Title: Prevalence and intensity of avian malaria in a quail hybrid zone

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Authorship Contributions

AMR performed data analyses, constructed the first draft of the manuscript, and revised the manuscript based on coauthors' suggestions. JMG established the study system of hybridizing quail, the study site and field methodology, collected blood samples, identified individuals as California quail, Gambel's quail, or hybrid quail, and collated the precipitation data. JBW conducted parasite quantification. CNK created figures. JMG and JBW were responsible for the initial project conceptualization, under the supervision of Rosemary Grant. All authors were involved in the later stages of project development and revision of the written manuscript.

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Prevalence and intensity of avian malaria in a quail hybrid zone

Abstract

Hybridization is a common and important stage in species formation in plants and animals. The evolutionary consequences of hybridization depend not only on reproductive compatibility between sympatric species, but also on factors like vulnerability to each other's predators and parasites. We examine infection patterns of the blood parasite *Haemoproteus lophortyx*, a causative agent of avian malaria, at a site in the contact zone between California quail (*Callipepla californica*) and Gambel's quail (*C. gambelii*). We tested whether species identity, sex, and year predicted infection status and intensity. While we found no effect of sex on the status or intensity of infection, we found differences in infection status and intensity across species and between years. The prevalence of infection in California and hybrid quail was lower than in Gambel's quail. Once infected, however, California and hybrid quail had higher infection intensities than Gambel's quail. California and hybrid quail exhibited no significant differences in prevalence or intensity of infection. These findings suggest that infection by *H. lophortyx* has the potential to influence species barrier dynamics in this system, however, more work is necessary to determine the exact evolutionary consequences of this blood parasite.

Keywords: *Haemoproteus lophortyx*, blood parasite, *Callipepla*, Galliform, species barriers

Introduction

Understanding the proximate and ultimate mechanisms underlying the maintenance of species barriers in localities where closely related congeners overlap is a major focus of evolutionary biology. Recently, parasitism has been put forth as a mechanism that may work to promote the formation, preservation, or breakdown of species barriers (reviewed in Theodosopoulos et al. 2019). Although tests of this hypothesis may be conducted in the lab (e.g., Goldberg et al. 2005; Hedrick et al. 2006; Brucker and Bordenstein 2013; González et al. 2014; Liang et al. 2018), hybrid zones provide scientists the opportunity to study the effects of factors influencing gene flow, such as parasitism, in a natural setting (Harrison and Larson 2014; Kenney and Sweigart 2016; Mořkovský et al. 2018; Theodosopoulos et al. 2019). Because hybridization may have major implications for both host species as well as their parasites, it is important to examine coevolutionary dynamics between the two across hybrid zones (Hafner et al. 1998; Tompkins et al. 2003; Reullier et al. 2005; Theodosopoulos et al. 2019).

From the viewpoint of parasites, host hybrid zones can function as population sinks, population sources, or as "bridges" that enable the colonization of a new host species (Whitham 1989; Floate and Whitham 1993; Strauss 1994). Parasite specificity can either limit or facilitate expansion of a parasite's range into a new host species, with parasites with lower specificity moving more easily between host species (Bensch et al. 2000; Ricklefs and Fallon 2002; Ricklefs et al. 2004; Reullier et al. 2005). Nevertheless, because hybrids may have a range of phenotypes intermediate to that of their parental species, hybrid zones may allow parasites of one parental species to evolve to colonize the other parental species, even in cases where host specificity is relatively high (Floate and Whitham 1993).

From the viewpoint of hosts, parasites may alter the direction and magnitude of gene exchange between two hybridizing species, and differential parasitism between hybrids and their parental species may either reinforce or degrade host species barriers (e.g., Derothe et al. 2001; Parris 2004; Goldberg et al. 2005; Hedrick et al. 2006; Brucker and Bordenstein 2013; González et al. 2014; Guttel and Ben-Ami 2014; Maynard et al. 2016; Eastwood et al. 2017; Liang et al. 2018; reviewed in Theodosopoulos et al. 2019). If hybrids experience reduced fitness, due to higher susceptibility or exposure to parasites than parental species, backcrossing, resulting in introgression of parental genes, should be minimized, leading to reinforcement of species barriers (Moulia 1999; Grant and Grant 2008; Theodosopoulos et al. 2019). On the other hand, in instances where no other factors work to maintain species barriers, these barriers may be eroded if hybrids have intermediate or greater fitness than parental species, due to reduced susceptibility or exposure to parasitism (Theodosopoulos et al. 2019). Past work has provided support for both parasitic driven maintenance (e.g., Derothe et al. 2001; Parris 2004; Goldberg et al. 2005; Brucker and Bordenstein 2013; González et al. 2014) and erosion (e.g., Hedrick et al. 2006; Guttel and Ben-Ami 2014; Maynard et al. 2016; Eastwood et al. 2017; Liang et al. 2018) of species barriers, across a range of animal taxa, with ca. 37% of studies examined in a 2019 review suggesting that hybrids are more negatively affected by parasites, and ca. 41% of studies suggesting that hybrids are less negatively affected by parasites, compared to parental species (Theodosopoulos et al. 2019).

