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Prevalence and evolutionary relationships of haematozoan parasites in native versus introduced populations of common myna *Acridotheres tristis*

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The success of introduced species is frequently explained by their escape from natural enemies in the introduced region. We tested the enemy release hypothesis with respect to two well studied blood parasite genera (*Plasmodium* and *Haemoproteus*) in native and six introduced populations of the common myna *Acridotheres tristis*. Not all comparisons of introduced populations to the native population were consistent with expectations of the enemy release hypothesis. Native populations show greater overall parasite prevalence than introduced populations, but the lower prevalence in introduced populations is driven by low prevalence in two populations on oceanic islands (Fiji and Hawaii). When these are excluded, prevalence does not differ significantly. We found a similar number of parasite lineages in native populations compared to all introduced populations. Although there is some evidence that common mynas may have carried parasite lineages from native to introduced locations, and also that introduced populations may have become infected with novel parasite lineages, it may be difficult to differentiate between parasites that are native and introduced, because malarial parasite lineages often do not show regional or host specificity.

Keywords: *Acridotheres tristis*; common myna; enemy release hypothesis; *Haemoproteus*; introduced; native

1. INTRODUCTION

Release from parasites, predators or competitors are considered likely reasons for the success of introduced species in their introduced regions by the 'enemy release hypothesis' (Torchin *et al.* 2001, 2003; Clay 2003; Font 2003; Mitchell & Power 2003; Poulin & Mouillot 2003; Tsutsui *et al.* 2003). Conversely, in the process of introduction, a species may transport a subset of its native parasites to the new range (Delvinquier & Freeland 1988; Torchin *et al.* 2003) and become infected by local parasite faunas in their new range (Font & Tate 1994; Torchin *et al.* 1996).

Vector-transmitted diseases such as malaria provide an alternative parasite model for examining this hypothesis since parasite abundance depends on both the presence of an appropriate host as well as a competent vector (Bennett *et al.* 1974; Apanius *et al.* 2000; Sol *et al.* 2000). In birds, *Plasmodium* spp. and *Haemoproteus* spp. have been shown to be pathogenic, and exhibit varying host specificity and modes of transmission (Atkinson & van Riper 1991). Recently, it has become possible to characterize evolutionarily distinct parasite lineages with molecular techniques (polymerase chain reaction, PCR; Bensch *et al.* 2000; Fallon *et al.* 2003*a*; Beadell *et al.* 2004). Owing to its wide geographical and host distribution (Bensch *et al.* 2000; Ricklefs & Fallon 2002; Waldenström *et al.* 2002) and potentially deleterious effects on health (Caum 1933; Warner 1968; Atkinson & van Riper 1991; Bennett *et al.* 1993) and reproductive success (e.g. Marzal *et al.* 2005) of hosts, avian malaria makes an interesting system for testing the enemy release hypothesis.

In this study, we assess the prevalence and distribution of malarial parasite lineages in the common myna *Acridotheres tristis* (family: Sturnidae), native to southern Asia (Ali & Ripley 1972) that has been introduced to many parts of the world. The common myna was primarily introduced as a control for insect pests during the period from 1862 to 1900, but also could have been secondarily introduced via accidental escapes or releases of cagebirds, and is now found throughout much of the Old World and on several Pacific islands (Long 1981). Dates of introduction of common mynas are well documented (table 1), but not the inoculum numbers and regional sources

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Table 1. Prevalence of *Plasmodium* and *Haemoproteus* in native and introduced myna populations. (Prevalence data include infection recorded by the polymerase chain reaction using three screening primers, including restriction enzyme primers.)

