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RESEARCH ARTICLE

The *Polistes* war: weak immune function in the invasive *P. dominulus* relative to the native *P. fuscatus*

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Abstract Invasive species are of growing ecological concern, in part because of conflicts arising with native congeners. The European paper wasp *Polistes dominulus* was first introduced to North America in the 1970s, and may be displacing at least one native species, *P. fuscatus*. Previous reports indicate that in native territories over half of *P. dominulus* colonies are infected by Strepsipteran parasites, which decrease host fitness. In North America, *P. fuscatus* are parasitized to a lesser degree (approximately one-third), but no infected colonies of invasive *P. dominulus* have been reported. Because immune function is an indicator of susceptibility to parasitism, we quantified activated levels of immune function by measuring the encapsulation response and phenoloxidase activity and then compared these levels between species. Counterintuitively, our results indicate that *P. dominulus* has lower levels of both mechanisms of immunity. Additionally, *P. dominulus* displayed less self-grooming activity than *P. fuscatus*. We briefly discuss possible immunological explanations for this invasion success, including the selective expression of low immunocompetence.

Keywords Invasion biology · Enemy-release · Ecological immunology · Competition · Hymenoptera

Introduction

The ecological impact of exotic and invasive species is of growing importance. The rate at which species are transported to novel habitats is increasing, fueled by human activity (Levine and D'Antonio, 2003) and global climate change (Simberloff, 2000). Successful regional establishment may be facilitated by many factors, including a release from enemies (Porter et al., 1997; Holway and Suarez, 1999; Hänfling and Kollmann, 2002). Changing landscapes create opportunities for habitat expansions, forcing previously allopatric species into novel sympatric environments. For congeneric species that share a habitat, inevitable battles for niche occupation ensue. One recent example is the invasion of *Polistes dominulus* in North America. *P. dominulus* is currently expanding its range in North America from its native Europe and North Africa. Since their introduction, at least one native congener, *P. fuscatus*, has been outcompeted for nesting sites; its former nesting sites are now being occupied by *P. dominulus* (Gamboa et al., 2002, 2004).

Recently, *Polistes* wasps have been used as a model system to study invasion biology (see Cervo et al., 2000; Gamboa et al., 2002, 2004, 2005; Silagi et al., 2003; Johnson and Starks, 2004; Liebert et al., 2006). *P. dominulus* was first documented in the USA in 1978 in Cambridge, Massachusetts (MA) (Eickwort, 1978; Hathaway, 1981). Over the subsequent three decades, *P. dominulus* has been documented across the USA (Cervo et al., 2000) and is established across the northern, eastern, and western portions of the country (reviewed in Liebert et al., 2006). The nesting ecology of *P. dominulus* overlaps directly with other native paper wasps, with multiple observations of displacement events of natives (Gamboa et al., 2002, 2004; Liebert et al., 2006). In contrast, *P. dominulus* is found living sympatrically with at least three congeners in its native Europe, with no reports of competitive displacement.

Multiple hypotheses exist for why *P. dominulus* may be so successful in North America (reviewed in Liebert et al., 2006). Cervo and colleagues (2000) proposed that in their invasive range, *P. dominulus* might be unaffected by their

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naturally associated European parasites. This putative release from enemies might improve their invasion success (Cervo et al., 2000). In 1998 and 1999, Pickett and Wenzel (2000) noted New York populations of *P. fuscatus* were infected by an obligate parasite within the genus *Xenos* (Order Strepsiptera; Kathirithamby, 1998, 2009). Parasites from this genus commonly infect *P. dominulus* in its native Old World habitat; however, invasive *P. dominulus* populations were not infected (Pickett and Wenzel, 2000). Gamboa and colleagues (2004) further tested this hypothesis in Michigan, USA, by surveying 28 *P. fuscatus* and 30 *P. dominulus* colonies for Strepsipteran infection. Eleven *P. fuscatus* nests were infected, but no *P. dominulus* nests were infected. The reason why invasive populations of *P. dominulus* appear to avoid infection by *X. vesparum* remains unclear. To our knowledge, no reports of the Old World species of *Xenos*, *X. vesparum*, exist documenting the parasite in the New World, neither in an infected host nor in free-living form.