Differences in parasite susceptibility, exposure, or fitness costs may arise between hybrids and their parental species due to physiological and/or ecological factors (Le Brun et al. 1992; Baack and Rieseberg 2007; Nadachowska-Brzyska et al. 2012; Grossen et al. 2014; Guttel and Ben-Ami 2014; Delmore et al. 2016; Wyman et al. 2016; Zhang et al. 2017; Theodosopoulos

et al. 2019). In terms of physiology, hybrids may be better or worse equipped than parental species to fight off infection. On the one hand, hybrid vigor may exist with respect to immune function, and thus parasite resistance, due to the admixture of locally adapted alleles conferring resistance from both parental species, the generation of transgressive phenotypes for resistance (i.e., hybrids may possess phenotypes that are extreme compared to parental phenotypes), interactions between hybrid immune systems and other transgressive traits such as body size, and/or higher MHC diversity (Rieseberg et al. 1999; Baack and Rieseberg 2007; Nadachowska-Brzyska et al. 2012; Grossen et al. 2014; Guttel and Ben-Ami 2014; Zhang et al. 2017; Theodosopoulos et al. 2019; although it is important to note that increased MHC diversity does not always result in heightened immune response; Sommer 2005; Sommer et al. 2014). On the other hand, hybrids may have decreased immune responses, or it may be more costly for hybrids to mount an immune response, compared to parental species, due to a higher stress response, fewer resources, and/or because genetic mixing may lead to debilitated immune function and/or metabolic processes (Dupont and Crivelli 1988; Moulia 1999; Theodosopoulos et al. 2019). Hybrids may also have immune functions intermediate to that of their parental species if they possess intermediate MHC diversity or inherit a combination of alleles related to immunity (Theodosopoulos et al. 2019). In addition to differences in host-parasite interactions arising from physiological factors, hybrids may have an ecology that is distinct from their parental types, such as different intra- and interspecific social relationships, food resources, and habitat use, which lead to higher or lower parasite transmission or intensity of infection in hybrids, compared with parental species (Le Brun et al. 1992; Guttel and Ben-Ami 2014; Delmore et al. 2016; Wyman et al. 2016; Theodosopoulos et al. 2019).

Using four years of data, we explored infection patterns of *Haemoproteus lophortyx*, an intraerythrocytic parasite, at a site in the northern region of the contact zone between California quail, *Callipepla californica*; Shaw, 1798 and Gambel's quail, *Callipepla gambelii*; Gambel, 1843. The hybrid zone is located along an ecological transition between the relatively the xeric habitat of Gambel's quail to the relatively mesic habitat of California quail, and local gene exchange occurs frequently in disjunct patches of species overlap. (Gee 2003, 2004). We compared the status and intensity of *H. lophortyx* infection in California quail, Gambel's quail, and their hybrids. We also considered whether year and sex might influence infection status and intensity.

Methods

Study System and field methods

California quail and Gambel's quail are sister species that are medium-sized (ca. 150-200g), highly social, sexually dichromatic, nonmigratory New World quail (Odontophoridae; Leopold 1977; Zink and Blackwell 1998; Hosner et al. 2015; Gee 2003; 2004). California quail are native to the western United States and Baja California, preferring chaparral and semiarid scrub, while the natural ranges of Gambel's quail span the Mojave and Sonoran Deserts, with these quail preferring more arid environments (Leopold 1977). These sister taxa hybridize readily under captive and natural conditions, and there is no evidence for assortative mating in the hybrid zone (Johnsgard 1971, Gee 2003; 2004; 2005). The hybrid zone straddles a narrow ecotone (roughly 20-30 km), and even across that distance, habitat differences between the species are striking. Hybrids of all classes are present in the quail hybrid zone and hybrids can

easily comprise at least 20% of the population, depending upon the ecological conditions (Gee 2004).

The genus *Haemoproteus* is a diverse group which parasitizes a range of avian species (e.g., Ricklefs et al. 2005; Valkiūnas et al. 2007; 2010; 2013; Iezhova et al. 2011; Levin et al. 2012; Swanson et al. 2014; Ayadi et al. 2018). Along with parasites of the genus *Plasmodium*, *Haemoproteus* can cause avian malaria, however, unlike *Plasmodium*, *Haemoproteus* lineages appear to have high host specificity (Atkinson and Van Riper 1991a; Bensch 2000; Clark and Clegg 2017; Loiseau et al. 2017; Ayadi et al. 2018). *Haemoproteus lophortyx*; O'Roke, 1929 may cause anemia, prostration, and death in various quail species, including California quail, Gambel's quail, and bobwhite quail, *Colinus virginianus*; Linnaeus, 1758 (O'Roke 1928; 1930; 1932; Herman et al. 1942; Tarshis 1955; 1958; Gullion 1957; Cardona et al. 2002; Mullens et al. 2006; Samour 2016). There exists no evidence for sex differences in infection in California quail (Herman and Glading 1942). Past work has demonstrated that, in quail, *H. lophortyx* may be spread by several vectors including hippoboscid flies (*Lynchia hirsuta*; Ferris 1927 and *Stilbometopa impressa*; Bigot, 1885; both of which are obligate ectoparasites) and biting midges (*Culicoides* spp. – especially *C. bottimeri*; Wirth, 1955 (Tarshis 1955; 1958; Mullens et al. 2006; Samour 2016).

JMG and student assistants trapped adult quail using seed-baited, walk-in funnel traps from January-September in 1998-2001 at a site called Royal Carrizo (33.6410° N, 116.4253° W), which consists of pinyon-juniper woodland habitat at ca. 3000' in Southern California. Hybrid reproductive success at this sympatric study site is moderate, based on comparisons of clutch size and hatching success with the two parental species in allopatry (Gee 2003). We assigned birds as California quail, Gambel's quail, or hybrids, based on morphological features which are

tightly correlated with genotype (Figure 1; Gee 2004). At the time of first capture, we ringed birds for individual identification and collected blood samples to test for *H. lophortyx* infection. We collected blood from the left brachial vein into a microcapillary tube. We smeared the blood onto a glass slide, which we air-dried and fixed in 95% ethanol before Giemsa-staining. This work was conducted under California State Fish and Game permit SC 949 and was approved by Princeton University's Institutional Animal Care and Use Committee.

