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Invited Review

The things they carried: The pathogenic effects of old and new parasites following the intercontinental invasion of the Australian cane toad (*Rhinella marina*)

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ABSTRACT

Brought to Australia in 1935 to control agricultural pests (from French Guiana, via Martinique, Barbados, Jamaica, Puerto Rico and Hawai'i), repeated stepwise translocations of small numbers of founders enabled the cane toad (*Rhinella marina*) to escape many parasites and pathogens from its native range. However, the infective organisms that survived the journey continue to affect the dynamics of the toad in its new environment. In Australia, the native-range lungworm *Rhabdias pseudosphaerocephala* decreases its host's cardiac capacity, as well as growth and survival, but not rate of dispersal. The lungworm is most prevalent in long-colonised areas within the toads' Australian range, and absent from the invasion front. Several parasites and pathogens of Australian taxa have host-shifted to cane toads in Australia; for example, invasion-front toads are susceptible to spinal arthritis caused by the soil bacterium, *Ochrobactrum anthropi*. The pentastome *Raillietiella frenata* has host-shifted to toads and may thereby expand its Australian range due to the continued range expansion of the invasive toads. Spill-over and spill-back of parasites may be detrimental to other host species; however, toads may also reduce parasite loads in native taxa by acting as terminal hosts. We review the impact of the toad's parasites and pathogens on the invasive anuran's biology in Australia, as well as collateral effects of toad-borne parasites and pathogens on other host species in Australia. Both novel and co-evolved pathogens and parasites may have played significant roles in shaping the rapid evolution of immune system responses in cane toads within their invaded range.

Contents

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1. Introduction

The native range of the cane toad (*Rhinella marina*) (Fig. 1) extends from southern Texas and western Mexico to central Brazil (Zug and Zug, 1979; Acevedo et al., 2016). Cane toads were brought from Guyana (directly) and French Guiana (via Martinique) to Barbados in the mid 1800s (to control pest beetles that were consuming farmed sugarcane (Easteal, 1981; Turvey, 2013), then translocated from Barbados to Puerto Rico (some directly, and some via Jamaica) in 1920 (Turvey, 2009). In 1932, 149 toads were brought from Puerto Rico to Oahu, Hawai'i in order to control cane beetles (Turvey, 2009). Over the following two years, more than 100,000 individuals were distributed across the Hawai'ian Islands (Turvey, 2009). In 1935, 101 Hawai'ian cane toads were brought to Queensland, Australia and bred in captivity; their offspring were later released along the Queensland coast (Turvey, 2009).

Cane toads rapidly spread across tropical and subtropical Australia (Urban et al., 2008), and have had major ecological impacts on Australian native fauna (Shine, 2010). The chemically distinctive toxins of *R. marina* (Hayes et al., 2009) are fatal if ingested by many Australian predators (which lack a history of evolutionary exposure to bufonid anurans, and thus to their toxins) (Llewelyn et al., 2009, 2014; Shine, 2010; Llewelyn et al., 2014). As a result, the continuing spread of cane toads has caused massive declines in populations of anuran-eating predators both in tropical and temperate Australia (Letnic et al., 2008; Shine, 2010; Brown et al., 2011; Jolly et al., 2015; Jolly et al., 2016).

Although scientific studies on the ecological impact of cane toads in Australia have focused on lethal toxic ingestion as the primary mechanism of impact, and have looked primarily at effects on top-order predators, other mechanisms of toad impact may be important also. For example, invaders may carry with them parasites from the native range that can severely affect other host species in the invaded range (Raffel et al., 2008). Also, invaders may act as additional competent hosts for parasites from the invaded range, thereby increasing infection rates of native species via spillback mechanisms (Kelly et al., 2009b). On the other hand, invaders may reduce rates of parasitism in native hosts by removing infective stages of the parasite life cycle from the environment and becoming parasite sinks (Heimpel et al., 2003; Kelly et al., 2009a; Lettoof et al., 2013; Nelson et al., 2015a). In the current review, we summarise available information on the pathogens and parasites of cane toads in Australia (compared to those in the toads' native range), and the interaction between invasion dynamics and pathogenic effects as immune function evolves in the toads.

Fig. 1. Cane toad (*Rhinella marina*), a large bufonid anuran invasive to Australia. Photo taken by Dr. Matt Greenlees.

2. Impact of translocation to Australia on parasites and pathogens of the cane toad

2.1. The enemy release hypothesis

When organisms are translocated to new areas, they may escape from co-evolved competitors, predators, parasites, and pathogens ('enemy release hypothesis,' or ERH) (Colautti et al., 2004). The low number of host individuals transferred to the introduced range diminishes the probability of native pathogens/parasites being represented (Lewicki et al., 2014). Pathogens and parasites that do accompany the invasive host often face barriers to transmission such as low host density (Arneberg et al., 1998; MacLeod et al., 2010; Blakeslee et al., 2012), and a lack of vectors or intermediate hosts needed to complete their life cycles in the introduced range (Blakeslee et al., 2012; Lewicki et al., 2014). For "enemy release" to be realized, several conditions must be met. First, co-evolved pathogens and parasites (specialized to the host in its native range) must be absent from the introduced range (Keane and Crawley, 2002; Prenter et al., 2004; Liu and Stiling, 2006). Second, host-switching of pathogens and parasites from native taxa in the introduced range to the invasive host should be uncommon (Keane and Crawley, 2002; Prenter et al., 2004; Liu and Stiling, 2006). Third, enemies in the introduced range should be less pathogenic to the invasive host than to native taxa (Keane and Crawley, 2002; Prenter et al., 2004; Liu and Stiling, 2006). If these conditions are met, enemy release may enable the invasive species to thrive in its introduced range (Colautti et al., 2004). The ERH has been supported by many studies on plants (DeWalt et al., 2004; Blumenthal, 2006), but relatively few on animals (Torchin et al., 2002; Torchin, 2003; Roy et al., 2011).