In this study, we investigate the immune function of these two recently sympatric, congeneric, social insects. We hypothesized that there is a difference in the activated immune response between invasive *P. dominulus* and native *P. fuscatus* populations, in conjunction with the differential pathogen pressure in North America. To test this hypothesis, we quantified two levels of innate immunocompetence (IC), defined as the ability of an organism to mount an immune response (Wilson-Rich et al., 2009).

Materials and methods

Specimen collection

All wasps were collected from 9–16 August 2006 from standard wooden nest boxes at the Cummings School of Veterinary Medicine at Tufts University in Grafton, MA, USA. Wasp nests were transported to Tufts University in Medford, MA for analyses of immune function. We collected 1–7 individual female *P. dominulus* (mean ± SD 2.6 ± 2.4 wasps) per colony ($N = 26$ *P. dominulus* individuals from 10 colonies). Because *P. fuscatus* were more prevalent at our collection site in 2006, we collected one individual female wasp from each colony ($N = 26$ *P. fuscatus* individuals from 26 colonies). Importantly, no parasitism or outward sign of disease was noted on any wasp or nest collected.

Encapsulation response

The ability to encapsulate a novel foreign body is effective against relatively larger pathogens, including parasitoids (Carton and David, 1983; Kraaijeveld et al., 2001a, b), parasites (Doums and Schmid-Hempel, 2000), and host cells infected with viruses (Washburn et al., 1996; Trudeau et al., 2001). A modified encapsulation response assay (König and Schmid-Hempel, 1995) was performed to quantify the innate cellular immune response.

Each wasp was ice anesthetized and implanted with a sterile 1–2 mm nylon monofilament ventrally between fourth and fifth abdominal sternites. A very tiny portion of the monofilament remained outside the abdomen to facilitate its removal for later analysis. The monofilament approximates the presence of a parasite protruding through the intersegmental membrane, as in female *Xenos* spp. After implantation, specimens were placed individually in 1.5 ml microcentrifuge tubes (Fisherbrand, USA) to protect the implant from grooming activity. After 4 h, the monofilament was removed (now termed 'explant') and mounted in glycerol on a glass slide. Digital images of explants were captured at 40× magnification using an Olympus VX40 fluorescence-detecting microscope and image-capturing software (Optronics Magna Fire-SP v1.0 ×5). The autofluorescent properties of melanin enabled us to capture images through a multi-wavelength filter and detect only particles emitted within a confined spectra and control for non-melanized debris accumulated on the monofilament.

Melanin deposition was assessed using digital images captured from three focal depths (top, mid-section, and bottom). The mean gray value (MGV) of each experimental and one unimplanted control monofilament was quantified using ImageJ (v. 1.34s, NIH, USA; Rasband, 1997). The control MGV was subtracted from the MGV of each experimental implant, and then converted to optical density (OD) units using a step-function calibration curve generated through ImageJ. Two explants from *P. fuscatus* not different from control were not used for further analysis, as these likely did not puncture the intersegmental membrane (Wilson-Rich et al., 2008). Additionally, nine explants were found outside the body cavity after the 4 h period and also were not further analyzed.

Morphometric analyses

Digital images of a forewing, a proleg, and the head were taken for each specimen under 10× magnification. The following measurements were quantified using ImageJ: first discoid cell in wing length, total forewing length, proleg tarsus and tibia length, and head width (Field et al., 1998; Cervo et al., 2004; Tibbetts and Curtis, 2007). Whole weight and abdominal weight were also recorded for each specimen.

Hemolymph isolation

Hemolymph samples are very difficult to collect from *Polistes*; the conventional 'poke-and-bleed' technique

commonly performed in other insects is not reliably carried out, presumably do to low hydration states of wasps. Instead, we employed a reliable method of hemolymph collection by modifying a technique described by Korner and Schmid-Hempel (2004). First, we thawed frozen abdomens and then homogenized each by hand in phosphate-buffered saline (PBS) using sterile 1.5-ml pellet pestles (Kontes). Next, we centrifuged the homogenate at 4°C for three minutes at 2,000 × *g*, to pull soluble hemolymph proteins to the supernatant and apart from the cuticle. Finally, we transferred supernatant samples to fresh PBS and refroze each at −20°C for later use in the phenoloxidase assay.