Parasite quantification

Blood smears consisted of ca. 30,000 erythrocytes per slide. Criteria for *H. lophortyx* identification were based on the morphological descriptions given by O'Roke (1928) and Atkinson and Van Riper (1991b). We examined a minimum of 30 fields of view at 100x magnification, using an oil immersion lens. At this magnification, a mean ± SD of 184 ± 73 erythrocytes per field of view per sample was examined. We obscured all information about the sample before parasite quantification. We recorded the number of infected and uninfected erythrocytes in each sample.

We collected 208 blood samples across 4 years (1998: N = 61; 1999: N = 32; 2000: N = 102; 2001: N = 13; see Table S1). *H. lophortyx* was present in 69 of these blood samples.

Statistical methods

We used R 3.5.2 (R Core Team 2018) for all analyses and examined the factors affecting the status (i.e., 1= one or more H. lophortyx were found in the sample, 0 = no H. lophortyx were found in the sample) and intensity (i.e., proportion of infected erythrocytes) of *H. lophortyx* infection. To examine infection status, we used a generalized linear mixed model with a binomial

family and the status of infection as the response. To examine infection intensity, we ran a similar model, but instead, included the ratio of infected to uninfected erythrocytes as the response. For this analysis, we only examined the subset of individuals that were infected. For both sets of analyses we included species (i.e., California quail, Gambel's quail, or hybrids), sex, and year as fixed effects, and individual identity and month as random effects. We included month as a random effect given that past work has demonstrated seasonal fluctuations in *H. lophortyx* infection in quail (Tarshis 1955; Cardona et al. 2002). Given that species was a categorical variable, we examined whether the overall effect of species was significant by comparing models with and without species, using likelihood ratio tests. Similarly, given that year and sex were also categorical, we used likelihood ratio tests to compare a model with and without year and to compare a model with and without sex to determine the overall effect of these variables.

Results

We tested for the presence of *H. lophortyx* in 193 quail (72 California quail, 27 Gambel's quail, and 94 hybrids), 13 of which were sampled twice across years (3 California quail, 3 Gambel's quail, and 7 hybrids), and one California quail which was sampled thrice, for a total of 208 blood smears. Of the 208 blood smears examined, 69 (~33%) showed signs of *H. lophortyx* infection (24 California quail smears (~31%), 16 Gambel's quail smears (~53%), and 29 hybrid smears (~29%)). Of the 69 blood smears in which infection was observed, infection intensity ranged from 4.700e-5 – 0.013 *H. lophortyx*/cell with a mean ± SD of 0.002 ± 0.002 *H. lophortyx*/cell (California quail: range = 5.710e-5 – 0.013 *H. lophortyx*/cell, mean ± SD = 0.003 ± 0.003 *H. lophortyx*/cell; Gambel's quail: range = 6.000e-5 – 0.001 *H. lophortyx*/cell, mean ± SD = 3.686e-

2164 ± 2.689 e-4 *H. lophortyx*/cell; hybrids: range = 4.700e-5 – 0.009 *H. lophortyx*/cell, mean ± SD
217= 0.001 ± 0.002 *H. lophortyx*/cell).

218 For the analysis examining infection status, we found that significantly more Gambel's
219quail were infected than either California or hybrid quail, but we found no significant difference
220between hybrids and California quail (i.e., Gambel's quail > hybrid quail = California quail;
221Table 1; Figure 2a). In contrast, for the analysis examining the intensity of infection in the subset
222of individuals that were infected, California and hybrid quail had significantly higher proportions
223of *H. lophortyx*/cell than Gambel's quail (Table 2; Figure 3a). Again, there was no significant
224difference between hybrids and California quail (i.e., Gambel's quail < hybrid quail = California
225quail, Table 2; Figure 3a). There was a significant overall effect of species on *H. lophortyx*
226infection status (Chi-squared = 8.997, df = 2, p = 0.011) and intensity (Chi-squared = 8.162, df =
2272, p = 0.017). Furthermore, there was a significant overall effect of year on infection status (Chi-
228squared = 21.560, df = 3, p < 0.001), with significantly less individuals infected in 1999 than in
2291998 or 2000 (Table S2; Figure 2b). Although we also found a significant overall effect of year
230on infection intensity (Chi-squared = 17.367, df = 3, p = 0.001), with a significantly higher
231intensity of infection in 1999 compared to 2000 (Table S3; Figure 3b), it is important to note that
232we had a limited sample size for both 1999 and 2001, as we only used the subset of data with
233infected individuals for this analysis. Given this, these results should be taken with caution. We
234found no significant overall effect of sex on infection status (Chi-squared = 0.225, df = 1, p =
2350.635) or intensity (Chi-squared = 0.491, df = 1, p = 0.484).

236 In order to check the robustness of our results, we reran analyses using a subset of data
237where at least 10,000 erythrocytes were examined for each sample (as opposed to examining 30
238fields of view, which sometimes resulted the examination of <10,000 erythrocytes; Rätti et al.

2391993; Staats and Schall 1996; Kelly et al. 2016; Shurulinkov et al. 2018). This reduced our 240overall sample size from 208 to 189 blood smears and our infected sample size from 69 to 63 241blood smears. Our results for these follow-up analyses were qualitatively similar to our original 242results (Appendix 1; Tables S6 and S7), suggesting that our findings were robust to different 243sampling methods.