			Plasmodium		*Haemoproteus*	
regions	period of introduction	sample size	no.	infected (%)	no.	infected (%)
introduced populations						
Australia	1862–1872	26	12	46	5	19
Fiji	1890–1900	10	0	0	0	0
Hawaii	1865	92	9	10	0	0
New Zealand	1895	41	22	54	3	7
South Africa	1888–1900	10	8	80	0	0
Cook Islands	1920 (from NZ)	22	9	40	4	18
native populations						
India		96	48	50	15	1
Haryana		15	10	66	3	20
Uttar Pradesh		10	3	30	0	0
Gujarat		1	0	0	1	100
Madhya Pradesh		8	5	63	2	25
Maharashtra		8	5	63	1	13
West Bengal		9	3	67	0	0
Orissa		10	4	40	1	10
Andhra Pradesh		10	5	50	1	10
Karnataka		10	8	80	4	40
Tamil Nadu		10	4	40	1	10
Assam		5	1	20	1	20

(Long 1981). All of the introduced populations in our comparison (Australia, Fiji, Hawaii, New Zealand and South Africa), except the Cook Islands populations, which were introduced from New Zealand, were founded directly with Indian stock (Long 1981). Thus, they provide reasonable samples for comparative analysis of haematozoan parasites between native (India) and six introduced populations. To our knowledge, this is the first comparative study conducted on blood parasites in an avian host that has been introduced to multiple regions from a common native region. In this study, we explore, using molecular methods, (i) whether parasite prevalence differs among native and introduced populations of mynas, (ii) whether we can differentiate between Indian origin and local origin parasites in introduced myna populations, (iii) to what extent malarial lineages are widespread and generalized in terms of host specificity and geography and (iv) whether parasite lineages acquired by introduced mynas are more genetically similar to parasite lineages encountered in their native range?

2. MATERIAL AND METHODS

(a) *Sample collection and preparation*

We analysed samples ($n=96$) from 11 myna populations in India (see figure 1). These included tissue samples ($n=76$) collected in 1980s by A. J. Baker and blood samples ($n=20$) collected in 2003 by F. Ishtiaq (there was no difference in prevalence between the two types of sample: $\chi^2_1=0.14$, $p=0.70$). Samples were collected at sites throughout India, including historic export points of birds (Baker & Moeed 1987). We also analysed samples from six introduced populations: tissues from Australia ($n=26$), Fiji ($n=10$), Hawaii ($n=92$), New Zealand ($n=41$) and South Africa ($n=10$) (Baker & Moeed 1987; Fleischer *et al.* 1991), and dried blood smears from the Cook islands ($n=22$; Steadman *et al.* 1990). We extracted DNA using DNeasy kits (Qiagen) as per the manufacturer's instructions. Each extraction included a negative control, which was screened to detect potential contamination.

(b) *Molecular analysis*

Samples were screened with two sets of screening primers (F2/R2 (91 bp) and 850F/1024R (167 bp); Beadell *et al.* 2004) designed to amplify small fragments of haematozoan parasite mtDNA (cytochrome *b* gene and *COIII* gene, respectively). We also screened these samples with another set of primers 213F (5′-GAG CTA TGA CGC TAT CGA-3′) and 372R (5′-GGA ATG AGA GTT CAC CGT TA-3′), to amplify a 160 bp fragment of DNA encoding two restriction sites, which are diagnostic for *Plasmodium* and *Haemoproteus* (Beadell & Fleischer 2005). Mixed infections were revealed by restriction patterns showing two or more bands or by multiple peaks in sequence chromatograms. Screening with three different primer sets increased the likelihood of detecting diverse parasite lineages. To help insure that failure to detect a parasite was not due to poor DNA extractions, we amplified a small fragment (347 bp) of avian cytochrome *b* DNA using primers cytb1 and cytb2, following the methods described in Kocher *et al.* (1989). These PCRs were successful in all cases.

