. There were no significant differences between the two populations (Kruskal–Wallis chi-squared test)

for ITdom, therefore our number of replicates was appropriate. We found eight triungulins that were on the larva cuticle but not entered in USdom and two in ITdom, while the number of encapsulated triungulins (the result of an effective immune response) was five for both groups. Parasite success after entering the host was also lower in USdom larvae, though again this was not significant: only 11 out of 87 triungulins performed the first moult while for ITdom the ratio was 16 out of 72 (Kruskal–Wallis chi-squared = 1.60, $df = 1$, $P=0.20$).

Colony-level response to *X. vesparum* in terms of hygienic behaviour towards infected larvae was higher in USdom at all three timepoints (Fig. 3). This difference was not significant at days 1 and 3 (odds ratios: $P=0.11$ and 0.17, respectively), but was significant at day 7, with 72 out of 102 USdom larvae removed compared to only 42 larvae out of 97 in ITdom (Pearson's Chi-squared = 92.17, $df = 7$; odds ratios: $P<0.0001$). Interestingly, USdom also exhibited a higher tendency to remove larvae in controls, though in this case the difference between the two populations was not significant (odds ratios: $P=0.20$).

### Individual-level response to the pathogen *E. coli*

No bacteria were detected in the hemolymph of control or PBS-injected samples. For the *E. coli*-infected specimens, bacterial load was higher in USdom than ITdom larvae (GLM chi-squared = 28.41, $df = 10$, $P=0.0016$). Larvae of fourth and fifth instar from the two populations were of comparable size. We did not observe any correlation between the larval stage and the amount of bacteria in the hemolymph. There were no significant differences in bacterial loads in adult wasps (GLM chi-squared = 1.94, $df = 11$, $P=0.99$). On average, larvae were able to reduce the bacterial infection from $1\times10^4$ *E. coli* cells (injection dose) to approximately $1\times10^2$ (CFU counted on the plate) while adults reduced bacterial loads from $2\times10^5$ to $1–4\times10^4$ (Fig. 4). Thus, wasp larvae were capable to kill thousands of bacterial cells while adult wasps cleared hundreds of thousands: therefore, the anti-bacterial response or bacterial clearance was higher in adults than larvae.

### Colony-level response to the pathogen *B. bassiana*

The statistical analysis included treatment (infected and control), time after challenge (days 2 and 7) and population of origin as main factors with equivalent relative importance (Fig. 5a). There was a significant effect of treatment ($b = -0.96$, $z = -2.81$, $P<0.01$), with both populations removing more infected larvae than controls (Fig. 5b). There was a significant effect of time, with more manipulated larvae removed at days 7 vs. 2 ($b = -3.01$, $z = -9.25$, $P<0.0001$). Finally, there was a significant effect of population, with USdom having reduced hygienic behaviour, i.e. more manipulated larvae were left on the nest ($b = 1.21$, $z = 3.75$, $P<0.001$).

## Discussion

In this study we extended the "evolution of increased competitive ability" hypothesis to a social insect model which has both physiological and behavioural responses to immune challenges, and is a host to a coevolved parasite which was lost during invasion. Our results suggest that the tested *P. dominula* population from the invasive range differs significantly from the tested wasp population from the

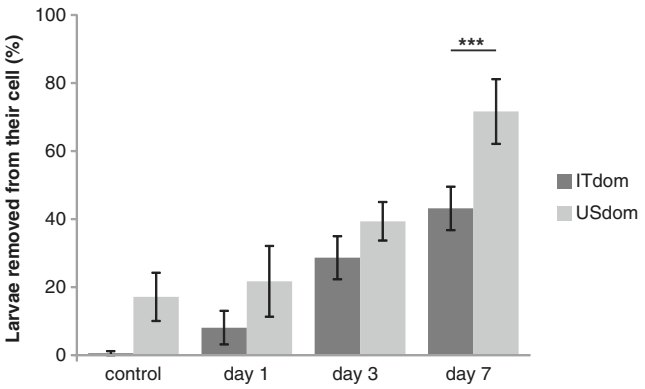


**Fig. 3** Colony-level response to *X. vesparum*. *Bars* indicate the average percentage±S.E. of infected larvae and controls that were removed in ITdom and USdom colonies at three different timepoints post-infection in four separate trials (***=$P<0.001$, Pearson's chi-squared test with odds ratios)

