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Specialist enemies, generalist weapons and the potential spread of exotic pathogens: malaria parasites in a highly invasive bird ☆

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ABSTRACT

Pathogens can influence the success of invaders. The Enemy Release Hypothesis predicts invaders encounter reduced pathogen abundance and diversity, while the Novel Weapons Hypothesis predicts invaders carry novel pathogens that spill over to competitors. We tested these hypotheses using avian malaria (haemosporidian) infections in the invasive myna (*Acridotheres tristis*), which was introduced to southeastern Australia from India and was secondarily expanded to the eastern Australian coast. Mynas and native Australian birds were screened in the secondary introduction range for haemosporidians (*Plasmodium* and *Haemoproteus* spp.) and results were combined with published data from the myna's primary introduction and native ranges. We compared malaria prevalence and diversity across myna populations to test for Enemy Release and used phylogeographic analyses to test for exotic strains acting as Novel Weapons. Introduced mynas carried significantly lower parasite diversity than native mynas and significantly lower *Haemoproteus* prevalence than native Australian birds. Despite commonly infecting native species that directly co-occur with mynas, *Haemoproteus* spp. were only recorded in introduced mynas in the primary introduction range and were apparently lost during secondary expansion. In contrast, *Plasmodium* infections were common in all ranges and prevalence was significantly higher in both introduced and native mynas than in native Australian birds. Introduced mynas carried several exotic *Plasmodium* lineages that were shared with native mynas, some of which also infected native Australian birds and two of which are highly invasive in other bioregions. Our results suggest that introduced mynas may benefit through escape from *Haemoproteus* spp. while acting as important reservoirs for *Plasmodium* spp., some of which are known exotic lineages.

1. Introduction

To the detriment of native species, invaders can have competitive advantages in their introduced range that lead to rapid range expansions and the exclusion of native species (DeWalt et al., 2004; Lake and Leishman, 2004). The mechanisms proposed to explain this competitive advantage include release from predators, rapid adaptation to new environments, and changes in host-pathogen interactions (Keane and Crawley, 2002; Lee, 2002). Host-pathogen interactions in particular can influence the way introduced species establish in non-native ranges (Schuler et al., 2012; Adlard et al., 2015), with two primary hypotheses explaining how pathogens might influence invasion success. The Enemy Release Hypothesis predicts invaders encounter a reduced abundance and diversity of pathogens in the introduced range, allowing the avoidance of costly infections and facilitating ecological release (Keane and Crawley, 2002; Dunn and Hatcher, 2015). The Novel Weapons Hypothesis predicts that invaders carry novel pathogens to the introduced range that can subsequently spill over to immunologically naïve competitors (Callaway and Ridenour, 2004; Adlard et al., 2015).

Haemosporidian malaria parasites (*Plasmodium* and related *Haemoproteus* spp.) are vector-borne blood parasites found in birds across the globe (Valkiūnas, 2005; Clark et al., 2014a). Haemosporidian infections in birds can have a range of fitness impacts, from reduced locomotion and fecundity to mortality (Valkiūnas, 2005; Palinauskas et al., 2009; Asghar et al., 2015). Infections are particularly harmful in naïve island hosts, raising

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important concerns for conservation of native avifauna (van Riper III et al., 1986). Recent studies of parasite DNA sequences provide evidence that introduced birds can carry invasive haemosporidian lineages that are detrimental to native birds (Ewen et al., 2012; Hellgren et al., 2014), supporting the Novel Weapons Hypothesis. However, evidence also suggests that introduced birds sometimes encounter a depauperate set of haemosporidian lineages in their introduced range (e.g. house sparrow, *Passer domesticus*; Marzal et al., 2011), supporting the idea for a competitive edge through infection avoidance. Importantly, the two haemosporidian genera display different specificity strategies, with *Plasmodium* lineages often infecting a range of species (generalists) while *Haemoproteus* lineages are more specific (Olsson-Pons et al., 2015). This difference in life history strategies could lead to different capabilities of parasite genera to exhibit Enemy Release or to act as Novel Weapons, particularly in Australia where many birds often carry a high prevalence of *Haemoproteus* but a low prevalence of *Plasmodium* spp. (Adlard et al., 2004; Clark et al., 2015). Due to their cosmopolitan distribution and the existence of molecular techniques to characterise infections, avian haemosporidian parasites present a tractable system to study the influences of pathogens in the spread of invaders.

The common myna (*Acridotheres tristis*) is considered by the International Union for Conservation of Nature (IUCN) as one of the world's most invasive avian species (www.issg.org). Introduced mynas are known to spread weeds, damage crops and aggressively defend feeding locations (Pell and Tidemann, 1997). Increases in myna abundance often correlate with declines of native species, possibly due to aggressive competition for roosting and feeding sites (Grarock et al., 2012, 2014) or to the myna's innovative ability to occupy diverse ecological niches (Sol et al., 2011; Griffin and Diquelou, 2015). Following their primary introduction to southeastern (SE) Australia from India between 1862 and 1872, common mynas from the introduced stock were secondarily introduced along the eastern Australian coast, from where they have spread rapidly (see Supplementary Fig. S1 for map of introduced ranges; Martin, 1996). This sequential pattern of introductions and subsequent expansions may have presented multiple opportunities for mynas to undergo Enemy Release. Due to this expansion and due to their detrimental impacts on native birds, mynas are an ideal study group to investigate mechanisms driving the spread of invaders (Ishtiaq et al., 2006; Grarock et al., 2013).