For those samples that were positive for parasites based on the above tests, we amplified a larger fragment (351 bp) using primers FIFI (5′-GGG TCA AAT GAG TTT CTGG-3′) and 4292 RW2 (5′-TGG ACC AAT ATG TAR AGG AGT-3′) at an annealing temperature of 51 °C, or primers F2 and 4292RW2 (256 bp) at 52 °C. We purified the largest of the PCR products available using Qiaquick kits (Qiagen) and sequenced the fragment on an ABI 3100 Sequencer (Applied Biosystems, Inc.). Sequences were assembled, aligned and edited using SEQUENCHER v. 4.1.

(c) *Phylogenetic analysis*

We estimated the parasite phylogenetic relationships among parasite lineages using samples for which we had at least 256 or 351 bp of cytochrome *b* sequence. Based on the phylogeny in

region	total samples
Haryana	15
Uttar Pradesh	10
Gujarat	1
Madhya Pradesh	8
Maharashtra	8
West Bengal	9
Orissa	10
Andhra Pradesh	10
Karanataka	10
Tamil Nadu	10
Assam	5

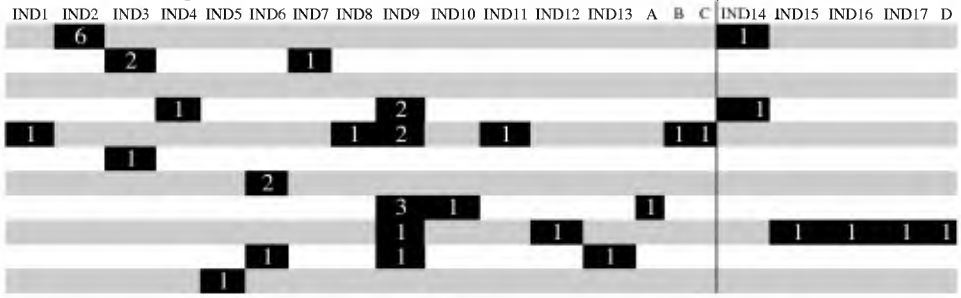

Figure 1. Common myna sampling sites in India (on map). Below is the distribution of *Plasmodium* and *Haemoproteus* lineages based on mtDNA sequence across the sampling sites in India.

Perkins & Schall (2002), we used mammalian *Plasmodium* sequences as outgroups for rooting the tree (GenBank accession numbers AY06914, AF069624, AF055587, AY099051, AY283019 and AF069610). We used the neighbour-joining method with a Kimura-2-parameter distance matrix in MEGA 2.1 (Kumar *et al.* 2001). The inclusion of lineages from previous studies of avian malaria parasites with known morphology (Bensch *et al.* 2000; Beadell *et al.* 2004; Beadell & Fleischer 2005) allowed us to define *Plasmodium* and *Haemoproteus* clades, but we did not include these sequences in final trees. We used MEGA 2.1 (Kumar *et al.* 2001) to estimate pairwise distances between populations.

To further explore the pattern of distribution of parasite lineages between native and introduced populations, we constructed a tree of parasite lineages in the common myna, along with parasite lineages from 105 host species ($n=428$ birds) in Australia (Beadell *et al.* 2004), 16 host species ($n=171$) in Southern Africa (R. C. Fleischer *et al.*, unpublished; Beadell *et al.*, submitted), 112 species ($n=323$) in Uruguay, 53 species ($n=195$) in Guyana (Durrant *et al.*, in press), 37 species ($n=183$) in India, 129 species ($n=344$) in Myanmar and 46 species ($n=181$) in South Korea (Ishtiaq *et al.* submitted) and 81 species ($n=299$) in continental Africa and Indian Ocean Islands (Ishtiaq *et al.*, in preparation).