The translocation of the cane toad to Australia seems to conform to the ERH. Many pathogens (such as bacteria and fungi) from the toad's native range seem to have been lost along its journey to Australia (Speare, 1990), filtered by the stepwise invasion process. There are two possible exceptions to this, but both are uncertain. The first is a gram-negative bacillus which causes granulomatous lesions in the livers of toads in New South Wales (Speare, 1990). However, this strain of bacteria has not been definitively identified, so its origin remains uncertain (Daszak et al., 1999). The second is the fungus *Mucor amphibiorum*, which has been found in free-ranging toads throughout Queensland and can cause fatal septicaemia in anurans (Speare et al., 1994). This fungus has not been detected in native Australian anurans, suggesting that it may have

been introduced with the cane toad (Speare et al., 1994). However, the same fungus has been detected in the platypus in Tasmania (Speare et al., 1994), a part of Australia to which toads were not introduced. This suggests that the parasitic fungus may be endemic to Australia (Speare et al., 1994). Regardless, the toads are suitable hosts for *Mucor amphibiorum*, and have the potential to amplify the fungal parasite's numbers.

Viruses seem to follow a similar pattern, although there are few studies documenting them in the toad throughout its worldwide distribution (Speare, 1990). *Ranavirus* (family Iridoviridae) infects amphibians and fish (Hyatt et al., 2000). Although six species of *Ranavirus* have been isolated in native-range cane toads in Venezuela (Hyatt et al., 2000), none have been found in Australian toads (Zupanovic et al., 1998). However, antibodies against *Ranavirus* were detected in blood serum from both Venezuelan and Australian toads (albeit at low prevalence in Australia), suggesting some exposure to *Ranavirus* in Australia (Whittington et al., 1997; Zupanovic et al., 1998). The viruses encountered by Australian toads may originally have come from South America, or may have host-shifted from Australian hosts (Zupanovic et al., 1998). Cane toads may amplify and disseminate *Ranavirus* to other susceptible Australian anurans (Zupanovic et al., 1998).

2.2. Protozoans

Within their native South American range, cane toads contain many species of endemic protozoans, reviewed by Delvinquier and Freeland (1988). To test the ERH in Australian cane toads, protozoan parasite load was surveyed in toads of multiple life stages across Queensland (the original site of introduction: Turvey, 2009) in the 1980s, approximately 50 years after toads were introduced (Delvinquier and Freeland, 1988b). Only three species out of approximately sixty documented South American parasites were found to infect Australian toads (Table 1), indicating that the majority of native-range pathogens had indeed been lost (Delvinquier and Freeland, 1988b). Blood parasites were completely lost, possibly due to their absence from the small founder population, or a lack of vectors in the introduced range (Delvinquier and Freeland, 1988b).

However, introduction to Australia has also exposed toads to new protozoan parasites, several of which have been acquired from native anurans (Delvinquier and Freeland, 1988b) (Table 2). When an invasive species is physiologically similar to native hosts, it has the potential to amplify parasite numbers (Barton, 1997). This process, called spill-back (Kelly et al., 2009a, b), may consequently increase incidence of parasitism in native hosts (Lang and Benbow, 2013). Spill-back is a more common phenomenon than its converse, spill-over, whereby invaders carry with them novel parasites (Torchin, 2003) that subsequently infect native hosts (Hartigan et al., 2011).

Other protozoans found to infect Australian toads include *Saccamoeba* and flagellates (Diplomonadida and Trichomonadida) (Freeland et al., 1986). Most of these protozoans have not been shown to exert pathogenic effects on their hosts (Delvinquier and Freeland, 1988b), but infections by *Zelleriella* and *Saccamoeba* are associated with greater susceptibility to other parasites (Freeland et al., 1986). Host-shifting of *Zelleriella* from cane toads to native anurans has been unsuccessful, as the parasite is only able to survive for a short time within these unfamiliar hosts (Delvinquier and Freeland, 1988a).

Table 2
Species of novel protozoan parasites acquired by the Australian cane toad in Queensland. Prevalence of each parasite within the sample populations of tadpoles, juveniles, and adults are reported as percentages. All data from Delvinquier and Freeland (1988b).

Species	Bodily location	Prevalence in Australian populations(%)		
		Tadpoles	Juveniles	Adults
Chilomastix caulleryi	Intestine, cloaca	0	0	6
Retortamonas dobelli	Intestine, cloaca	0	0	3
Giardia agilis	Intestine, cloaca	30	0	<1
Spironucleus elegans	Intestine, cloaca	42	0	61
Monocercomonas batrachorum	Intestine, cloaca	0	0	21
Protoopalina australis	Intestine, cloaca	58	0	11
Protoopalina hylarum	Intestine, cloaca	0	0	<1
Protoopalina raffae	Intestine, cloaca	0	0	4
Nyctotheroides species	Cloaca	63	0	27
Trichodina species	Tadpole skin	92	0	0

2.3. Metazoans

In Australia, the cane toad has also escaped from native-range arthropod parasites. Although several species of ticks that infect cane toads in South America have been lost, local mites and mosquitoes utilize cane toads as hosts in Australia (Speare, 1990). The same trend is observed in myxozoan parasites, though some South American parasites were thought to have been introduced to Australia with the toad (Delvinquier, 1986). When *Myxidium* parasites were detected in the gall bladders of both invasive Australian toads and native Australian anurans, the parasite was thought to be *Myxidium immersum* (Delvinquier, 1986), which infects cane toads in Brazil (Lutz, 1889). Inspection of anuran museum samples also revealed that no *Myxidium* were detected in native Australian anurans collected prior to the arrival of toads in Australia (Hartigan et al., 2010). However, museum specimens collected after the toad introduction revealed that the parasite was found in native Australian anurans from areas that the toads had not yet invaded (Hartigan et al., 2010). Subsequent phylogenetic analyses revealed that there were actually two species of *Myxidium* parasites infecting Australian anurans, and both were distinct from the morphologically similar *Myxidium immersum* found in Brazilian toads (Hartigan et al., 2011). Moreover, neither of the two Australian *Myxidium* species were found in Hawai'ian toads, further refuting the idea that they came to Australia with the cane toads (Hartigan et al., 2011). Nonetheless, the toads may have amplified numbers of

Table 1
Species of native-range protozoan parasites retained by the Australian cane toad in Queensland. Prevalence of each parasite within the sample populations of tadpoles, juveniles, and adults are reported as percentages.

