Evolutionary ecology of microsporidia associated with the invasive ladybird *Harmonia axyridis*

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**Abstract** Invasive species are characterized by the rapid growth and spread of their populations after establishing a foothold in new habitats, and there are now many examples of such species negatively affecting biodiversity and the economy. It is unclear why some species can become successful invaders, whereas most (even if closely related) remain noninvasive. We previously proposed a hypothesis that parasites associated with invading species can promote their invasive success if they are harmless toward the invaders but harmful to their competitors and/or predators in the newly colonized habitat. Here we discuss whether microsporidia that have recently been discovered in the invasive ladybird *Harmonia axyridis* contribute to its invasive success. We show that all *H. axyridis* beetles sourced from diverse collection sites all over the world carry abundant microsporidia. This suggests that both native and invasive *H. axyridis* populations are associated with these tolerated parasites, which were likely to have existed in native populations before expansion rather than being acquired in newly colonized areas. We describe the pathogenesis of the microsporidia during different developmental stages of *H. axyridis* and we address the possibility that the predation of its infected eggs and larvae by competing native ladybird species may lead to their infection and ultimately to their decline. Finally, we discuss our initial hypothesis: microsporidia that are tolerated by an invasive vector insect can be active against susceptible native competitors and/or predator species.

**Key words** Coccinellidae; evolutionary ecology; *Harmonia axyridis*; innate immunity; intraguild predation; invasion biology; microsporidia

**Introduction** Biological invasions have a negative impact on biodiversity and economy on a global scale (Wagner & van Driesche, 2010). The increasing spread of invasive species by human activities such as international trade and agriculture has promoted a new research discipline called invasion biology or invasion ecology to address these concerns (Heger et al., 2013). The latter seeks to understand why some species become successful invaders after their establishment in new habitats, whereas others, even if closely related, remain noninvasive. The harlequin ladybird *Harmonia axyridis*, which is also known as the multicolored ladybird or Asian ladybird, is indigenous in eastern Asia, and has emerged as a model species for invasion biology. It feeds efficiently on aphids and scale insects and has thus been introduced as a biological control agent in Europe and North America (Koch, 2003). However, it has
spread beyond these areas and has now been found as far as South Africa and parts of South America.

The genetic analysis of different *H. axyridis* populations has identified 5 important invasion routes (Lombaert *et al.*, 2010, 2011). This invasive species can successfully outcompete native ladybird species in newly colonized habitats, prompting researchers to investigate the mechanisms underlying its invasive success (Evans *et al.*, 2011; Guillemaud *et al.*, 2011). Invasive populations of *H. axyridis* have been reported to change their reproductive strategies, for example, invasive males have higher reproductive success, and invasive females reproduce sooner and yield more eggs, which are fertilized by larger number of fathers (Laugier *et al.*, 2013). A comparison of native and introduced *H. axyridis* populations showed that only the former suffer from inbreeding depression, whereas inbred individuals are as fit as outbred ones within invasive populations (Facon *et al.*, 2011). Furthermore, the absence of inbreeding depression seems to be linked to a population bottleneck of intermediate size that purges deleterious mutations in the first stages of the invasion process (Facon *et al.*, 2011). The invasive populations therefore have a higher mean fitness than the native populations and this may have promoted the establishment and spreading of the invasive species in new areas (Facon *et al.*, 2011). *H. axyridis* is also well adapted to a broad temperature range, thus allowing long migrations to overwintering sites (Majerus *et al.*, 2006). The aggregation behavior at overwintering sites may also reflect adaptation of invasive populations to areas with cold winters (Durieux *et al.*, 2013).