244

245**Discussion**

246 The hybridization of two host species can have complex effects on host-parasite 247interactions. We found that while there was no significant difference between hybrid California × 248Gambel's quail and California quail in *Haemoproteus lophortyx* infection status and intensity, 249both species had lower infection prevalence but higher infection intensity than Gambel's quail. 250Our results suggest that, in this Galliform host system, infection by the blood parasite *H.* 251*lophortyx* has the potential to impact species barrier dynamics.

252 We found that there were fewer *H. lophortyx* infected California and hybrid quail than 253Gambel's quail, but upon infection, California and hybrid quail had higher infection intensities 254compared to Gambel's quail. This may appear counterintuitive, as one might expect relationships 255involving infection status and intensity to be similar in directionality. For example, a study 256conducted on three species of wild doves found that species which had higher likelihoods of 257*Haemoproteus columbae* infection had higher, rather than lower, infection intensities (Adriano & 258Cordeiro 2001). There are two possible scenarios that may lead to the patterns observed in our 259study (see Table S4 for a complete summary of these scenarios and their potential implications).

260 First, it is possible that host behavior or vector preference causes Gambel's quail to 261experience higher rates of exposure to *H. lophortyx*, compared to California quail, leading to

higher infection prevalence in the former. For example, the frequency, type, network structure, or duration of social interactions in Gambel's quail may be more amenable to the direct horizontal transmission of *H. lophortyx* carrying hippoboscid flies, compared to the social interactions of California quail. Furthermore, biting midges have been implicated in the transmission of *H. lophortyx* in bobwhite quail and may also transmit *H. lophortyx* in the quail species examined in our study (Mullens et al. 2006). Gambel's quail could be more likely than California quail to use habitats where biting midges are more prevalent, leading Gambel's quail to experience greater exposure to *H. lophortyx*. If Gambel's quail experience higher rates of *H. lophortyx*, compared to California quail, due to their ecology, this may indicate a longer coevolutionary history between Gambel's quail and *H. lophortyx* than between California quail and *H. lophortyx*, leading Gambel's quail to have evolved higher resistance to *H. lophortyx* infection (i.e., they are better able to fight off infection), explaining their relatively low infection intensities. From the parasite's perspective, if Gambel's quail have a longer coevolutionary history with *H. lophortyx* than California quail, it is possible that the hybrid zone may have acted as a bridge for *H. lophortyx* to expand its range from Gambel's quail to California quail.

Hybrid quail may have inherited behavioral traits (e.g., habitat use and/or social behavior) more similar to parental California quail, making them less likely to acquire the parasite than Gambel's quail. Furthermore, hybrid quail may not have inherited resistance alleles from parental Gambel's quail. If Gambel's quail have lower infection intensities than California and hybrid quail, due to higher resistance to infection, and if hybrid quail have reduced fitness compared to Gambel's quail, as a result of higher infection intensities, we would expect species barriers to be maintained. Parasitism has been demonstrated to aid in the maintenance of species

barriers in a number of species (e.g., Derothe et al. 2001; Parris 2004; Goldberg et al. 2005; Brucker and Bordenstein 2013; González et al. 2014; reviewed in Theodosopoulos et al. 2019).

Second, Gambel's quail may actually have *lower* resistance to *H. lophortyx* than California quail, causing them to exhibit a higher prevalence, but lower intensity of infection, compared to California quail. In this scenario, Gambel's quail may experience similar rates of exposure to *H. lophortyx* as California quail. However, Gambel's quail may be more likely to become infected and less likely to completely clear their system of the parasite, once infected, compared to California quail, leading to a relatively high prevalence of low-intensity, chronic infections. Because infection intensities are usually highest during the early stages of infection (e.g., Ahmed & Mohammed 1978; Cepeda et al. 2019), the few individuals with relatively high infection intensities seen in California quail may simply reflect a handful of newly infected individuals.

In this case, hybrid quail may have inherited resistance alleles from parental California quail. Chronic *Haemoproteus* infections have been demonstrated to have fitness costs in other host systems (Puente et al. 2010; Asghar et al. 2011; 2015; but see Ortego et al. 2008). If chronic infections in Gambel's quail lead individuals to have lower fitness compared to hybrid quail, hybridization and backcrossing may facilitate the introgression of resistance alleles, and we would expect species barriers to be eroded. This aligns with numerous studies that have suggested the occurrence of parasite-mediated breakdowns of species barriers (e.g., Hedrick et al. 2006; Guttel and Ben-Ami 2014; Maynard et al. 2016; Eastwood et al. 2017; Liang et al. 2018; reviewed in Theodosopoulos et al. 2019).

While our results were robust across various sampling methods, more work is needed to tease apart the two mutually exclusive interpretations of our results presented above. Although

past work has shown that *H. lophortyx* can have high fitness costs for quail, even resulting in death (O'Roke 1930; 1932; Cardona et al. 2002; Mullens et al. 2006), we did not examine the relative fitness costs of this parasite for each host species or the potential costs of chronic, low-intensity infections. Given this, while we can infer the potential for *H. lophortyx* to influence species barrier dynamics, we cannot draw any definitive conclusions regarding the parasite-driven maintenance or breakdown of species barriers, and future work should assess the relative fitness costs and course of infection of *H. lophortyx* across this hybrid zone. Furthermore, our study possesses several other limitations that future studies may work to address. For example, we scored individuals as either hybrids or parental species (Figure 1), however, because hybrids are not truly a single class, differences may exist between hybrids in parasite susceptibility or fitness costs, thereby affecting the influence of parasitism on gene flow (Derothe et al. 2004; Goldberg et al. 2005; Theodosopoulos et al. 2019). For example, if most of the hybrids sampled in our study were highly backcrossed to California quail, this could explain the observed similarities between hybrid and California quail in prevalence and intensity of *H. lophortyx* infection. Furthermore, because interactions between different parasites may not be additive, coinfection dynamics can have drastic consequences for the effects of parasitism on gene flow, and studies which examine a single parasite may only capture a portion of the story (Telfer et al. 2010; Bordes and Morand 2011; Johnson and Hoverman 2012; Rynkiewicz et al. 2015; Vaumourin et al. 2015; Theodosopoulos et al. 2019). Lastly, we assumed California quail, Gambel's quail, and their hybrids were all infected by a single lineage of *Haemoproteus* in this study. However, a drawback of blood smears is that different lineages may appear morphologically identical, while remaining genetically distinct (Bensch et al. 2000). This is especially relevant given the high host specificity of *Haemoproteus* lineages, and it is possible