(d) *Statistical analysis*

To assess differences in prevalence of *Haemoproteus* and *Plasmodium* among native and introduced populations, we performed an ANOVA (GLM in SAS v. 8.2; SAS Institute) on arcsine square-root transformed prevalence. We estimated the proportion of variance attributable to different populations using the NESTED procedure in GLM in SAS, nesting different sampling sites within each country. We also used contingency table analysis to compare frequency differences, using χ^2 or Fisher's exact tests to determine significance. ESTIMATES v. 7.0 (Colwell 2004) was used to calculate a rarefaction (species–area) curve and the non-parametric Chao2 estimator. The Chao2 estimator uses the observed number of species in a sample, combined with the number of species appearing in only one and two samples (see Colwell & Coddington 1994) to estimate the number of species expected in the sample. It has been shown to have excellent predictive power when data are in the form of presence–absence and sample sizes are small.

3. RESULTS

(a) *Parasite prevalence*

We screened 297 common mynas from all regions. In India, 63 of 96 (65%) individuals tested positive for

malarial infection. Prevalence of parasites in Indian populations with greater than five individuals sampled, however, varied from 30 to 80% for *Plasmodium* spp. and 0 to 40% for *Haemoproteus* spp. (table 1). Nevertheless, among-population prevalence did not vary significantly by contingency table analysis (for *Plasmodium* $\chi^2_8=9.5$, $p=0.30$; for *Haemoproteus* $\chi^2_8=10.1$, $p=0.26$).

In Australia, 17 of 26 (65%) individuals tested positive for malarial infection, and in New Zealand, 25 of 41 (61%) mynas tested positive. In South Africa, 8 of 10 (80%) tested positive, all with *Plasmodium* spp. and in Hawaii 9 of 92 (10%) mynas were positive, and all were infected with *Plasmodium*. In the Cook Islands, 16 of 22 (72%) tested positive. We found no infection in 10 mynas from Fiji. Based on the nested ANOVA, prevalence of parasites did not vary among countries (*Haemoproteus* spp. $F=1.38$, d.f.$=5$, $p=0.282$; *Plasmodium* spp. $F=2.52$, d.f.$=5$, $p=0.072$). However, country grouping explained 32% of the total variance in *Plasmodium* prevalence among different regions. The overall parasite prevalence in India (65%) was significantly higher than prevalence in Hawaii (10%; $\chi^2=62.0$, $p<0.0001$) and Fiji (0%; Fisher's exact test $p<0.001$), but not in Australia, New Zealand, South Africa or Cook Islands ($\chi^2=1.7$, $p=0.61$). Prevalence by parasites is shown in table 1. There was no significant difference in the proportion of infections that were *Haemoproteus* versus *Plasmodium* between Indian (15%) and introduced population samples (13.6%; $\chi^2=0.07$, $p=0.79$).

We constructed a rarefaction curve based on Colwell & Coddington (1994) and Gotelli & Colwell (2001) for Indian common myna populations (see electronic supplementary material, appendix 2) to estimate the accumulation of unique haplotypes. The mean Chao2 estimate for 16 *Plasmodium* lineages in India based on 33 sequences was 27.9 ± 15.16, and the Chao2 estimate for five lineages of *Haemoproteus* was 6.40 ± 3.95 based on six sequences. Chao2 values indicate the richness of lineages expected in a sampled population. For *Plasmodium*, the Chao2 estimate of 27.9 is almost double the number of lineages ($n=16$) found in the myna populations in India. This discrepancy suggests that additional sampling would be required to exhaustively determine the number of *Plasmodium* lineages in India.