A prominent feature of predatory ladybirds is that they also feed on the eggs and larvae of other ladybirds. This so-called intraguild predation has been recognized as a major selective force among competing ladybird species, and a number of studies provide evidence that *H. axyridis* is a more successful intraguild predator than ladybird species that are indigenous to Europe, such as the 7-spotted ladybird *Coccinella septempunctata* and the 2-spotted ladybird *Adalia bipunctata* (previewed by Pell *et al.*, 2008; Gardiner *et al.*, 2011; Roy *et al.*, 2012). Overwintering *H. axyridis* are also less susceptible than *C. septempunctata* and *A. bipunctata* to infection with the entomopathogenic fungus *Beauveria bassiana* (Roy *et al.*, 2008). The resistance of *H. axyridis* against fungal pathogens has recently been attributed to the expression of antifungal peptides, particularly coleoptericins discovered among its immune-inducible genes which displayed potent activity against entomopathogenic fungi (Vilcinskas *et al.*, 2013a). The latter study identified more than 50 predicted different antimicrobial peptides in *H. axyridis*, the highest number reported to be produced by an animal thus far. These findings support the hypothesis that successful invasive species have a superior immune system to closely related noninvasive species, because in the colonized habitats they encounter new pathogens and parasites (Lee & Klasing, 2004; Vilcinskas, 2013).

The relatively new discipline of invasion immunology aims to determine the role of immunity in invasion biology, and *H. axyridis* has emerged as a fruitful model in this context (Schmidtberg *et al.*, 2013; Vilcinskas *et al.*, 2013a). Its ability to defend against pathogens and parasites successfully can be expanded beyond its remarkably broad spectrum of antimicrobial peptides, which are induced and secreted in its hemolymph following immune activation, to include also a chemical weapon that is constitutively present in the hemolymph. The alkaloid harmonine ((17R,9Z)-1,17-diaminoctadec-9-ene) was found to increase in abundance as eggs develop into larvae, reaching their highest levels in the hemolymph of adult beetles (Schmidtberg *et al.*, 2013). This is particularly interesting because harmonine was found to display antibacterial as well as antiparasitic activity and therefore may complement the protection provided by antimicrobial peptides (Röhrich *et al.*, 2012).

An independent study confirmed the presence of harmonine in *H. axyridis* eggs (Kajita *et al.*, 2010), and the authors postulated that this compound protect the eggs from predation by native ladybird species because *C. septempunctata* exhibited high mortality when fed with *H. axyridis* eggs, but the reciprocal situation was non-lethal. However, the hypothesis that harmonine may serve as a chemical defense compound against intraguild egg predation has been tested by challenging *C. septempunctata* beetles with synthetic harmonine, and even high concentrations of this compound injected into the beetles did not increase mortality compared to controls injected with buffer. Instead, the injection of *H. axyridis* hemolymph into *C. septempunctata* beetles was lethal, but not if the hemolymph was heated first (Vilcinskas *et al.*, 2013b). These observations led to our hypothesis that *H. axyridis* carries another lethal factor in its eggs and in its hemolymph.

**Detection of microsporidia in *Harmonia axyridis***

Our analysis of hemolymph samples isolated from *H. axyridis* led to the discovery of abundant microsporidia among the hemocytes. The beetles showed neither signs of infection nor impaired performance in terms of aphid predation or fecundity despite carrying a high microsporidial load. Microsporidia are spore-forming obligate parasites found only in animal hosts, and frequently associated
Evolutionary ecology of microsporidia

Fig. 1 Microsporidia in the hemolymph of *H. axyridis* sampled from different geographic regions. Fresh hemolymph samples from adult *H. axyridis* females showing the high concentration of spores between the larger hemocytes (arrowheads). Scale bars = 20 μm unless stated otherwise. The samples were collected from populations at different geographical sites: (A) Beijing, China; (B) Brussels, Belgium; (C) Santiago, Chile; (D) Fuchu, Japan (scale bar = 50 μm in the main image, and 20 μm in the inset image); (E) Cuneo, Italy; (F) Korea; (G) Novosibirsk, Russian Federation; (H) Brookings, USA.

with insects. They reproduce within host cells following the injection of their germ plasma through extrudable germ tubes, which we also observed in fixed specimens (Vilcinskas et al., 2013b). All *H. axyridis* beetles collected from different locations around Germany were found to carry microsporidia, and the same was true of hemolymph samples from female *H. axyridis* beetles collected from different geographic sampling sites, ranging from China, Japan, and Korea (the native range) through to the invasive populations of Europe, and North and South America (Fig. 1). This was a significant finding because until our report there was little information about the distribution of microsporidia in native and invasive *H. axyridis* populations (Osawa, 2011).