Species	Bodily location	Native countries	Prevalence in Australian populations (%)		
			Tadpoles	Juveniles	Adults
Trichomitus batrachorum	Cloaca	Costa Rica, Colombia	22	0	77
Zelleriella antilliensis	Intestine, Cloaca	Jamaica, Bermuda, Brazil, Mexico, Costa Rica, Venezuela, Colombia, Fiji	75	100	38
Hyalodaktylethra renacuajo	Cloaca	Argentina	26	0	14

All data from Delvinquier and Freeland (1988b).

these Australian parasites, accounting for their lack of detection before 1966 (Hartigan et al., 2011).

2.3.1. Lungworms

Although helminths parasitize toads in South America (Speare, 1990; Campiao et al., 2014), only one species (*Rhabdias pseudosphaerocephala*) has been shown to persist in Australian populations (Dubey and Shine, 2008). Initially, the nematode found in the lungs of Australian cane toads was identified (based on morphometrics) as an endemic Australian species, *Rhabdias* cf. *hylae* (Barton, 1997). However, subsequent mitochondrial and nuclear genetic analyses identified the lungworm as the American taxon *R. pseudosphaerocephala,* indicating that this parasite had indeed persisted through several serial translocations (Dubey and Shine, 2008). Confusingly, *R. pseudosphaerocephala* has not been found in Hawai'ian cane toads (Barton and Pichelin, 1999; Barton and Riley, 2004; Marr et al., 2010), the source of the toads brought to Australia (Barton, 1994). The lungworm may have gone extinct in the Hawai'ian Islands prior to sampling.

Adult *Rhabdias pseudosphaerocephala* attach to the lung epithelium of the host and consume erythrocytes (Colam, 1971; Barton, 1996). The parasite's life cycle begins in the host's lungs, where adult worms lay eggs which are carried on a mucous ladder up the trachea to the throat (Baker, 1979; Anderson, 2000). Eggs are then swallowed into the digestive system, and passed into the environment through host faeces (Pizzatto et al., 2010). Newly hatched larvae escape from faeces into the soil, where they moult several times and develop into free-living sexually reproducing adults (Baker, 1979; Anderson, 2000) over the span of 24–48 h (Kelehear et al., 2012a). Offspring develop within the free-living mother and eventually consume her (4–10 days after toad defecation: Kelehear et al., 2012a), and enter the environment as infective third-stage larvae (L3) (Baker, 1979; Anderson, 2000). Once the L3 are able to locate a host, they burrow through its skin around the eye socket (Kelehear et al., 2011a, b, c). The larvae then migrate through host tissues to reach the lungs, where they mature into hermaphroditic adults and attach to the epithelia in order to reproduce and repeat the life cycle (Pizzatto et al., 2010).

The prevalence (percentage of hosts infected) and intensity (number of parasites per infected host) of *R. pseudosphaerocephala* infections in Australian toads vary seasonally (Pizzatto et al., 2013). During the wet season, when the soil is saturated with water, the mobility of larvae is limited due to their poor swimming abilities (Pizzatto et al., 2013). Additionally, toads are less likely to aggregate during the wet season, as hydration and shelter sites are widespread (Pizzatto et al., 2013). During the dry season, however, toads are forced to aggregate around receding sources of moisture, increasing their density and facilitating parasite transmission (Pizzatto et al., 2013).

L3 of *R. pseudosphaerocephala* commonly enter the toad host by burrowing through epidermis around the eye (Kelehear et al., 2011a, b, c). Infiltration of helminths through this location may render toads susceptible to eye infections and neurological complications, as these effects have been observed by rhabditid nematodes in Asian horned frogs (*Megophrys montana*) (Imai et al., 2009). Although toads attempt to dislodge larvae crawling on their skin (e.g. by kicking, tongue-flicking, and blinking), these measures are largely ineffective (Kelehear et al., 2011a, b, c). Furthermore, toads do not actively avoid helminth larvae (Kelehear et al., 2011a, b, c). Rather, they approach L3 larvae and attempt to consume them (Kelehear et al., 2011a, b, c).

Toads can also acquire *R. pseudosphaerocephala* through cannibalism because larger toads often prey upon smaller ones (Pizzatto and Shine, 2011c). Within their new host, these lungworms are able to survive, continue their life cycle, and reduce host mobility (Pizzatto and Shine, 2011c). Unable to eliminate traveling larvae once inside the body, the toads' histiocytes nonetheless sometimes isolate the pathogen by forming granulomas, which resemble cysts (Pizzatto et al., 2010).

Rhabdias pseudosphaerocephala larvae reduce the survival, growth rate, locomotor activity, and feeding rate of metamorph cane toads (Kelehear et al., 2009). This could be through a number of proposed mechanisms, including the consumption of host erythrocytes, physical obstruction of the lung surfaces, and initiation of energetically costly immune responses. Alternatively, these impediments may be caused by the imposition of large L3 crawling through tiny metamorph bodies, but the exact mechanism is still not fully known (Kelehear et al., 2009). Metamorphs are particularly vulnerable due to their poor locomotor skills and immunocompromised physiology during this period of development (Kelehear et al., 2011a, b, c), but they are not the only vulnerable life-stage (Kelehear et al., 2011a, b, c). Infection with *R. pseudosphaerocephala* was also associated with reduced growth rates of adult toads, both in the wild and in captivity (Kelehear et al., 2011a, b, c). Such impacts of parasites on their hosts are often attributed to changes in host diet or the parasite's directly pathogenic effects (Kelehear et al., 2011a, b, c). However, adult toads infected with lungworms did not change their feeding rates (Kelehear et al., 2011 a, b, c) nor exhibit declining growth rates with increasing intensity of infection (Kelehear et al., 2011a, b, c). If energy depletion arising through erythrocyte consumption by *R. pseudosphaerocephala* was responsible for decreasing growth rates, more parasites would be expected to produce greater growth reduction (Kelehear et al., 2011a, b, c). Because infection intensity did not affect growth, the negative impact likely was caused by the costs of mounting an immune response and the mechanical damage associated with L3 migration (Kelehear et al., 2011a, b, c).