(b) *Phylogenetic analysis*

Of 138 infections detected in myna samples, we obtained lineage information from 94. In the remaining cases ($n=44$), we were not able to identify lineages due to non-amplification of large pieces, poor sequence or multiple infections ($n=6$). Note that samples for which we could differentiate the parasite genera by restriction enzyme digestion were used to calculate the prevalence data in table 1; here only sequenced lineages are compared. Among infections with readable sequence, we identified 33 unique mitochondrial lineages across all countries (GenBank sequences in electronic supplementary material, appendix 1); 7 *Haemoproteus* and 26 *Plasmodium* (figure 2). In 22 cases, sequences obtained from primers F2/R2 (91 bp) were used to identify the parasite lineage, but were not used in the phylogenetic analysis. We found 16 *Plasmodium* lineages and 5 *Haemoproteus* lineages in mynas from India. In Australia, we recovered four *Plasmodium* lineages and one *Haemoproteus* lineage. We found only *Plasmodium* lineages in South African mynas (five lineages) and New Zealand (seven lineages, figure 2). We found six *Plasmodium* and two *Haemoproteus* lineages in the Cook Islands and several individuals were infected with multiple haplotypes which were retrieved by different primer sets and amplifications. Hawaiian mynas harboured only a single mtDNA lineage (IND9/GRW4 AY099041; Bensch *et al.* 2000), the *Plasmodium* haplotype that predominates in Hawaii (Beadell *et al.* submitted). We also found this lineage in India ($n=9$), Australia ($n=3$), New Zealand ($n=1$) and Cook Islands ($n=5$). Six additional lineages were detected, each of which were shared by mynas in India and populations in New Zealand (IND 10), South Africa (IND 2), Australia (IND 1) or the Cook Islands (IND 8, IND 11, IND 15)

The phylogenetic tree differentiates *Haemoproteus* and *Plasmodium* into separate clades, although bootstrap support is low (figure 2). In addition, there are two major clades within *Plasmodium*, but again, bootstrap support is low. A well supported topology is not necessary for our analyses. Sequences from both Indian and introduced populations occur in each of these clades, and there is no evidence of geographical structuring of parasite lineages (although we do obtain evidence of some geographical structure in other studies, e.g. Beadell *et al.* submitted).

In figure 2, we show the distribution of the myna parasite lineages in other host species in India, Australia, South Africa and Hawaii (see electronic supplementary material, appendix 1, for distribution in other regions). We found IND 15, IND 16 and IND 17, the three *Haemoproteus* lineages, were shared by four host species in South Africa, Australia and India (figure 2). In the *Plasmodium* clade, IND 2, IND 3, IND 4, IND 7, IND 8, IND 9, IND 10, IND 11 and NZ 2 were shared by other host species in more than one region.

To evaluate the differentiation between parasite lineages found in mynas within India and between mynas in India and introduced populations, we calculated average pairwise genetic distances within and between populations. In general, the average genetic distance between parasites found in India and those found in introduced populations (0.014–0.052) was no greater than the average pairwise distance of parasites found within India (0.054 ± 0.008; table 2).

4. DISCUSSION

(a) *Prevalence of parasites*

The prediction of the enemy release hypothesis of reduced parasite prevalence in introduced populations appears only to hold for two isolated island populations (Hawaii and Fiji), and not for two continental and two additional oceanic island populations. We had the same number of samples ($n=10$) from Fiji and South Africa, but had statistically significant differences in prevalence between the two. This suggests that birds in Fiji may not be exposed to haematozoan infection as much as those in South Africa. However, increased sampling would provide more strength to our findings, as previous studies have demonstrated temporal and spatial variations in malaria infection (Schall & Marghoob 1995; Bensch & Akesson 2003). The absence of blood parasites in mynas in Fiji could be attributed to the lack of established or appropriate vector populations there, which appears to