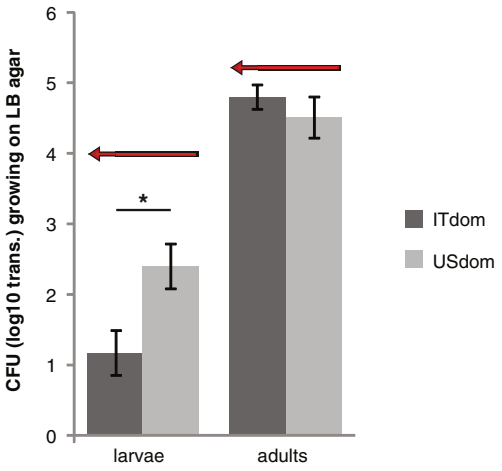


**Fig. 4** Individual-level response to *E. coli*. *Bars* indicate the $\log_{10}$ transformation of the average number of bacterial colonies ± S.E. that were still detectable after 24 h in the hemolymph of immature and adult wasps from ITdom and USdom colonies (*=$P<0.05$, GLM). *Arrows* represent the bacterial dose initially injected in the wasps

**a)**

| | Estimate | Std. Error | Z-value | Pr(>\|z\|) | |
|---|---|---|---|---|---|
| (Intercept) | 1.6579 | 0.3798 | 4.365 | 0.0000127 | *** |
| USdom vs. ITdom | 1.2113 | 0.3227 | 3.753 | 0.000174 | *** |
| day 7 vs. day 2 | -3.0108 | 0.3254 | -9.253 | <2.00E-16 | *** |
| FNG vs. CON | -0.9651 | 0.3435 | -2.81 | 0.004958 | ** |

**b)**

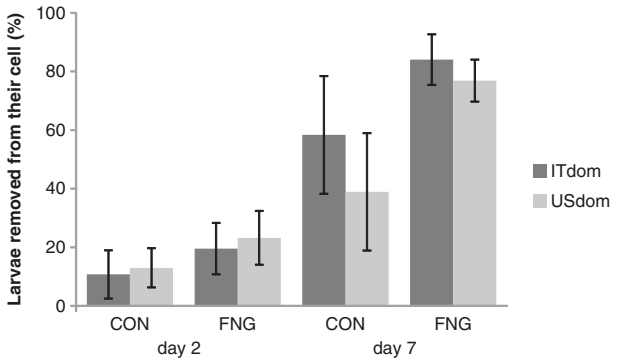


**Fig. 5** Colony-level response to *B. Bassiana*. **a** Output of statistical analysis performed using a GLM with Multiple Logistic Regression. *z* values represent the chances to find the larva in the nest: if $z>0$, the first term of the comparison removed less larvae than the second; the opposite when $z<0$. **b** Extent of the hygienic behaviour performed after the fungal challenge. *Bars* indicate the average percentage ± S.E. of challenged larvae and controls that were removed in ITdom and USdom colonies at two different timepoints. *CON*, control; *FNG*, application of spores of *B. bassiana*

native range for both individual and colony-level immune defences. At the colony level (i.e. hygienic behaviour), the USdom paper wasps have significantly lower susceptibility to the coevolved parasite *X. vesparum*, but significantly higher susceptibility towards a general pathogen (*B. bassiana* fungi). At the individual level, the USdom and ITdom populations do not differ significantly in terms of resistance to *Xenos*, but USdom larvae have a reduced immune response to general pathogens (*E. coli* bacteria, in this case) while no difference was detected in adult workers. Thus, overall in the invasive population we observed a reduction in the investment in immunocompetence towards general pathogens but a stronger behavioural response to a coevolved parasite. It must be noted, however, that this study used one population of invasive wasps and one population of wasps from their native range; further studies would be needed to determine if these phenotypic traits were consistent across the global invasive range.