Rhabdias pseudosphaerocephala infection has other impacts on host physiology also. It is believed that the lungworm impedes the process of blood oxygenation in its host (Pizzatto et al., 2012a,b). Reduction of cardiac capacity by the lungworm also has implications for host behavior (Pizzatto et al., 2012a,b; Heise-Pavlov et al., 2013). Although feeding rate is not influenced by infection status or intensity, diversity of prey items decreases with increasing parasitism (Heise-Pavlov et al., 2013). Because foraging can be physically demanding, individuals with incapacitated cardiovascular systems are more limited in their pursuits. Prey items whose capture pose a greater challenge are likely only attainable to uninfected toads (Heise-Pavlov et al., 2013). Nonetheless, if prey is plentiful, all toads may obtain roughly equal quantities of food (Heise-Pavlov et al., 2013).

2.3.1.1. Spill-over. Cane toads overlap with many Australian frogs in diet and shelter-site selection, creating opportunities for parasite transfer among host species. However, there are no reports of the lungworm *R. pseudosphaerocephala* infecting native anurans under natural conditions (Pizzatto et al., 2012a,b); instead, they are commonly infected by another helminth of the same genus, *R. hylae* (Pizzatto et al., 2010). Laboratory studies have shown that infective larvae of *R. pseudosphaerocephala* can penetrate the bodies of at least some species of native frogs (Pizzatto et al., 2010), but most may be dead-end hosts (the nematodes are not retained in the lungs) (Pizzatto and Shine, 2011a). Frogs mounted faster immune responses than did toads, indicating that the specialization of this helminth for its preferred host includes evasion strategies for its physiological defences (Pizzatto et al., 2010). Among the seven species of native frogs tested in that study, none exhibited significant declines in growth, mobility, or survival when exposed to *R. pseudosphaerocephala* (Pizzatto and Shine, 2011a).

However, a follow-up study found that *R. pseudosphaerocephala*

is deadly to at least one species of native frog (*Litoria splendida*) (Pizzatto and Shine, 2011b), diminishing the hope that the parasite could be used as a control agent for toads without compromising the safety of native anurans (Pizzatto and Shine, 2011b). Clearly, the lungworm's impact on frogs differs among species (Pizzatto and Shine, 2011b).

2.3.1.2. Spill-back. Some Australian helminths from native anurans have capitalized on the toad's introduction (Table 3) (Freeland et al., 1986). However, host-switching of parasites from native frogs to introduced toads appears to be rare at the original location of introduction (Townsville area: Freeland et al., 1986; Dubey and Shine, 2008), and native lungworms are not known to have host-shifted into toads (Pizzatto et al., 2012a,b). Laboratory experiments suggest that the immune systems of native frogs can recognise and destroy larvae of *R. pseudosphaerocephala* (Nelson et al., 2015b).

In general, gastrointestinal parasitism by Australian helminth larvae is more prevalent in toads than in native anurans (Kelehear and Jones, 2010), possibly because toads are larger than frogs, and infection rates tend to be positively correlated with body size (Kelehear and Jones, 2010). Toads may also feed upon a broader range of prey items, increasing the risk of exposure to new parasites (Kelehear and Jones, 2010). Among Australian frogs, parasite loads were higher in species that were larger or experienced more niche overlap with toads (Kelehear and Jones, 2010). Although the overall incidence of parasitism was higher in toads than in frogs, parasites in toads were frequently found encapsulated in cysts made up of toad immune cells, which potentially diminish parasite viability (Kelehear and Jones, 2010). In contrast, coevolution between Australian frogs and their parasites has allowed parasites to become specialized for the physiological conditions of the available hosts' bodies (Kelehear and Jones, 2010).

Also reflecting a lack of long-term coevolution, native helminths elicited stronger histological immune reactions in toads than they did in native frogs (Kelehear and Jones, 2010). The systemic arm of the vertebrate immune system, which deals with newly encountered pathogens, comprises the most inflammatory and stressful immune responses (Janeway et al., 2001).

Cross-infection experiments on toads and several species of native frogs, with the toad lungworm (*R. pseudosphaerocephala*) and the frog lungworm (*R. hylae*), showed that each parasite was more successful at reaching the target tissue in its respective traditional host; toads exhibited superior resistance to the frog lungworm than did frogs to the toad lungworm (Nelson et al., 2015b). Mirroring earlier findings, *R. pseudosphaerocephala* produced illness in toads, while *R. hylae* did not induce obvious pathogenic effects in the frogs (Nelson et al., 2015b). Toads have likely evolved a strong immune response which is also stimulated by infection with *R. hylae* (Nelson et al., 2015b).

All of the parasites (except the native Australian helminths) mentioned above utilize cane toads as a definitive host, meaning that they attain sexual maturity within the toad and thus do not depend upon any subsequent hosts (Hechinger and Lafferty, 2005). However, some parasites require an intermediate host in which they pass through one or more asexual life stages before moving onto their definitive host (Hechinger and Lafferty, 2005). In these systems, transmission between hosts frequently occurs through predation (Hechinger and Lafferty, 2005). Toads are unlikely to be intermediate hosts in Australia (Kelehear and Jones, 2010) because they are eaten by relatively few species of Australian predators (Cabrera-Guzmán et al., 2012, 2014, 2015). Toads are thus a "dead-end" for several parasites (Kelehear and Jones, 2010; Nelson et al., 2015a). The decline of one native Australian proteocephalid tapeworm has been attributed to increases in toad densities (Freeland, 1994).