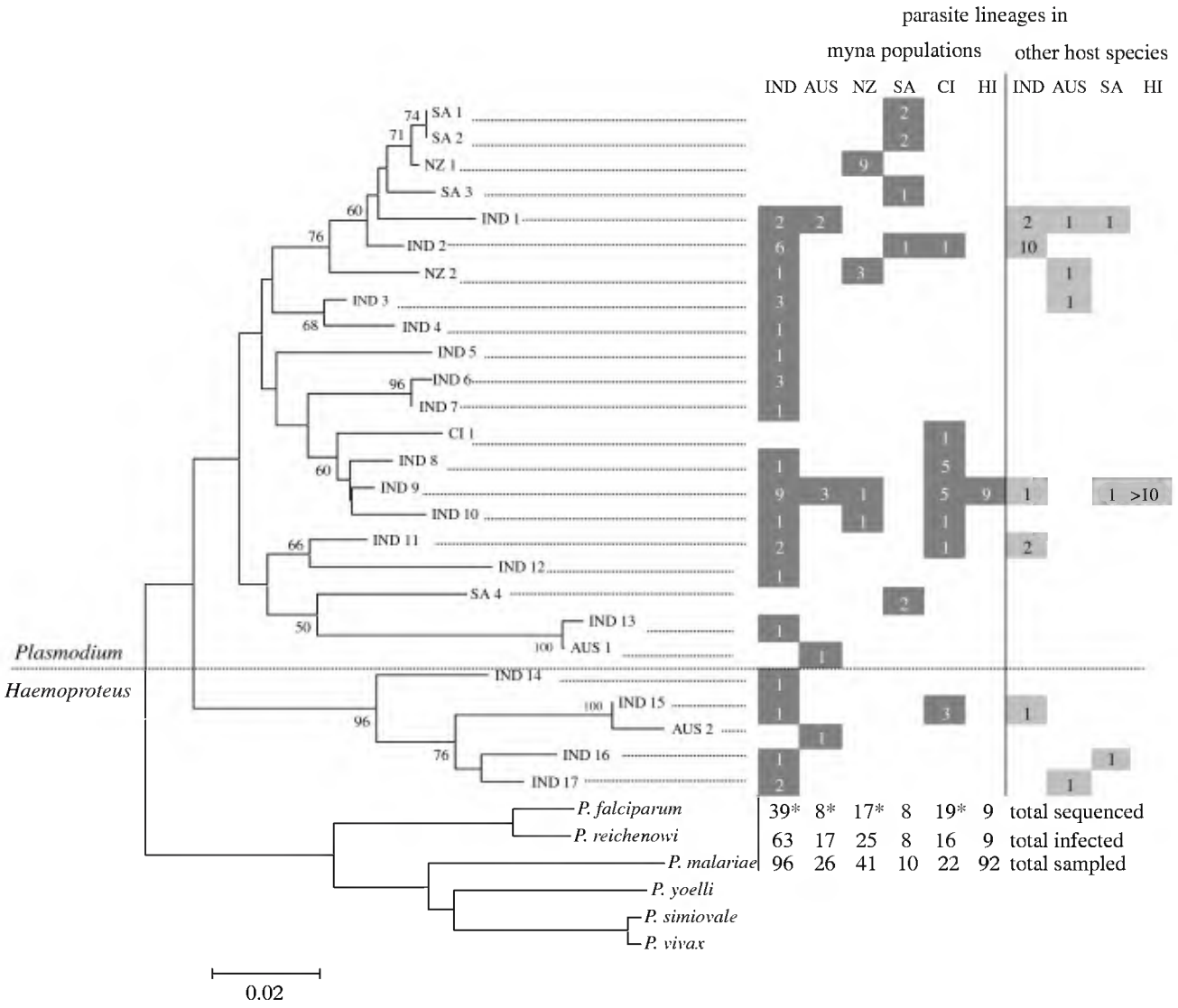

Figure 2. Neighbour-joining tree for the unique haplotypes ($n=37$) of avian malaria parasites in common myna *Acridotheres tristis* with their distribution in native and introduced regions. IND, India; AUS, Australia; SA, South Africa; NZ, New Zealand; CI, Cook Islands; HI, Hawaii. *Total number of independent sequences obtained from infected individuals in India, Australia, New Zealand and Cook Islands, including several 91 bp sequences which are not used phylogenetic analysis. For Cook Islands and IND 1, individuals were infected with two different haplotypes. Note: none of the birds in Fiji ($n=10$) showed *Plasmodium* or *Haemoproteus* infection. SA1 and SA2 differ by one base pair.

Table 2. Uncorrected pairwise genetic distances of *Plasmodium* lineages among regions. (Values in bold show within-population distances (mean and s.e.)