Phenoloxidase (PO) activity

Phenoloxidase is an enzyme of vital importance to the invertebrate immune response (Hoffman, 2003). This enzyme is effective against relatively small-scale pathogens, including viruses (Wilson et al., 2001; Beck and Strand, 2007), bacteria (Pye, 1974; Ashida and Brey, 1997), fungi (Ochiai and Ashida, 1988), and parasites (Leonard et al., 1985, Paskewitz and Riehle, 1994; Gorman et al., 1996; Siva-Jothy, 2000). PO plays an important role in both cellular and humoral immune defense; however, in this test, we quantified PO activity in a cell-free environment so as to measure its humoral activity (Rantala et al., 2003; Wilson-Rich et al., 2009).

To quantify activity of the PO enzyme, we first added a tyrosine-derived substrate, L-dopa (Thermo Sci Acros Organics, Fair Lawn, NJ, USA) to thawed hemolymph samples, to reach a final concentration of 0.03 M. Next, we measured the rate of melanin production using spectrophotometer (Bio-Rad microplate reader, model 450) at 490 nm every 2 min for 30 min. Last, we graphically determined the linear reaction phase for each sample, which typically occurred during the first 10 min after substrate addition. The slope of this line was used to determine the maximum reaction velocity (V_{max}).

Total protein concentration was determined for each individual using a standard Bradford protein assay (see methods described in Wilson-Rich et al., 2008). PO activity was divided by total protein concentration for final data analysis. This additional step allowed us to control for differences in hydration state between individuals.

Statistical analyses

Encapsulation response and PO activity were compared between species using a univariate general linear model (GLM; SPSS for Windows, v.16.0). The original GLM incorporated species as the independent variable, immunity (either encapsulation response or PO activity) as discrete dependent variables, colony of origin nested under species as a random factor, and size (head width) as a covariate fixed factor.

Results

Encapsulation response

The ability to encapsulate a novel foreign object was quantified by calculating the optical density (OD) of explants. The net, mean OD of explants was low in *P. dominulus* compared to *P. fuscatus* (Fig. 1). Colony of origin was not a significant factor influencing encapsulation response ($F = 1.710$, $df = 21$, $P = 0.160$). Likewise, size (head width) was not a significant covariate with encapsulation response ($F = 0.361$, $df = 1$, $P = 0.259$). As such, both were removed from the final GLM. After ensuring that differences were not driven by colony of origin or size, results showed that the difference in encapsulation response between species was significant ($F = 8.621$, $df = 1$, $P = 0.006$).

Putative grooming response

An unexpected observation occurred during the encapsulation response experiment. After 4 h, all monofilaments implanted in *P. dominulus* remained in situ. Surprisingly,

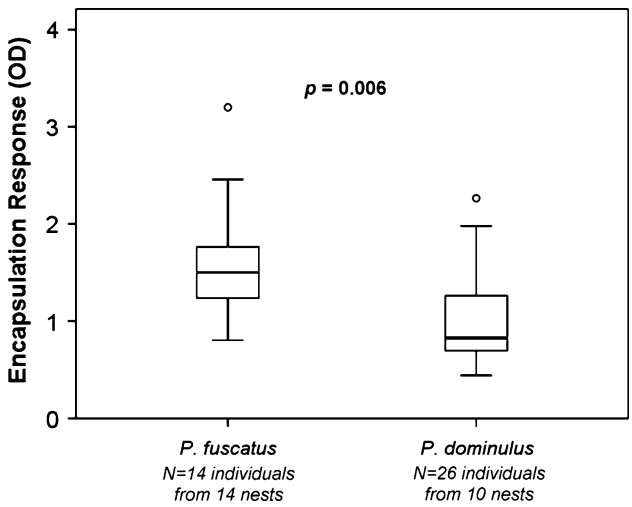

Fig. 1 Native *P. fuscatus* have a stronger encapsulation response than invasive *P. dominulus*. A standard encapsulation response assay was used as a direct measure of the innate, cellular immune response. *Boxes* show first and third interquartile range (middle 50% of individuals); *lines in middle of boxes* indicate median values. *Whiskers extending from boxes* encompass 95% of individuals, beyond which outliers reside. Statistics were calculated using a general linear model (see "Methods")

nine monofilaments (34.6%) were completely removed from *P. fuscatus* abdomens and found at the bottom of the isolation tubes. The difference in implant displacement frequency between species was significant ($\chi^2 = 8.33$, $df = 1$, $P < 0.01$).