that different lineages may have disparate pathologies (Atkinson and Van Riper 1991a; Bensch 2000; Clark and Clegg 2017; Loiseau et al. 2017; Ayadi et al. 2018).

Lastly, we found that patterns of infection varied across years; however, the cause(s) of these differences remains unclear. Increases in precipitation have been found to either increase host exposure rates (O'Connor et al., 2007, 2008; Bohrer et al., 2014; Shearer and Ezenwa 2020) or decrease host susceptibility to parasitism (Ezenwa 2004; Thurber et al. 2011; Masi et al. 2012; Shearer and Ezenwa 2020) in other systems. Although El Niño Southern Oscillations caused precipitation to fluctuate across years, in our study, it is unlikely that the differences in infection status and intensity seen between years were related to variation in precipitation. (See Table S5 for a summary of how year relates to precipitation, infection status, and infection intensity.) Nevertheless, because hybridization has been shown to increase with increased precipitation (Gee 2004), precipitation may work in tandem with *H. lophortyx* to mediate species barrier dynamics.

In summary, we found that infection prevalence was higher, while infection intensity was lower, in Gambel's quail, compared to hybrid and California quail, suggesting that *H. lophortyx* infection has the potential to influence species barrier dynamics in this system. Future work should focus on examining the fitness consequences and course of infection of *H. lophortyx*, as well as the diversity of *Haemoproteus* spp. and their distribution across this quail hybrid zone.

Data Accessibility Statement

The data associated with this manuscript are available on Figshare (https://doi.org/10.6084/m9.figshare.12217958)