The increased hygienic behaviour of the USdom wasps to the *Xenos* parasitized larvae (Fig. 3) is somewhat surprising. Based on the EICA hypothesis, it would be assumed that invasive populations would be more susceptible to parasites. There are several possible explanations for the observed phenomenon. First, it is possible that only highly hygienic and therefore parasite-free wasp populations successfully relocated to the US. Thus, the lack of *Xenos* in the US could be due to selection for healthy wasp colonies during the invasion process. However, given that USdom colonies were less hygienic in response to fungal infection than ITdom (Fig. 5a) this does not seem to be a likely scenario. Unfortunately, the paucity of data of population genetics about the European source for wasp populations that invaded the Northeast of the US does not allow us to test this hypothesis directly (for data about the genetic comparison of American and European populations of *P. dominula* see Johnson and Starks 2004; Liebert et al. 2006; Stahlhut et al. 2006). Second, USdom adults may be more sensitive to the triungulins themselves, therefore performing higher hygienic behaviour if they perceive large numbers of triungulins on the nest (Manfredini et al. 2010c). Lastly, *P. dominula* wasps might display tolerance towards *Xenos* in the native range, where the pressure of the parasite on host populations is high. Such a strategy would reduce the costs of continuously mounting immune defenses: since only some of the individuals in a colony are usually parasitized, this strategy allows adult wasps to invest more resources in rearing a larger amount of brood during the incipient phase of colony growth in order to compensate for the losses due to parasitism, therefore achieving high levels of colony productivity even in the presence of the parasite (David Hughes personal communication). The molecular and physiological mechanisms mediating tolerance to parasites and pathogens have not been well characterized yet, but tolerance appears to be a common host defense strategy that reduces the negative impact of infections on host fitness (Medzhitov et al. 2012). It is possible that maintaining tolerance is costly and thus in the US populations, without the selective pressure of active *Xenos* parasitization over the course of a 30-year period (i.e. 30 consecutive generations), tolerance was lost. Therefore, upon infection by *Xenos* parasites, the individual USdom larvae could respond with either a large immune reaction or no response at all, which would make them sick or alternatively highly parasitized. In both cases, infection by *Xenos* would result in significant physiological changes, which in turn are detected by the adults in the colony (likely through chemical cues) and result in the removal of these larvae. Our studies on the effects of *Xenos* parasitization of individual larvae (Fig. 2) lend some support to the last hypothesis, in which USdom larvae—as well as adults—are less tolerant towards *Xenos*. Infected USdom larvae were more likely to die than ITdom larvae (though this was not significant), and triungulins in USdom larvae were less likely to successfully enter and moult (though again this was not significant). This might suggest that a reduction in the host–parasite compatibility is occurring, which could lead to a breakdown in the coevolutionary mechanism of reciprocal adaption of the two organisms in the future.

For general pathogens, USdom wasps displayed lower hygienic behaviour after infection with fungi and lower

bacterial clearance in larval individuals. This is in agreement with the EICA, since invasive species are expected to have lower levels of immunocompetence in comparison to conspecific populations from the native range and sympatric competitors in the invasive range (Lee and Klasing 2004). Indeed, USdom has also proven to be less immunocompetent than the sympatric congeneric *P. fuscatus*, in terms of encapsulation response and phenoloxidase activity (Wilson-Rich and Starks 2010). With reference to the different hygienic behaviour after a fungal challenge, two alternative mechanisms could possibly justify the observed phenomenon, rather than the proposed reduction in immunocompetence for USdom. First, USdom larvae could be more resistant to fungi thus they are not removed because they do not get sick: however, in our preliminary tests of fungal infection outside the nest in larvae from both populations, we did not observe any difference in susceptibility to *B. bassiana* spores. Second, it is also possible that USdom adults perform higher hygienic behaviour in terms of removing fungal spores from the nest, therefore fewer larvae get sick than in ITdom. This is a possibility that deserves further investigation.

These findings seem to support the existence of separate immune pathways in *P. dominula* depending on the level of specificity of the challenge. The core innate immune response pathways that respond to general bacterial pathogens appear to be more effective in the ITdom wasps but weakened in the USdom, while the pathways responsible for mediating the interaction with *Xenos* appear to be differentially regulated. The lack of difference in bacterial clearance in adults (Fig. 4) is surprising, and future studies using a dose response of varying bacterial concentrations, multiple bacterial species or a time-course may be necessary to determine if there are differences in antibacterial activity in these two populations. Furthermore, there may be differences in immunocompetence between the populations under conditions of stress, such as food deprivation.

In order to fully evaluate the relevance of the EICA in this invasive species, it will be necessary to test additional populations from throughout North America and the native range, and to determine if USdom colonies do capitalize on their reduced immune function—and perhaps tolerance—by investing more in reproduction than native species. Furthermore, it will be interesting to investigate whether USdom allocate different resources depending on the nature of the immune response, i.e. constitutive or induced (Schmid-Hempel and Ebert 2003), such that they can reduce constitutive responses to improve growth and fecundity but they can still produce an induced specific immune response when needed. Finally, it is required to examine USdom susceptibility to native parasites from North America, such as other native *Xenos* species (Kathirithamby and Taylor 2005), the lepidopteran brood ectoparasitoid *Chalcoela iphitalis* (Madden et al. 2010), calliphorid flies of the genus *Lespesia* (Amy Toth, personal observation), and other yet-to-be identified flies that emerge from wasp late pupal instars (personal observation). While the ERH predicts that invasive species are initially less susceptible to local enemies in the new range because they are not identified as suitable targets, the "increased susceptibility hypothesis" and the "exotic prey naïveté hypothesis" (Li et al. 2011) predict that they may be more susceptible. Further studies will be necessary to determine if one, or all, models are at play in mediating the invasion of *P. dominula* in North America and around the world.

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**Ethical standards** The authors declare that the experiments performed in this study comply with the current laws of the USA.

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