Phenoloxidase (PO) activity

The ability to inactivate pathogens through the PO cascade was quantified by determining the linear phase of PO activity, which occurred during the first 10 min of reaction (*P. dominulus*, $V_{max} = 11.57$ μM/min; *P. fuscatus*: $V_{max} = 15.73$ μM/min). PO activity (V_{max}) was then divided by total protein concentration (mg/ml). The mean PO:total protein concentration ratio was low in *P. dominulus* compared to *P. fuscatus* (Fig. 2; median ± SE, 8.68 ± 1.03 V_{max}*ml/mg and 13.95 ± 2.18 V_{max}*ml/mg, for each species, respectively). Colony of origin was not a significant factor influencing PO activity ($F = 1.082$, $df = 31$, $P = 0.558$). Likewise, size (head width) was not a significant covariate with PO activity ($F = 0.361$, $df = 1$, $P = 0.558$). As such, both were removed from the final GLM. After ensuring that differences were not driven by colony of origin and size, results showed the difference in PO activity between species was significant ($F = 6.540$, $df = 1$, $P = 0.014$).

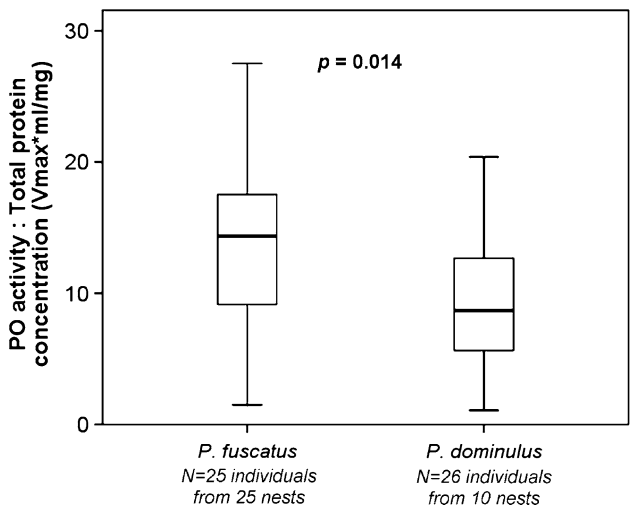

Fig. 2 Native *P. fuscatus* have greater phenoloxidase (PO) activity than invasive *P. dominulus*. A standard PO activity assay was performed as a direct measure of the innate, humoral immune response. Total hemolymph protein concentration was corrected for in post hoc analysis to control for variation in individual hydration state. *Boxes* show first and third interquartile range (middle 50% of individuals); *lines in middle of boxes* indicate median values. *Whiskers extending from boxes* encompass 95% of individuals, beyond which outliers reside. Statistics were calculated using a general linear model (see "Methods")

Morphometric analyses

All seven morphometric measurements (length of first discoid wing cell, total wing length, femur length, tibia length, whole weight, abdominal weight, and head width) correlated with one another (1-tailed Pearson correlation; $P < 0.001$ for all pairs). Overall, *P. dominulus* was smaller than *P. fuscatus* for all morphometric measurements (Kruskal–Wallis test $P < 0.001$ for all seven measurements). Despite the smaller body size of *P. dominulus*, morphometric differences between species were not significant covariates with either measure of immune function (see "Statistical analyses" in "Methods").

Discussion

We report two direct measures of IC in a successful invasive species (Liebert et al., 2006). Our data indicate that the cellular and humoral innate immune response of an invasive population of *P. dominulus* is significantly lower than that of a native sympatric population of *P. fuscatus*. Subsequent data from implant displacement rates suggest *P. dominulus* groomed out a foreign body from the ventral abdomen significantly less frequently than *P. fuscatus*. This observation is unexpected and surprising, given that *P. fuscatus* have a larger body size and therefore less room to maneuver within the confined chamber than did the smaller *P. dominulus*. Although direct grooming out of these implants was not observed, this finding warrants further investigation into differential grooming rates between these species as a measure of behavioral IC.