2.3.1.3. Pentastomes. One parasite of cane toads is an arthropod that is neither an Australian native nor brought by the toads. Rather, the pentastome *Raillietiella frenata* was introduced to Australia at least 40 years ago inside the Asian house gecko (*Hemidactylus frenatus*) (Kelehear et al., 2013). This pentastome has been detected in both native anurans and toads in Australia (Kelehear et al., 2011a, b, c). As in the case of *R. pseudosphaerocephala*, the pathogenesis of *R. frenata* includes the consumption of blood cells in the lungs (Kelehear et al., 2012b). Accumulation of these pentastomes can cause lung punctures, pneumonia, or blockage of respiratory airways (Kelehear et al., 2014). Prevalence of pentastomes was found to be higher in male toads than in females, possibly due to sex differences in microhabitat use or diet (Kelehear et al., 2012b). Toads of intermediate body size exhibited the highest prevalence of pentastome infection, suggesting that older individuals may develop adaptive immune defences against pentastomes (Kelehear et al., 2012b). Although *R. frenata* infection is correlated with reduced fat stores in the Mediterranean house gecko (*Hemidactylus turcicus*), this trend was not observed in toads, suggesting minimal energetic costs in this novel host (Kelehear et al., 2012b). There is also a significant association between increasing pentastome intensity and declining metabolic rate in active house geckos, although the same association is not significant in resting house geckos (Caballero et al., 2015). This relationship has not yet been tested in toads.

Table 3
Novel helminth parasites acquired by the Australian cane toad from native anurans.

Agent	Bodily location	Subgroup
Acanthocephalid cysts	Not stated	Acanthocephala
Proteocephalid cysts	Not stated	Cestoda
Maxvachonia flindersi	Intestine	Nematoda
Parathelandros sp.	Intestine	Nematoda
Nematodes (mainly Parathelandros)	Intestine	Nematoda
Trematodes (mainly Mesocoelium and Lecithodendriidae)	Intestine	Trematoda
Spirometra mansoni	Small Intestine	Cestoda
Mesocoelium mesenibrinum	Intestine	Digenea
Dolichosaccus symmetrus	Intestine	Digenea
Dolichosaccus juvenilis	Intestine	Digenea
Zeylanurotrema spearei	Intestine	Digenea
Parathelandros mastigurus	Intestine	Nematoda
Johnpearsonia pearsoni	Intestine	Nematoda
Porrorchis hylae larvae	Intestine	Acanthocephala
Neniatotaenia hylae	Intestine	Cestoda

All data from Freeland et al. (1986) and Barton (1997).

Previously, *R. frenata* was confined to a highly limited range in Australia due to the restriction of the Asian house gecko to widely separated urban areas (Kelehear et al., 2013). However, infected toads could serve as an additional host that carries the pentastome across the toad's entire Australian range, allowing the parasite to infect host species with which it did not previously share an overlapping range (Kelehear et al., 2013).

2.4. Summary

Overall, most pathogens and parasites from the toads' native range have failed to remain with their hosts during their translocation to Australia, presumably because of the multiple sequential founder effects involved in that process (Fig. 2) (Easteal, 1981; Barton, 1997). Not only were the numbers of founders small (e.g., 101 toads came from Hawai'i to Australia), but it was the progeny of those 101 toads that were released rather than the adult toads (Turvey, 2013). This precaution was taken to prevent the introduction of pathogens and parasites (Barton, 1997), but in fact the initial adult toads were kept in a single large enclosure with their offspring (Turvey, 2013), thereby allowing parasite transfer between generations.

Intuitively, generalist parasites and those with direct life cycles are more likely to become established because they do not require intermediate hosts (Lymbery et al., 2014). This bias may explain why macroparasites (e.g., helminths, arthropods) are generally more successful than microparasites (e.g., viruses, bacteria, protozoans) during invasion (Barton, 1997, 1999), although there are a substantial number of cases in which parasites with indirect life cycles successfully become established in other systems (Lymbery et al., 2014). Because only one helminth species was retained in the toads (Dubey and Shine, 2008), it is unsurprising that no native-range viruses, pathogenic bacteria, or fungi have been documented in Australian populations of the invader.

3. Impact of range expansion on parasites and pathogens of the cane toad in Australia

Release from co-evolved predators, competitors, pathogens, and parasites may have enhanced the cane toad's ability to spread through Australia. Rapid evolution of life-history traits and dispersal ability has enabled toad populations to expand through Queensland (Urban et al., 2008) and the Northern Territory (Covacevich and Archer, 1975), and into New South Wales (Easteal, 1981) and Western Australia (Rollins et al., 2015) (Fig. 3). As a result of novel evolutionary forces, and potentially also of genetic drift, phenotypic characteristics have diverged between toads in Queensland and those on the invasion front in western regions (Rollins et al., 2015). Compared to Queensland toads, western toads are larger in body size and relative size of the parotoid gland (Phillips and Shine, 2005), and have longer legs (Phillips et al., 2006). Behaviourally, invasion-front toads have more dispersive tendencies (Alford et al., 2009; Lindstrom et al., 2013). The phenotypic differences between toads in the range core and those on the range edge appear to be due to a combination of natural selection (higher fitness of faster dispersers) and spatial sorting (a non-adaptive process whereby genes for rapid dispersal accumulate at the range-edge because of interbreeding among the fastest-dispersing animals in each generation (Shine et al., 2011; Perkins et al., 2013).

3.1. Intermediate population disadvantage

Surveys of different populations of cane toads in Australia provide evidence on how parasite loads change with time since colonisation of an area. Parasite prevalence was low in the youngest (Westmoreland Station, 2 years old) and oldest (Townsville, 47 years old) populations, whereas intermediate-age populations (Burketown and Normanton, 4–19 years old) experienced greater parasitism (Freeland et al., 1986). Similarly, rates of parasitism by *R. pseudosphaerocephala* were lower in the oldest and youngest populations across eight field sites along the expanding range (Brown et al., 2015a). Interestingly, toads from populations with higher parasite prevalence also had smaller spleens and fat bodies, suggesting immune and energetic costs associated with parasite prevalence (Brown et al., 2015a).