	Hawaii	India	Australia	New Zealand	South Africa	Cook Islands
Hawaii	**0**					
India	0.032 (0.007)	**0.054 (0.008)**				
Australia	0.036 (0.008)	0.046 (0.009)	**0.054 (0.010)**			
New Zealand	0.048 (0.013)	0.042 (0.009)	0.046 (0.009)	**0.021 (0.008)**		
South Africa	0.050 (0.012)	0.047 (0.009)	0.051 (0.010)	0.032 (0.006)	**0.036 (0.008)**	
Cook Islands	0.014 (0.004)	0.037 (0.007)	0.043 (0.008)	0.049 (0.011)	0.052 (0.011)	**0.026 (0.007)**

be the reason for the lack of haematozoa in birds living in the Arctic tundra (Bennett *et al.* 1992; Earlé & Underhill 1993).

Microscopic examination of blood smears by Steadman *et al.* (1990) (used for PCR in this study) revealed no parasites in introduced or indigenous birds on the Cook Islands. This could be due to low-intensity peripheral parasitemia and is consistent with some previous studies comparing PCR and blood smears (Cooper & Anwar 2001; Jarvi *et al.* 2002; Richard *et al.* 2002). The Jarvi *et al.* (2002) study is particularly relevant, as these authors found a haematozoan prevalence of 59% of 188 birds of eight species in Samoa versus and 0% from 214 smears evaluated. In natural populations of birds, individuals

sampled showing no infection either have not been infected with malarial parasites or have completely cleared infection, and those showing low parasitemia level are often in a chronic stage. The high prevalence we found using our sensitive PCR test on the Cook Islands smears is likely a result of chronic infections, as there is documentation of appropriate native mosquito and other haematozoan vectors in the archipelago (Steadman *et al.* 1990).

Prevalence was low in Hawaii (10%) with only a single lineage (IND9/GRW4) found. Only a single species, *Plasmodium relictum*, is reported from Hawaii (Laird & van Riper 1981 in Shehata *et al.* 2001), and likely only a single vector (*Culex quinquefasciatus*; Fonseca *et al.* 2000). Oddly, the prevalence of the *Plasmodium* lineage in Hawaii is almost identical to its prevalence in India (9.4%).

(b) *Differentiating between Indian and local parasites in introduced myna populations*

We found more mtDNA lineages (16 *Plasmodium* and 5 *Haemoproteus*) in India than in any single region. However, we screened 96 mynas in India, and, other than Hawaii, no more than 41 individuals in any other sample; thus, the difference in number of lineages could be due to differences in sampling effort. Supporting this, we found 17 unique lineages of *Plasmodium* and 3 of *Haemoproteus* among 99 individuals sampled in all the introduced populations combined (except Hawaii). In total, common mynas were infected by 37 different mtDNA lineages of haematozoan. This is a high number of lineages in comparison to other intensively studied bird species (e.g. 5–8 lineages per species in four Caribbean bird hosts, Fallon *et al.* 2003*b*; nine lineages in *Luscinia svevica*, Hellgren 2005; 12 lineages in *Dendroica caerulescens*, Fallon *et al.*, submitted). Yet, our rarefaction curve and Chao2 estimator indicated that we have not likely sampled all of the lineages expected to occur in common mynas in India, suggesting the need for additional sampling.

The phylogenetic tree does indicate possible transmission from native to introduced regions as shown by four *Plasmodium* lineages that are shared between the native Indian and six introduced populations. The presence of the Hawaiian lineage in India (IND 9), which is the most common lineage sampled in five Indian populations (nine individuals) and also in Australia, New Zealand and Cook Islands, could indicate that common mynas were the introduced species that carried this lineage to Hawaii in 1865. However, historical data suggest that the parasite came much later in Hawaii (van Riper *et al.* 1986; Atkinson & van Riper 1991; Fleischer *et al.*, unpublished).