Although as stated above, the abundant microsporidial spores do not harm *H. axyridis* populations, the injection
of microsporidia purified from *H. axyridis* hemolymph into *C. septempunctata* killed all beetles within 2 weeks and the cadavers were full of microsporidia. This observation led to our hypothesis that the microsporidia carried by *H. axyridis* could be responsible for the mortality of *C. septempunctata* following the injection of *H. axyridis* hemolymph or the consumption of *H. axyridis* eggs (Vilcinskas et al., 2013b). Given that (i) intraguild predation is a major selective force among competing ladybirds (Gardiner et al., 2011), (ii) horizontal transmission of microsporidia occurs among ladybird species through intraguild predation (Saito & Bjørnson, 2006, 2008), and (iii) that *C. septempunctata* beetles die when challenged with microsporidia from *H. axyridis* (Vilcinskas et al., 2013), we postulated that these parasites may contribute to the worldwide invasive success of *H. axyridis*. Therefore, *H. axyridis* and its microsporidia provide an ideal model to analyze the role of parasites in the interactions between insect species and their competitors and predators. However, quantitative data are required to determine the impact of parasites on communities, and a combination of theoretical and empirical studies is needed to examine how the effects of parasitism scale up to the community level (Hatcher et al., 2006). To address the potential contribution of *H. axyridis* microsporidia to such community-level processes, we first review what is known about microsporidia in beetles (particularly Coccinellids), then focus on the pathogenesis of *H. axyridis* microsporidia, and finally consider the potential of these parasites as bioweapons that promote invasive success.

**Microsporidia in the Coleoptera**

Microsporidia are obligate, intracellular parasites that are highly specialized relatives of fungi (Keeling & Fast, 2002; Agnew et al., 2003). More than 1 200 species have been described, and most infect insects, including beetles (Smith, 2009), usually following the oral uptake of mature extracellular spores that are protected by thick, chitinous cell walls. Host infection is achieved by the protrusion of a polar tube that injects the infectious contents of the spore (the sporoplasm) into the host cells, resulting in the formation of relatively simple structures known as meronts. These replicate and eventually differentiate into sporonts, which divide to form sporoblasts that ultimately form the mature spores, thus completing the phase known as sporogony (Keeling & Fast, 2002). Recent genome analysis has revealed evolutionary strategies that promote the rapid intracellular growth of microsporidia as a basis for their diversity, distribution among animals and successful control of host cells (Keeling & Slamovits, 2004; Corradi et al., 2007; Williams, 2009; Cuomo et al., 2012).

The search for natural pathogens or parasites of beetles with the potential to act as biological control agents has revealed a number of microsporidial species, such as those infecting the red flour beetle *Tribolium castaneum* (Fisher & Sandborn, 1962; Miller, 1971; Blaser & Schmid-Hempel, 2005), various bark beetles (Purriani & Weiser, 1985; Weiser et al., 1995, 1998, 2002, 2006, listed in Kohlmayr et al., 2003), Chrysomelidae such as *Phylloprocta armata* (Zhu et al., 2011), flea beetles such as *Phyllotreta atrata* (Yaman et al., 2005), and *P. undulata*, the Colorado potato beetle *Leptinotarsa decemlineata* and several curculinids (listed in Cali & Briggs, 1967; Świątek & Górkiewicz, 2006). Insects that prey on pest beetles, such as parasitic wasps, often carry microsporidia that also infect their prey (Chapman & Hooker, 1992). However, the use of microsporidia to control introduced pests is controversial (Solter & Maddox, 1988). Microsporidia are frequent parasites of ladybird beetles and the identification of novel species is far from complete (Table 1, listed in Bjørnson et al., 2011; Steele & Bjørnson, 2014). The release of ladybird species as biological control agents may have promoted the spread of microsporidia into new areas and potentially to new coccinellid hosts (Joudrey & Bjørnson, 2007; Bjørnson, 2008; Bjørnson et al., 2011).