Data collected from seven morphometric measures showed that *P. dominulus* is significantly smaller than *P. fuscatus*. These findings are consistent with previous reports of size differences between these recently sympatric congeners (reviewed in Liebert et al., 2006). Our data show that size was not a significant covariate of either encapsulation response or phenoloxidase activity, indicating differences in immunity are not due to size.

Immunological study of native *P. dominulus* populations is crucial before confirming the factors influencing relatively low IC. Without these data, at least three mutually exclusive hypotheses explaining low IC in invasive *P. dominulus* are conceivable: (1) there has been no change in IC from native to invasive, (2) IC in *P. dominulus* is a phenotypically plastic trait, or (3) IC in *P. dominulus* has evolved to lower levels. The first prediction, that native populations may have undergone no change in immune investment and IC subsequent to North American invasions, is the most parsimonious explanation. A key assumption of this hypothesis is that IC is a trait with little variation between environments. This assumption may not

hold true because of noted differences in pathogen pressure between Old and New World habitats. Variation in pathogen pressure may drive variation in immune function.

The second and third hypotheses are more plausible given the high invasion success and evidence of competitive ability of North American *P. dominulus*. The second hypothesis, that IC is a phenotypically plastic trait, does not make any assumptions about variation in alleles coding for IC. Instead, this hypothesis assumes variable phenotypic expression of IC, dependent upon environmental cues. Assuming mounting an immune response is costly, low IC should be expressed when high IC is wasteful. And indeed, there is likely a fitness cost for maintaining high levels of immunity (Hughes and Cremer, 2007).

The third hypothesis, that IC in *P. dominulus* evolved to lower levels in invasive populations, assumes variation in alleles coding for IC function. Low IC may occur in at least two ways: either through a genetic bottleneck in IC alleles in the invasive population that by happenstance rendered the new population with alleles for low IC (hereafter, the 'non-selective hypothesis'), or if individuals with alleles for low IC had a selective advantage (the 'selective hypothesis'). The non-selective hypothesis is unlikely in *P. dominulus* because this species has undergone multiple invasions with no evidence of a genetic bottleneck (Johnson and Starks, 2004; Liebert et al., 2006). Of these two sub-hypotheses, the selective hypothesis seems more likely, given the degree of genetic diversity in the population. Natural selection should favor individuals with inherently low IC when it is advantageous. Assuming IC is energetically costly, resources formerly invested in IC might be diverted to competitive life-history traits (Blossey and Nötzold, 1995; Hänfling and Kollmann, 2002; Lee and Klasing, 2004). The North American landscape may provide such an environment, with relatively low pathogen pressure and observed competition for nesting sites. Clearly, IC data from native *P. dominulus* populations are needed in order to discriminate between these three major hypotheses.

The enemy-release hypothesis suggests the establishment and spread of an introduced species is fueled in part by a release from the parasites, pathogens, and predators that were major ecological constraints in the native habitat (e.g., Porter et al., 1997). In the northern United States, between 12% and 39% of *P. fuscatus* are infected by *Xenos* spp. (Pickett and Wenzel, 2000; Gamboa et al., 2002, 2004). In Tuscany, Italy, 58% of *P. dominulus* are infected by *Xenos* spp. (Hughes et al., 2003); there have been no reports of the parasite in North American populations to date. It is likely that parasitoids will eventually recognize *P. dominulus* as a viable host (unpublished data), and should that occur, our data suggest that the parasitoid would be, at least initially, highly successful. Future study should investigate the identity of enemy populations and whether or not these enemies recognize *P. dominulus* as a host.

Invasive species are of growing ecological importance. Data relating to these animals provide researchers with valuable insight into what makes a non-native species successful. With the case of the *P. dominulus* invasion, our results demonstrate weak cellular and humoral IC (vis-à-vis encapsulation response phenoloxidase activity), and low rates of hygienic behavioral acts (self-grooming), compared to the native *P. fuscatus*. Counterintuitively, release from predators may be driving low IC, resulting in a highly successful, but immunocompromised, invasion.

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