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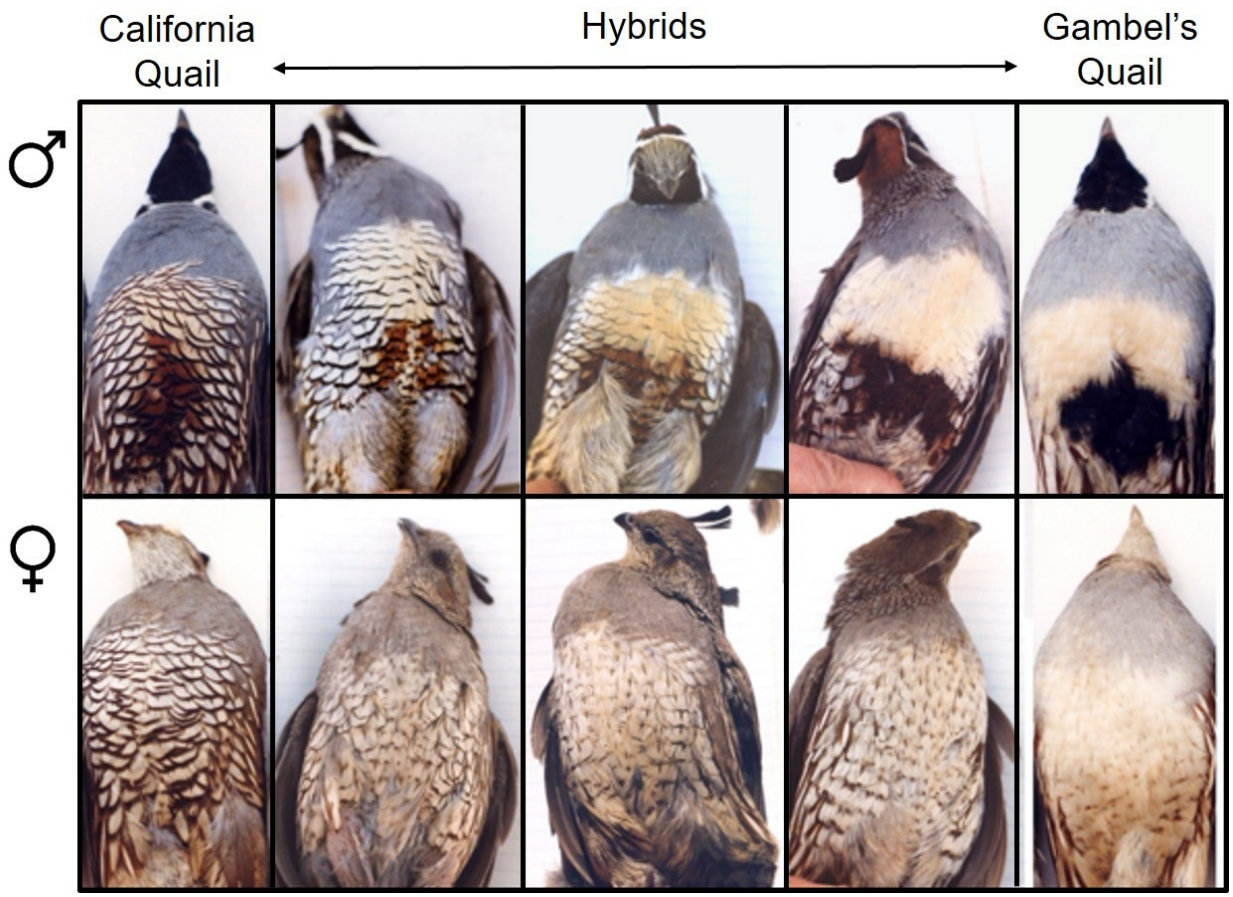
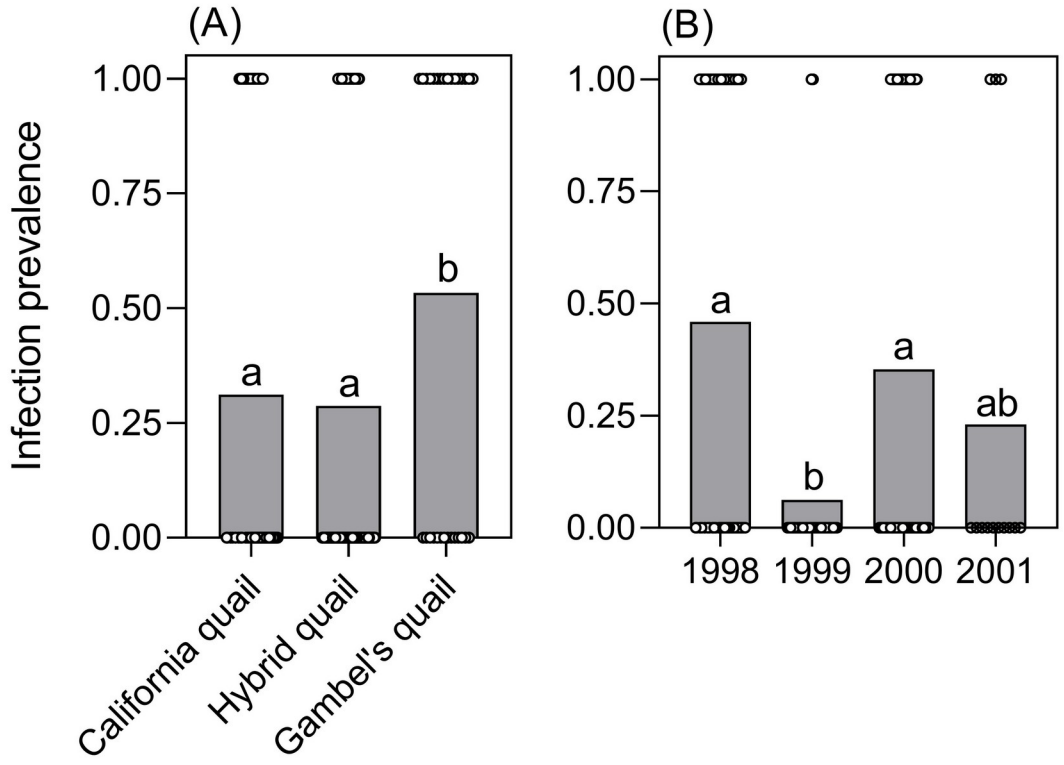
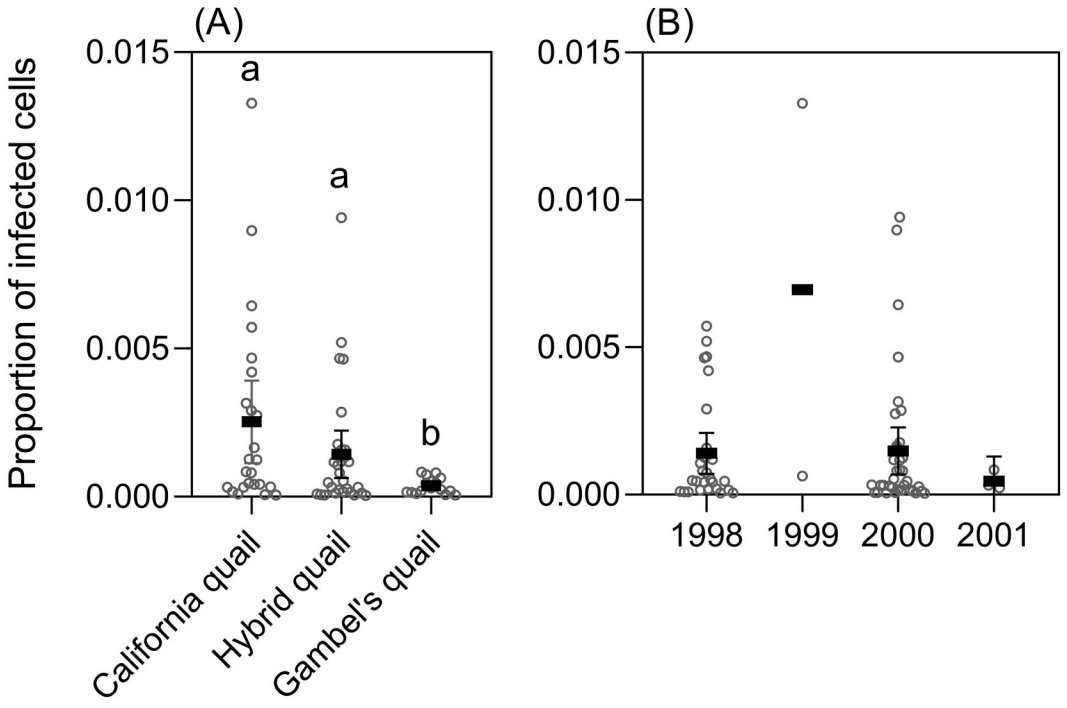


664
665**Figure 1.** The spectrum of morphological traits we examined when characterizing male (top
666row) and female (bottom row) birds as California quail (leftmost vertical panel), hybrid quail (3
667middle vertical panels), or Gambel's quail (rightmost vertical panel). Moving from California
668quail to Gambel's quail, the following patterns are observed: scaled breast, brown abdominal
669patch, overall blue body, chestnut colored cap and flanks, gray forehead and shorter plume
670versus the buffy clear breast, black belly patch, overall tan body, rust colored cap and flanks,
671blackish forehead and longer plume. Photos by JMG.