In *H. axyridis*, the amplification of small-subunit ribosomal RNA (rRNA) genes using a variety of primer sets resulted in the detection of a microsporidial 16S rRNA gene with ≥ 99% sequence identity to members of the Nosema/Vairimorpha clade, with *Nosema thomsoni* as the closest relative (Vilcinskas et al., 2013b,c). However, the relationship between the newly identified microsporidia and known pathogens will remain unclear until the genome sequence is available. The genus *Nosema* comprises 80 species (Kirk et al., 2008) including some that are common in coccinellid hosts (Cali & Briggs, 1967; Saito & Bjørnson, 2006; Bjørnson et al., 2011; Steele & Bjørnson, 2014). Several previous studies have identified 2 morphologically distinct microsporidial spore types (primary and environmental spores) with different functions (Weiser et al., 1998, 2006; Kohlmayr et al., 2003). The dense environmental spores are thought to promote infection, whereas the less dense primary spores are thought to inoculate different tissues in the infected host. Empty spores observed by microscopy may represent primary spores that have already extruded their contents or primary spores that did not survive fixation (Weiser et al., 1998).

The *H. axyridis* hemolymph appears to contain both weakly stained primary and intensely stained...
Table 1 Microsporidial species in different coccinellids (according to Saito & Bjørnson, 2006; Bjørnson et al., 2011; Steele & Bjørnson, 2013).

| Microsporidial species | Coleopteran species | Sites of infection | Spore form |
|------------------------|---------------------|-------------------|------------|
| *Nosema hippodamiae* (Lipa & Steinhaus, 1959) | *Hippodamia convergens* | Midgut, fat body | Ovoid |
| *Nosema tracheophila* (Cali & Briggs, 1967) | *Coccinella septempunctata* | Tracheal epithelium, hemocytes, connective tissue | Ovoid |
| *Nosema coccinellae* (Lipa, 1968) | *Adalia bipunctata* | Midgut epithelium, Malpighian tubules, gonads, nerves, muscle | Ellipsoidal |
| *Tubulinosema hippodamiae* (Saito & Bjørnson, 2006, 2008; Bjørnson et al., 2011) | *Hippodamia convergens* | Pyloric valve epithelium, hindgut epithelium, Malpighian tubules, connective tissue, ovary, fat body | Pyriform |
| *Nosema thomsoni-like microsporidium* (Vilcinskas et al., 2013) | *Harmonia axyridis* | Hemolymph (spores), hemocytes, fat body, ovaries, connective tissue sheaths surrounding midgut and muscles | Ovoid |
| *Nosema adalae* (Steele & Bjørnson, 2013) | *Adalia bipunctata* | Ovaries, testes, midgut epithelium, Malpighian tubules, hindgut, fat body, ventral nerve cord, muscle | Ovoid |

Environmental spores (Figs. 2B and D). Although many primary spores were detected, the discharge of the polar filament was only rarely observed after fixation (Fig. 2C) and never in fresh hemolymph samples. The visibility of the extruded polar tube may be species-dependent: it has never been observed in the case of *N. hippodamiae* during infections of *Hippodamia convergens* (Lipa & Steinhaus, 1959), but can be observed in *N. tracheophila* during infections of *C. septempunctata* (Cali & Briggs, 1967).

Hemolymph samples, prepared from late *H. axyridis* embryos isolated from eggs, and newly hatched larvae isolated before feeding on the egg shell, also contained spores that do not cause any visible symptoms of infection. Microsporidial infection usually modifies the host life cycle, in the most severe cases by killing the host and/or delaying normal larval development, thus preventing the onset of maturity (Blaser & Schmid-Hempel, 2005). In other cases, infection reduces fitness (Tanada & Kaya, 1993) and therefore influences the development, mortality, sex ratio, fecundity, and/or longevity of entire populations (Saito & Bjørnson, 2008; Joudrey & Bjørnson, 2007; Steele & Bjørnson, 2012). The ability of microsporidia to delay larval development has been described in several ladybird species (Saito & Bjørnson, 2006; Joudrey & Bjørnson, 2007; Steele & Bjørnson, 2012), although the impact on larval/adult mortality and egg production can vary considerably depending on the host (Joudrey & Bjørnson, 2007; Steele & Bjørnson, 2012). We were unable to investigate the impact of microsporidia on parameters such as mobility, mating, oviposition, mortality, and development in *H. axyridis* because we have not found microsporidia-free individuals, and nor have we been able to propagate captured beetles treated with the microsporidiacidal compound fumagillin.