Across the 11 populations sampled in India, we found 33 sequences and 16 distinct *Plasmodium* lineages. Of these, 81% of the infections could be assigned to lineages sampled only once across different populations, but three lineages were found in at least two distant regions. However, we do not have the statistical power to test whether lineage frequencies are different among regions. Based on our rarefaction analysis, some of the lineages found in the introduced populations may be undetected in India, and actually could have been carried by mynas. Thus, the four shared lineages must be considered a minimum estimate of the lineages transmitted from native to introduced regions. On the other hand, the absence of any lineage shared only between introduced populations and not with the native region suggests that India could have been the centre of transmission of these parasite lineages and the connecting point to the introduced regions. However, additional sampling and genetic data from the native myna populations are necessary to gain an estimate of the impact of missing parasites on questions about native range versus introduced range transmission. Lastly, in all of this we make the perhaps incorrect (e.g. Bensch & Akesson 2003) assumption that lineages of parasites found recently in common mynas in India reflect the lineages that were there at the time of introduction more than a 100 years ago.

(c) *Geographical limitations of particular malaria lineages*

Avian haematozoan parasite lineages appear to lack a well defined regional structure, unlike other parasite communities such as helminthes and *Wolbachia* (Torchin *et al.* 2003; Tsutsui *et al.* 2003) that provide insights into colonization by novel parasite lineages and extinction of native parasite lineages. To calculate the rate of colonization by new lineages and to assess missing lineages, it is important to have an idea of the number of lineages confined to one particular region. This possibility is not well supported by this and previous findings about the distributions of malarial parasites: geographical and temporal patterns of lineages observed in previous studies of wintering and breeding hosts (Bensch *et al.* 2000; Ricklefs & Fallon 2002; Waldenström *et al.* 2002) suggest that avian host species can acquire novel parasites at different locations and spread them widely across the world. Another factor is that our introduced myna populations went through population bottlenecks upon introduction (Baker & Moeed 1987) that could have impeded subsequent transmission of parasites present in the founder population (Ricklefs & Cox 1972; Torchin *et al.* 2003).

The distribution of the myna parasite lineages in other host species in India, Australia, South Africa and Hawaii emphasizes that there is little or no regional structure of parasite lineages and that they lack host specificity. Thus, it is difficult to be certain that mynas carried these native parasites with them versus the same lineages being local and infecting the introduced populations.

(d) *Are the novel parasite lineages more genetically like the parasite lineages encountered in the native range?*

If we assume that myna parasite lineages were transmitted from India to introduced regions, we would expect a lower genetic distance between myna parasite lineages of native and introduced regions. Average pairwise distances within native and introduced populations are similar to the between-population distances (table 2). This suggests that these myna parasite lineages are weakly differentiated. We calculated an average pairwise distance between parasite lineages encountered in India among other (non-myna) host species (Ishtiaq *et al.* submitted) and parasite lineages obtained in mynas in India. We found no difference in the genetic distance between the two groups (0.049 ± 0.008) and that within mynas (0.054 ± 0.008) or within other host species (0.049 ± 0.008). Lack of allozyme divergence among localities in the native populations (Baker & Moeed

1987) suggests a high level of connectivity among these populations and this connectivity probably explains low genetic distance in parasites within myna population in India.

5. CONCLUSIONS

Not all comparisons of introduced populations to the native population were consistent with expectations of the enemy release hypothesis. Native populations show greater overall parasite prevalence than introduced populations, but the lower prevalence in introduced populations is driven by two populations on oceanic islands. Parasite diversity in India is higher than any single introduced population, but differing sample sizes could compromise this finding. There is some evidence that common mynas carried parasite lineages from native to introduced locations and that introduced populations became infected with novel parasite lineages. However, it may be difficult to differentiate between parasites that are native and introduced because malarial parasite lineages often do not show regional or host specificity.

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