672
673**Figure 2.** Lowercase letters denote statistical differences. **a)** Infection status of *Haemoproteus*
674*lophortyx* in California quail, hybrid quail, and Gambel's quail when infection was determined
675by examining at least 30 fields of view at 100x magnification. **b)** Infection status of
676*Haemoproteus lophortyx* in 1998, 1999, 2000, and 2001 when infection was determined by
677examining at least 30 fields of view at 100x magnification.



678
679**Figure 3.** Lowercase letters denote statistical differences. a) Infection intensities of
680*Haemoproteus lophortyx* in California quail, hybrid quail, and Gambel's quail when infection
681was determined by examining at least 30 fields of view at 100x magnification. b) Infection
682intensities of *Haemoproteus lophortyx* in 1998, 1999, 2000, and 2001 when infection was
683determined by examining at least 30 fields of view at 100x magnification.

684**Table 1.** Effects of species (i.e., California quail, Gambel's quail, California × Gambel's quail
685hybrid), year (1998, 1999, 2000, 2001), and sex on *Haemoproteus lophortyx* infection status,
686when infection was determined by examining at least 30 fields of view at 100x magnification (N
687= 208).

Predictor	Est ± SE	Z	p
Reference Class: California Quail			
Species: Hybrid Quail	-0.012 ± 0.355	-0.033	0.973
Species: Gambel's Quail	1.394 ± 0.519	2.685	**0.007**
Year: 1999	-2.734 ± 0.817	-3.346	**0.001**
Year: 2000	-0.098 ± 0.374	-0.261	0.794
Year: 2001	-0.972 ± 0.764	-1.272	0.204
Sex: Male	0.173 ± 0.341	0.507	0.612
Reference Class: Hybrid Quail			
Species: California Quail	0.012 ± 0.355	0.033	0.973
Species: Gambel's Quail	1.406 ± 0.504	2.788	**0.005**
Year: 1999	-2.734 ± 0.817	-3.346	**0.001**
Year: 2000	-0.098 ± 0.374	-0.261	0.794
Year: 2001	-0.972 ± 0.764	-1.272	0.204
Sex: Male	0.173 ± 0.341	0.507	0.612
Reference Class: Gambel's Quail			
Species: California Quail	-1.394 ± 0.519	-2.685	**0.007**
Species: Hybrid Quail	-1.406 ± 0.504	-2.788	**0.005**
Year: 1999	-2.734 ± 0.817	-3.346	**0.001**
Year: 2000	-0.098 ± 0.374	-0.261	0.794
Year: 2001	-0.972 ± 0.764	-1.272	0.204
Sex: Male	0.173 ± 0.341	0.507	0.612

688The results presented in each subsection of this table represent the same model with different
689species coded as the reference class. Presenting the same model with each species coded as the
690reference class allows for a comparison in *H. lophortyx* infection status between each pair of
691species. For year, the reference class is 1998 (see Table S2 for a comparison between years). For
692sex, the reference class is female.

693**Table 2.** Effects of species (i.e., California quail, Gambel's quail, California × Gambel's quail
694hybrid), year (1998, 1999, 2000, 2001), and sex on the intensity of *Haemoproteus lophortyx*
695infection (i.e., proportion of infected erythrocytes), when infection was determined by examining
696at least 30 fields of view at 100x magnification (N = 69).

Predictor	Est ± SE	Z	p
Reference Class: California Quail			
Species: Hybrid Quail	-0.474 ± 0.374	-1.269	0.204
Species: Gambel's Quail	-1.416 ± 0.486	-2.913	**0.004**
Year: 1999	0.319 ± 0.305	1.045	0.296
Year: 2000	-0.088 ± 0.290	-0.304	0.761
Year: 2001	-0.770 ± 0.827	-0.932	0.351
Sex: Male	-0.244 ± 0.356	-0.686	0.493
Reference Class: Hybrid Quail			
Species: California Quail	0.474 ± 0.374	1.269	0.204
Species: Gambel's Quail	-0.942 ± 0.469	-2.010	**0.044**
Year: 1999	0.319 ± 0.305	1.045	0.296
Year: 2000	-0.088 ± 0.290	-0.304	0.761
Year: 2001	-0.770 ± 0.827	-0.932	0.351
Sex: Male	-0.244 ± 0.356	-0.686	0.493
Reference Class: Gambel's Quail			
Species: California Quail	1.416 ± 0.486	2.913	**0.004**
Species: Hybrid Quail	0.942 ± 0.469	2.010	**0.044**
Year: 1999	0.319 ± 0.305	1.045	0.296
Year: 2000	-0.088 ± 0.290	-0.304	0.761
Year: 2001	-0.770 ± 0.827	-0.932	0.351
Sex: Male	-0.244 ± 0.356	-0.686	0.493

697The results presented in each subsection of this table represent the same model with different
698species coded as the reference class. Presenting the same model with each species coded as the
699reference class allows for a comparison in *H. lophortyx* infection intensity between each pair of
700species. For year, the reference class is 1998 (see Table S3 for a comparison between years). For
701sex, the reference class is female.