Intermediate populations of a range-expanding host species may experience higher energetic and immune costs of parasitism (such as reduction in spleen size and fat bodies) because they have not adapted to adequately suppress parasitic infection. All intermediate populations are at one time invasion “front” populations, in which parasitic infection rates are lower because low host densities make parasite transmission more difficult (Brown et al., 2015a). However, as new toads arrive to the current front and move farther westward, host densities (and thus parasite infection rates) likely increase faster than the toads in the intermediate populations can adapt. Meanwhile, lower parasitism at the range core may occur through competitive exclusion of other parasites by those which are co-adapted with the host (Freeland et al., 1986).

3.2. Host-parasite lag

Although 100% of Queensland toads surveyed were parasitised by the lungworms, this proportion declined westward along the invasion transect, with only 60% in eastern Northern Territory and 43% in the Darwin area (Dubey and Shine, 2008). At the edge of the invasion front, lungworms were absent (Phillips et al., 2010). The lack of lungworm parasites in invasion-front populations of cane toads is likely because host densities are too low for effective transmission (Phillips et al., 2010).

Nonetheless, radio-tracking of infected and uninfected toads revealed that *R. pseudosphaerocephala* does not significantly reduce its host's dispersal rate in the wild; toads with lungworms actually dispersed more rapidly than uninfected conspecifics (Brown et al., 2015a). These results are puzzling because *R. pseudosphaerocephala* adversely affects the host's cardiovascular system (Pizzatto et al., 2012a,b). Additionally, by virtue of their pathogenic nature, the larvae provoke immune responses which can reduce movement by depleting host energy stores. It seems that neither of these detrimental effects is strong enough to restrict adult toad mobility.

Why would infected toads disperse more rapidly? The lungworm might somehow influence the toad to move further (Brown et al., 2015a), or (more likely) toads that are inherently more mobile may be more likely to encounter lungworm larvae, or more susceptible to infection due to immunocompromise associated with the strenuous dispersal process (Brown et al., 2015a).

3.3. Costs of dispersal

The advantage of staying ahead of parasites through constant movement does not come without drawbacks. Approximately 10% of large toads (>110 mm snout-urostyle length) on the invasion front are afflicted with spinal spondylosis (Brown et al., 2007). This condition is caused by *Ochrobactrum anthropi*, a species of soil bacteria in Australia that is otherwise only documented as rarely exerting pathogenic effects in immune-compromised humans (Brown et al., 2007). However, in frontal toads, the bacterium causes bony fusion of the synovial joints between spinal vertebrae, leading to arthritis (Brown et al., 2007). The frequency of infection is positively correlated with toad body size and movement rate

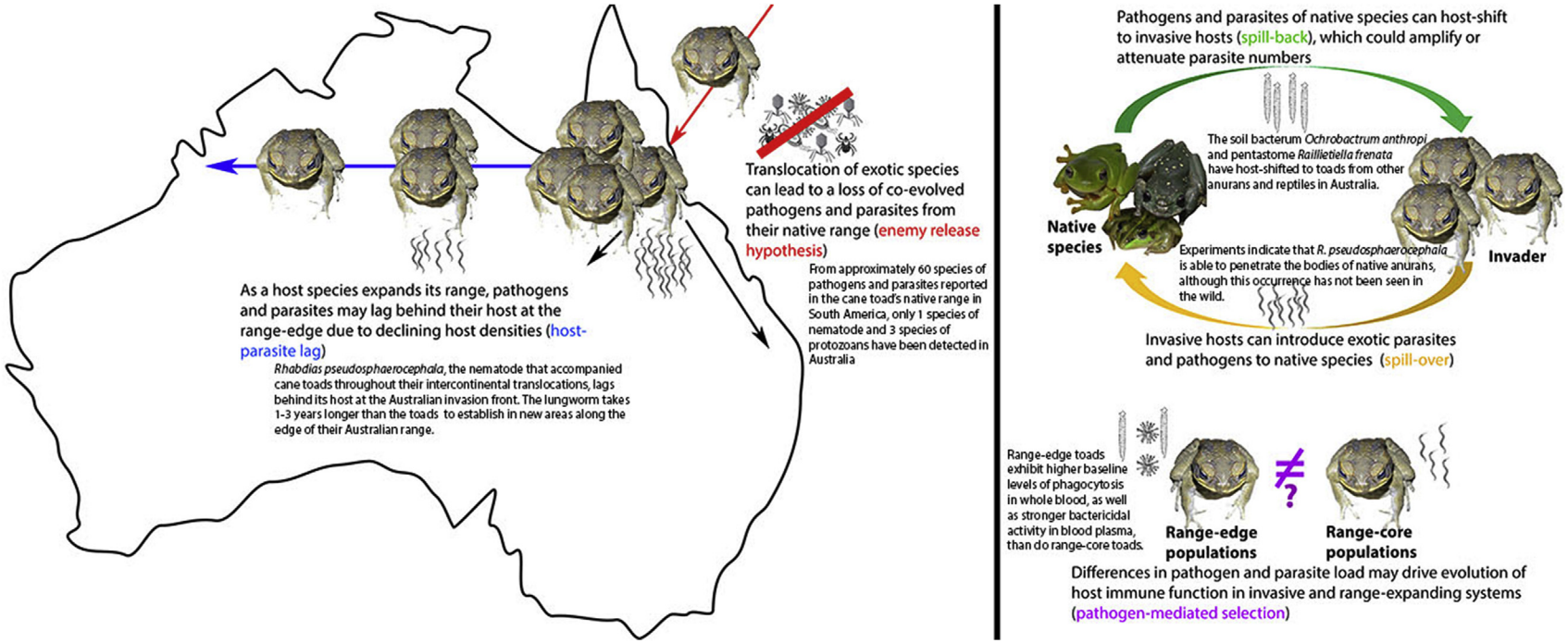

Fig. 2. Phenomena occurring in pathogen/parasite load during the introduction of exotic host species. All of these concepts are exemplified by the invasive cane toad model.