In its native range in India and SE Asia, the myna carries a high prevalence and diversity of haemosporidian parasites (Ishtiaq et al., 2006, 2007). Importantly, native-range mynas carry some of the most widespread and potentially invasive *Plasmodium* lineages currently known (Bensch et al., 2009). These include the parasite responsible for the decline of Hawaiian honeycreepers (*Plasmodium relictum* lineage GRW04; Beadell et al., 2006), as well as *P. relictum* lineage SGS1 and *Plasmodium elongatum* lineage GRW06, both of which are spread by introduced birds in New Zealand (Baillie et al., 2012; Ewen et al., 2012; Ha et al., 2013; Schoener et al., 2013). The potential for mynas to carry introduced haemosporidian parasites was highlighted by Ishtiaq et al. (2006), whose screening of 26 mynas collected from the primary introduction range in the early 1980s confirmed the first detection of lineage GRW04 in Australia. Nevertheless, efforts to identify introduced pathogens require robust phylogeographic analyses that can be hampered by a lack of adequate sampling in the introduced and/or native range (Ishtiaq et al., 2006; Hu et al., 2011), and we currently do not have detailed knowledge of the haemosporidian lineages infecting Australian native birds (Clark et al., 2014a, 2015). Moreover, studying the assembly of parasite communities in primary and secondary introduction ranges offers a unique opportunity to understand the processes involved during invasive species' range expansions.

Here, we screen introduced mynas and co-occurring native birds in the myna's secondary introduction range in Australia for avian haemosporidian infection. Our results are then combined with published data from the myna's primary introduction range (Ishtiaq et al., 2006) to assess the relative roles of the Enemy Release and Novel Weapons Hypotheses in contributing to the myna's invasion success. We characterised infections using parasite cytochrome-*b* (cyt-*b*) sequences, and we were able to discriminate between native and potentially introduced parasites by using published parasite cyt-*b* lineages from Australia and the introduced myna's native range to construct regional phylogenies. These data are used to test the following predictions. Enemy Release predicts: (i) lower parasite prevalence and diversity for introduced mynas compared with native mynas, and (ii) lower parasite prevalence in introduced mynas compared with native birds in Australia. Novel Weapons predicts: (i) introduced mynas act as a reservoir for introduced parasite lineages, some of which may be shared with native-range mynas, and (ii) some of these potentially introduced lineages will spill over to Australian native species.

2. Materials and methods

We captured native birds using mistnets at field sites in northern New South Wales (NSW) and southern Queensland (QLD), Australia between April 2012 and December 2013. Field sites covered a broad range of habitats including open Eucalypt forest and residential gardens, many of which overlapped with territories used by mynas. A blood sample was taken from the wing vein of each bird and stored in lysis buffer (1% SDS, 20 mM NaCl, 10 mM TRIS pH 8.0 and 10 mM EDTA pH 8.0). Individuals were banded with an Australian Bird and Bat Banding Scheme (ABBBS) metal band and released at the site of capture. A common myna culling program operates in NSW and QLD, and all mynas used for this study were caught as part of this program. Mynas were caught in the secondary introduction range in northern NSW and southern QLD using baited walk-in traps (see Supplementary Fig. S1 for sampling locations). Individuals were then transported to veterinary clinics where blood samples were collected prior to euthanisation by veterinarians.

2.1. Molecular methods and inclusion of published data

DNA was extracted from samples using ammonium acetate/ethanol precipitation (Richardson et al., 2001). We molecularly sexed each sample following the method of Griffiths et al. (1998) to confirm extraction quality and prevent false negatives. Samples were screened for avian haemosporidian DNA (*Plasmodium* and *Haemoproteus*) using nested PCR to amplify 479 bp of the parasite cyt-*b* gene (Waldenström et al., 2004; see Supplementary Table S1 for PCR conditions). Individual flip-cap PCR tubes were used to prevent contamination, and at least three positive and three negative controls were included in each PCR run. All samples were screened twice to reduce false negatives. Positive amplifications were sequenced at Macrogen (Seoul, South Korea). Parasite sequences were aligned in GENEIOUS 5.4 (Biomatters, Auckland, New Zealand) and identified by comparison with sequences on GenBank and the MalAvi database (Bensch et al., 2009). In addition to our sample of native forest birds, we aimed to improve phylogeographic resolution by including *Plasmodium* sequences that were amplified from migratory wader species that stopover in SE Asia before wintering in SE QLD (*Limosa lapponica* and *Calidris ruficollis*; Clark et al., unpublished data).

We combined our results with published data from Australia and India/SE Asia for comparisons of parasite prevalence, diversity