Microsporidial pathogenesis in *H. axyridis*

Coleopteran microsporidia do not generally appear to be tissue-specific (Weiser et al., 1998, 2006; Kohlmayr et al., 2003) and the infection rate (massive vs. scattered infection) in the same tissues may vary across different species. Light microscopic inspection revealed a high abundance of spores in the hemolymph of *H. axyridis*. The average concentration was $6.45 \times 10^6 \pm 7.82 \times 10^5$ in the 4th instar larvae and $13.34 \times 10^6 \pm 6.12 \times 10^6$ in beetles (average and standard deviation samples obtained from 30 individuals). We detected *H. axyridis* microsporidia in hemocytes, the fat body and ovary, as well as in the connective tissue surrounding the midgut, gonads, and muscle tissue (Fig. 2). These infection sites...
Fig. 2 Microscopic documentation of microsporidia infection in *Harmonia axyridis*. (A) Fresh ovoid spores in the hemolymph of adult *H. axyridis* collected in anticoagulant saline, scale bar = 20 μm. (B) Semithin section of hemolymph pellet reveals weakly stained primary spores (arrowheads) and intensely stained environmental spores, scale bar = 20 μm. (C) Scanning electron micrograph of spores with extruded polar filaments (white arrowheads) surrounding a hemocyte, scale bar = 5 μm. (D) Cross section through the abdomen of L2 instar larva reveals numerous densely stained environmental spores and weakly stained primary spores in the hemolymph and the hemocoel between the organs, scale bar = 20 μm. (E) Microsporidial spores (arrows) and presumptive sporoblasts (arrowheads) in the disintegrating fat body cells of adult beetles, scale bar = 20 μm. (F) Semithin section through the posterior glands of the female reproductive organ. Infected and partly disintegrating hemocytes (arrowheads) aggregate along the whole connective tissue sheath of the gland, scale bar = 20 μm. (G) Semithin section through the vitellariu of the *H. axyridis* ovary with spores in the peritoneal sheath surrounding the ovariole and few apparent sporoblasts in the ooplasm of young oocytes (arrowheads), scale bar = 20 μm. (H) Magnification of vitellariu. Environmental spores are distributed in the outer ovariole sheath (arrows) and apparent sporoblasts are found in small interstitial cells (arrowheads), scale bar = 20 μm. Key: c, cuticle; cs, connective tissue sheath; f, fat droplets; fb, fat body; g, gland; h, hemocoel; hc, hemocyte; oc, young oocytes; si, somatic interstitial cells. Further information on the material and methods used in this microscopic analysis can be found in the electronic supplement.
show minor differences in susceptibility among coccinellid species (Bjørnson et al., 2011). Despite the high spore count in the *H. axyridis* hemolymph, the spores in tissues were localized in spots and there was no evidence of significant tissue or organ damage.

In adult *H. axyridis*, spores were detected in the connective tissue sheaths surrounding muscles and the midgut epithelium, but the tissues themselves were devoid of spores and there appeared to be no impact on mobility. However, the 2nd instar larvae (Fig. 2D) contained densely stained inclusions within vesicles in the apical region of the midgut epithelium, potentially representing sporoblasts. In contrast, the intracellular infection of muscle fibers by microsporidia has been recorded in other beetles and this may compromise their mobility (Lipa, 1968; Kohlmayr et al., 2003; Weiser et al., 2006).