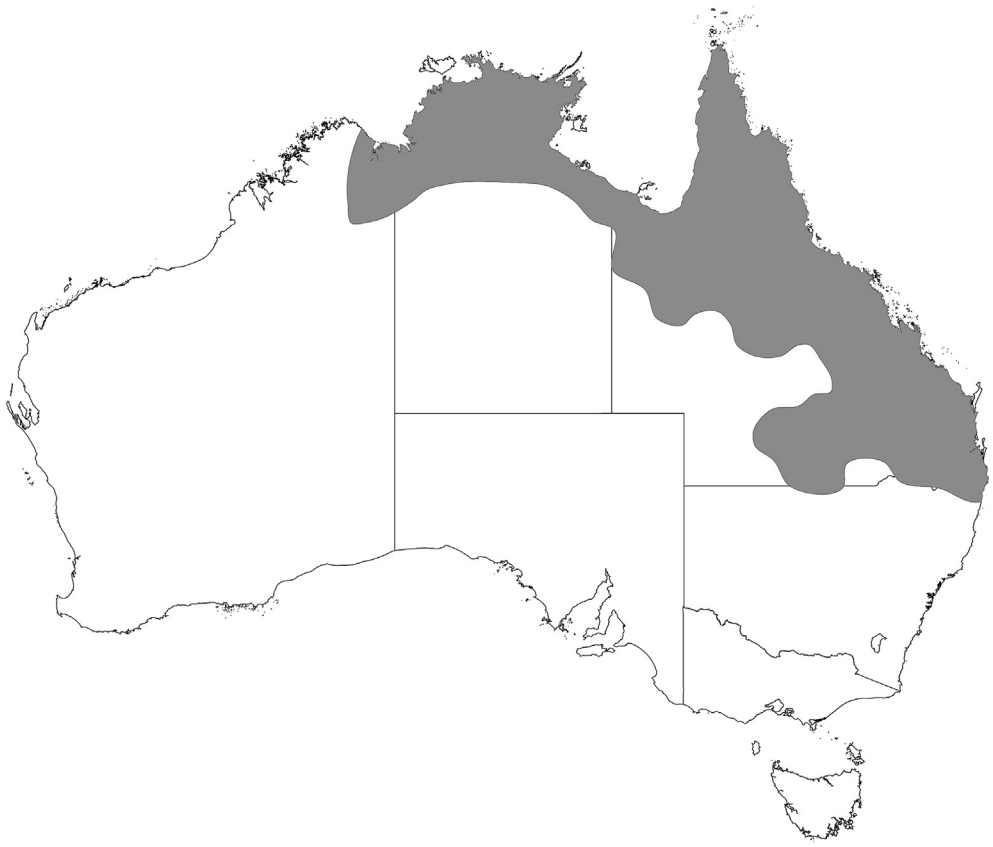

Fig. 3. Known distribution of the cane toad throughout Australia. Since arriving in Queensland, Australia in 1935, cane toads have further expanded their range through New South Wales, the Northern Territory, and into Western Australia. Map created by Georgia Ward-Fear (Tingley et al., In review).

(Brown et al., 2007). Thus, this unusual affliction appears to reflect the highly stressful lifestyle of dispersive toads and the phenotypic traits (such as long legs and high levels of activity) responsible for their high dispersal rate (Brown et al., 2007).

3.4. Flexibility in the immune system

How have these shifts in parasite load shaped the toads themselves? It is commonly proposed that individuals within range-expanding populations will be under selection to reduce investment in traits that do not directly benefit their ability to disperse (such as immune defences, because the body has limited energy stores: Lee and Klasing, 2004; Lee, 2006; Llewelyn et al., 2010). Nonetheless, down-regulation of immune capacity could imperil a founding population as it encounters new threats.

In laboratory trials, toads with longer legs had a reduced stress response (Graham et al., 2012). This is further supported by a positive linear relationship between population age and corticosterone levels following a stressful stimulus (Brown et al., 2015b), because toads at the invasion front tend to have longer hind legs than do those further behind the invasion front (Phillips et al., 2006). Lowered stress responses on the range edge may be adaptive, as over-reactions to common stressors would diminish rates of dispersal (Brown et al., 2015b).

In response to injection with lipopolysaccharide (LPS, an endotoxin found in bacteria), captive-bred toads whose parents originated from close to the invasion front exhibited smaller increases in metabolic rate than did those with parental origins from long-established populations (Llewelyn et al., 2010). Resting metabolic rate was approximately the same across populations (Llewelyn et al., 2010). These findings suggest heritable reduced investment in the immune response to LPS by frontal toads (Llewelyn et al., 2010).

To further clarify divergences in immune function, toads were collected from a field site in the Northern Territory and radio-tracked to quantify dispersal rates (Brown and Shine, 2014). With individuals differing in travel distances by up to 10-fold, immune assays were then conducted on the same toads (Brown and Shine, 2014). Corticosterone levels were not correlated with distance, but more mobile toads exhibited reduced complement-driven bacteria-killing and phagocytic capabilities, and enhanced phytohemagglutinin (PHA)-induced skin-swelling relative to their sedentary counterparts (Brown and Shine, 2014). The activities of complement proteins and phagocytes are part of innate systemic immunity (Ochsenbein and Zinkernagel, 2000), whereas PHA is a stimulant of cell-mediated action (Tella et al., 2008; Demas et al., 2011). These conflicting results require a more nuanced explanation than that of dispersal causing an overall reduction of investment in immunity. Energy is likely reallocated into different branches of the immune system based upon individual utility and cost (Brown and Shine, 2014); but we do not know if movement patterns have influenced immune function rather than the reverse (Brown and Shine, 2014).

More recently, in an attempt to minimize environmental confounds, toads from opposite ends of the species' current Australian range were bred in a "common garden" setting. Offspring with parental origins from the invasion front displayed higher complement-driven bacteria-killing and phagocytic capabilities, but no differences in PHA-induced skin-swelling (Brown et al., 2015c). These findings are discordant with the radio-tracking

experiment. Frontal toads are more dispersive, yet mobility was negatively correlated with the enhanced systemic immune responses that they exhibit (Brown et al., 2015c). One plausible explanation is that the captive-bred toads in the common garden experiment had not undergone the stress imposed by long-distance movements (Brown et al., 2015c). Thus, the systemic component may evolve to be up-regulated in frontal toads because it will be heavily exhausted during their lifetimes due to their arduous lifestyles (Brown et al., 2015c).

3.5. Immunogenetic comparisons across the range

Despite the phenotypic differences observable between Queensland and Western Australian toads, which are suggestive of rapid evolution, genetic diversity is low in all of these invasive populations (Rollins et al., 2015). The major histocompatibility complex (MHC) is a family of genes encoding glycoproteins which function to present foreign antigens that alert other immune effectors (Benacerraf, 1980). MHC class I products primarily target viruses that have infiltrated host cells, translocating viral peptides to the infected cell's surface (Fabre, 1991). This process triggers destruction of that entire cell by cytotoxic T-cells or natural-killer cells (Fabre, 1991). MHC class II products affect extracellular pathogens such as bacteria, which can become engulfed by host phagocytes (Ting and Baldwin, 1993). With the help of class II MHC glycoproteins, macrophages can display the antigens of their ingested targets, signalling for helper T-cells to assist them (Ting and Baldwin, 1993).

In the course of the toad's range expansion from Queensland to Western Australia, MHC class I has lost its remaining allelic diversity on the invasion front, likely owing to genetic drift rather than balancing selection (Lillie et al., 2014). Such relaxed selection could be due to the lack of viral challenges (Lillie et al., 2014). MHC class II diversity, however, has been maintained on the invasion front (Lillie et al., 2016). In this case, selection may be maintained by the plethora of extracellular pathogens or parasites (such as *O. anthropi* and *R. pseudosphaerocephala*) at or near the invasion front (Lillie et al., 2016). Both MHC classes contain very low allelic variation relative to that typically seen in MHC, archetypally demonstrating the effects of bottlenecks (Nei et al., 1975).

Low levels of genetic variation in Australian toads also manifest in their low microsatellite diversity (Leblois et al., 2000), as well as their lack of variation in mitochondrial haplotypes (Slade and Moritz, 1998). Because of these circumstances, it is unclear whether phenotypic differences among toads from different populations are underpinned by genetic variation, or if heritable epigenetic variation may play a role instead.

3.6. Range expansion of lungworms in cane toads

Toads are not the only organisms to have responded to the selection pressures (and other evolutionary forces such as spatial sorting: Shine et al., 2011) imposed by their dispersal across Australia. Comparisons of *R. pseudosphaerocephala* on opposite ends of the toads' Australian range revealed life-history differences associated with (and putatively driven by) variations in toad population density (Kelehear et al., 2012a). At free-living life stages (i.e., between successive hosts), worms closer to the invasion front had larger body sizes, increasing their chances of survival before their next toad encounter. That change may offset the density-imposed diminished likelihood of encountering a new host (Kelehear et al., 2012a). These lungworm populations also exhibited faster development and higher survival to adulthood (Kelehear et al., 2012a). Although range-edge worms laid small numbers of large eggs, range-core worms laid large numbers of small eggs (Kelehear et al., 2012a). Infection intensity, however, did not vary between populations (Kelehear et al., 2012a).

The possibility of lungworms and pentastomes expanding their Australian ranges via the toad raises conservation concerns as toads move farther westward and southward. Western Australia is home to many endemic species of frogs (Aplin and Smith, 2001), some of which are threatened (Hero and Roberts, 2004; Roberts and Hero, 2004). Because it is difficult to predict how successful these parasites are at infecting novel anuran hosts, the impact that they will have on endemic or endangered frog populations in Western Australia is unknown. Studies similar to those described in section 2.3.1.1, which examine the ability of lungworms or pentastomes to infect different frog species, would be a useful place to begin assessing potential damage. However, such studies would only be predictive of the frog species that are tested.

4. Conclusion

The "enemy release" hypothesis asserts that invasive species thrive because they escape from most of the co-evolved threats that they have faced in their native range, and face less threat from species that are indigenous to the introduced range. Many surveys of parasites have been conducted in cane toads around the world, and only one helminth and three protozoans have been documented to persist all the way through to Australia. Although the protozoans exhibit little pathogenicity, the exotic helminth (*Rhabdias pseudosphaerocephala*) significantly reduces toad growth and survival. Although it has yet to be shown to infect native anurans in the wild, laboratory studies have indicated that *Rhabdias pseudosphaerocephala* can infiltrate the bodies of native anurans, causing pathogenesis to widely varying extents in different species. Meanwhile, Australian lungworm parasites can also infiltrate toad bodies, but do not complete their life cycle due to encapsulation of larvae by toad immune defences. These advantages may have aided the successful establishment and massive range expansion seen in the cane toad in Australia.

The cane toad invasion of Australia provides many opportunities for study of the dynamics between introduced hosts, native hosts, introduced parasites, and native parasites. The rapid evolution of multiple phenotypic traits in Australian cane toads, apparently in response to evolutionary pressures on dispersal rate, also facilitate the exploration of how density-dependent disease transmission is affected by dispersal.

Phenotypically, a host's immune defences are moulded by the abundance and diversity of pathogens and parasites, as well as their infectivity. The invasive cane toad has demonstrated significant flexibility in its immune system within 80 years, indicating that rapid evolution has indeed taken place. Such rapid evolution seems paradoxical given low levels of genetic diversity in Australian cane toads, resulting from sequential introduction-imposed bottlenecks followed by expansion-driven drift. This situation suggests that more is at play than simply genetic variation. One logical next step is to investigate the role of epigenetic changes in driving rapid evolution of the cane toad's immune system. Such an approach may clarify the mechanisms by which the toad has thrived within its new home, and the nature of selective pressures imposed by enemies from the past and present.

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