The microsporidial infection of freely floating hemocytes was observed only rarely in *H. axyridis*, but plasmatocytes clumped together near strands of connective tissue were preferentially infected (Fig. 2). Laigo and Paschke (1966) described this process as the conversion of freely circulating hemocytes to sedentary cells during infection, and demonstrated that inoculation with *Nosema* spp. reduces the total hemocyte count in the host and may stimulate connective tissue growth. In this way, the microsporidia may recruit hemocytes as a vehicle for dispersal within the hemocoel (Cali & Briggs, 1967). Our experiments showed that this is also the case for microsporidia in *H. axyridis* (Fig. 2F). We also observed a limited infection of the fat body in larvae and adults (Fig. 2E). In the latter case, infection was restricted to individual lobes in which the fat body cells disintegrated and fat droplets and protein granules spread into the hemolymph. These structures contained numerous presumptive sporoblasts and spores. Fat body infection has been observed in several coccinellids containing microsporidia (Lipa & Steinhaus, 1959; Bjørnson et al., 2011; Steele & Bjørnson, 2013).

The presence of microsporidia in *H. axyridis* female gonad tissues suggests the possibility of maternal transmission, which is widespread among beetles (Weiser et al., 1998; Świątek & Górkiwicz, 2006). All microsporidial life stages in *H. axyridis* were observed in the ovariole sheath, trophic chambers, follicular cells, oocytes, and eggs, but oogenesis was generally not affected (Świątek & Górkiwicz, 2006). In the adult *H. axyridis* ovary, the infection sites appeared to be concentrated in the connective tissue of the peritoneal coat enclosing the trophic chamber and the oocyte (Fig. 2G). Microsporidia were only occasionally found in the trophocytes and in somatic interstitial cells of the trophic chamber (Fig. 2H). The infection process within *H. axyridis* ovarioles may therefore proceed as previously discussed (Świątek & Górkiwicz, 2006): microsporidia infect oocytes via the ovariole sheath and the follicular epithelium by polar tube extrusion, and then exploit intercellular connections between follicle cells and oocytes (Terry et al., 1997).

**Do microsporidia promote the invasive success of *H. axyridis***?

As stated above, our hypothesis is that the abundant microsporidia discovered in *H. axyridis* could act as a bioweapon against native intraguild predators. This was supported by injecting *C. septempunctata* beetles with microsporidia isolated from *H. axyridis* hemolymph, resulting in 100% mortality associated with the formation of novel microsporidial spores during pathogenesis (Vilcinskas et al., 2013b). We further postulate that indigenous ladybird species may be infected when they feed on *H. axyridis* eggs and larvae under natural conditions. Initial evidence showing that the ingestion of *H. axyridis* eggs is lethal for *C. septempunctata*, but not vice versa, was reported by Kajita et al. (2010). Further studies provide evidence for the transmission of microsporidia among different coccinellid host species that compete as intraguild predators, for example, egg cannibalism experiments revealed that microsporidia can be transmitted efficiently from *H. convergens* to *Adalia bipunctata*, *C. septempunctata*, *C. trifasciata perplexa*, and *H. axyridis* (Saito & Bjørnson, 2006, 2008). *Nosema tracheophila* is also known to infect *C. septempunctata*, affecting larval development and mortality, as well as adult fecundity and longevity (Cali & Briggs, 1967). However, it is unclear whether intraguild predation results in microsporidial transmission from *H. axyridis* to indigenous ladybird beetles in the field. Transmitted microsporidia need not necessarily be lethal towards indigenous intraguild predators, because it is still advantageous to *H. axyridis* if the microsporidia reduce the fecundity or fitness of the incumbent competitor. Microsporidia are maybe the only parasites that contribute to the invasive success of *H. axyridis*. Spiroplasma bacteria, which are common as so-called male killers in insects, have recently been reported to enhance body size and fecundity in infected *H. axyridis* (Elndagy et al., 2013). There are other examples in which microsporidia associated with nonnative insects appear to cause the decline of native insects. For example, the rapid and continent-wide collapse of some North American bumble bee populations has been linked to strains of the microsporidial species *Nosema bombi*. 

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that escaped from imported and commercially bred European bumble bees (Colla et al., 2006). Nosema ceranae, a microsporidial parasite of the Asian honey bee Apis ceranae, has crossed the species barrier to European honey bees (Apis mellifera) and may have contributed along with other factors to the colony collapse of this major pollinator (Chen & Huang, 2010).

It is currently unclear why H. axyridis is unharmed by its microsporidial cargo. However, at least two other features make H. axyridis unusual compared with other coccinellid species and even other insects (Fig. 3). First, the hemolymph in this species is characterized by potent, constitutive antimicrobial activity, which has been attributed to the chemical defense compound harmonine (Röhrich et al., 2012; Vilcinskas, 2013). Second, the genome of this species has undergone remarkable expansion in terms of the inducible antimicrobial peptide repertoire (Vilcinskas et al., 2013). Interestingly, the injection of bacteria results in a switch from constitutive chemical defense to an inducible innate immune response that may reflect a trade-off resulting from fitness-related costs associated with the simultaneous synthesis of harmonine and antimicrobial peptides (Schmidtberg et al., 2013). We speculate that harmonine may contribute to the tolerance of microsporidial parasites in H. axyridis, because the amount of harmonine in the hemolymph increases during development along with the abundance of the spores, and this compound displays in vitro activity against other parasites such as Plasmodium falciparum, the agent that causes malaria (Röhrich et al., 2012). Studies are under way to determine whether harmonine and/or some of the antimicrobial peptides in H. axyridis mediate its tolerance against microsporidia (Fig. 3). Little is currently known about the impact of insect defense molecules against endogenous microsporidial parasites (Texier et al., 2010).

However, microsporidia associated with Coccinellids have emerged as promising models to analyze the role of microorganisms in the interactions between insect species interacting in a competitive relationship such as intraguild predators. The cited studies which provide evidence that microsporidia can cross the species-barrier among ladybirds under experimental conditions call for field studies that explore the con- and heterospecific spread of these parasites under natural conditions. We postulate that the transmission rate depends on a number of factors such as the population density of the vector-ladybirds and the availability of alternative prey insects such as aphids if the dispersal of microsporidia is promoted through intraguild predation.

**Outlook**

This review focuses on the role of parasites in the invasive success of H. axyridis. Its resistance against its associated microsporidia and other pathogens and parasites is solely mediated by its superior immune system combined with chemical defense molecules such as harmonine. However, these factors alone do not satisfactorily explain its prominent ability to outcompete native ladybird species. H. axyridis displays fascinating features regarding its reproduction, population genetics, stress responses, and behavioral adaptations which have also been implicated in the literature to contribute to its performance as a successful invader (Table 2). We predict that the value of H. axyridis as model in invasion biology will increase with the application of genetic tools to compare both invasive and noninvasive populations as well as invasive and noninvasive ladybird species. Such comparative genomic analyses, which have recently begun, will ultimately help to decipher molecular traces of adaptations mediating invasiveness.
Table 2  Factors postulated in the literature to promote the invasive success of *H. axyridis*.

| Factor                  | Reference                                                      |
|-------------------------|----------------------------------------------------------------|
| Chemical defense        | Harmonine-mediated protection against parasites and pathogens   |
|                         | Harmonine-mediated protection of eggs against intraguild predation | Röhrich et al. (2012) |
| Immunity                | High resistance against entomopathogenic fungi which is conferred by a diverse spectrum of antinicrobial peptides | Kajita et al. (2010) |
| Parasites               | Tolerance against microsporidia which can infect native ladybird species | Vilcinskas et al. (2013a) |
|                         | Spiroplasma-infection increases body size and fecundity         | Elnagdy et al. (2013) |
| Reproduction            | Invasive males have higher reproductive success, and invasive females reproduce sooner and yield more eggs, which are fertilized by larger number of fathers | Laugier et al. (2013) |
| Population genetics     | Absence of inbreeding depression in introduced populations results in a population bottleneck of intermediate size that purges deleterious mutations in the first stages of the invasion process | Facon et al. (2011) |
| Stress adaptation       | Adaptation to a broad temperature range, thus allowing long migrations to over-wintering sites | Majerus et al. (2006) |
| Behavior                | Increased flight speed promotes dispersal in invasive populations | Lombaert et al. (2014) |
|                         | Aggregation of invasive populations at overwintering sites      | Durieux et al. (2013) |
|                         | Success in intraguild predation with native competitors         | Gardiner et al. (2011) |

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Disclosure

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**Supporting Information**

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Supplemental experimental section.