APPENDIX 1

When rerunning analyses using the subset of data where at least 10,000 erythrocytes were examined for each sample, our results were identical to the results from models where we examined at least 30 fields of view at 100x magnification, with one small exception (Tables S6 – S9). In the model that examined at least 30 fields of view, Gambel's and hybrid quail differed significantly in intensity of infection. In contrast, when we limited data to 10,000 or more erythrocytes, this difference was marginally nonsignificant ($p = 0.050$; Table S7). As in the models that used data where at least 30 fields of view were examined, when at least 10,000 erythrocytes were examined, there was an overall effect of species on infection status (Chi-squared = 7.778, df = 2, $p = 0.020$) and intensity (Chi-squared = 7.361, df = 2, $p = 0.025$), as well as an overall effect of year on infection status (Chi-squared = 18.778, df = 3, $p < 0.001$) and intensity (Chi-squared = 17.330, df = 3, $p = 0.001$). Sex did not appear to have an overall effect on either infection status (Chi-squared = 0.045, df = 1, $p = 0.832$) or intensity (Chi-squared = 1.087, df = 1, $p = 0.297$).

716**Table S1.** Breakdown of the number of samples obtained for each species in each year.

Predictor	California	Hybrid	Gambel's
1998	25	23	13
1999	6	19	7
2000	43	51	8
2001	3	8	2

717

718**Table S2.** Effects of species (i.e., California quail, Gambel's quail, California × Gambel's quail
719hybrid), year (1998, 1999, 2000, 2001), and sex on *Haemoproteus lophortyx* infection status,
720when infection was determined by examining at least 30 fields of view at 100x magnification (N
721= 208).

Predictor	Est ± SE	Z	p
Reference Class: 1998			
Species: California Quail	-1.394 ± 0.519	-2.685	**0.007**
Species: Hybrid Quail	-1.406 ± 0.504	-2.788	**0.005**
Year: 1999	-2.734 ± 0.817	-3.346	**0.001**
Year: 2000	-0.098 ± 0.374	-0.261	0.794
Year: 2001	-0.972 ± 0.764	-1.272	0.204
Sex: Male	0.173 ± 0.341	0.507	0.612
Reference Class: 1999			
Species: California Quail	-1.394 ± 0.519	-2.685	**0.007**
Species: Hybrid Quail	-1.406 ± 0.504	-2.788	**0.005**
Year: 1998	2.734 ± 0.817	3.346	**0.001**
Year: 2000	2.637 ± 0.805	3.276	**0.001**
Year: 2001	1.762 ± 1.050	1.679	0.093
Sex: Male	0.173 ± 0.341	0.507	0.612
Reference Class: 2000			
Species: California Quail	-1.394 ± 0.519	-2.685	**0.007**
Species: Hybrid Quail	-1.406 ± 0.504	-2.788	**0.005**
Year: 1998	0.098 ± 0.374	0.261	0.794
Year: 1999	-2.637 ± 0.805	-3.276	**0.001**
Year: 2001	-0.874 ± 0.765	-1.142	0.253
Sex: Male	0.173 ± 0.341	0.507	0.612
Reference Class: 2001			
Species: California Quail	-1.394 ± 0.519	-2.685	**0.007**
Species: Hybrid Quail	-1.406 ± 0.504	-2.788	**0.005**
Year: 1998	0.972 ± 0.764	1.272	0.204
Year: 1999	-1.762 ± 1.050	-1.679	0.093
Year: 2000	0.874 ± 0.765	1.142	0.253
Sex: Male	0.173 ± 0.341	0.507	0.612

722The results presented in each subsection of this table represent the same model with different
723years coded as the reference class. Presenting the same model with each year coded as the
724reference class allows for a comparison between each pair of years in *H. lophortyx* infection
725status. The reference class for species is Gambel's quail, and the reference class for sex is
726female.
727

728**Table S3.** Effects of species (i.e., California quail, Gambel's quail, California × Gambel's quail
729hybrid), year (1998, 1999, 2000, 2001), and sex on the intensity of *Haemoproteus lophortyx*
730infection (i.e., proportion of infected erythrocytes), when infection was determined by examining
731at least 30 fields of view at 100x magnification (N = 69).

Predictor	Est ± SE	Z	p
Reference Class: 1998			
Species: California Quail	1.416 ± 0.486	2.913	**0.004**
Species: Hybrid Quail	0.942 ± 0.469	2.010	**0.044**
Year: 1999	0.319 ± 0.305	1.045	0.296
Year: 2000	-0.088 ± 0.290	-0.304	0.761
Year: 2001	-0.770 ± 0.827	-0.932	0.351
Sex: Male	-0.244 ± 0.356	-0.686	0.493
Reference Class: 1999			
Species: California Quail	1.416 ± 0.486	2.913	**0.004**
Species: Hybrid Quail	0.942 ± 0.469	2.010	**0.044**
Year: 1998	-0.319 ± 0.305	-1.045	0.296
Year: 2000	-0.407 ± 0.101	-4.048	**<0.001**
Year: 2001	-1.089 ± 0.811	-1.343	0.179
Sex: Male	-0.244 ± 0.356	-0.686	0.493
Reference Class: 2000			
Species: California Quail	1.416 ± 0.486	2.913	**0.004**
Species: Hybrid Quail	0.942 ± 0.469	2.010	**0.044**
Year: 1998	0.088 ± 0.290	0.304	0.761
Year: 1999	0.407 ± 0.101	4.048	**<0.001**
Year: 2001	-0.682 ± 0.805	-0.848	0.397
Sex: Male	-0.244 ± 0.356	-0.686	0.493
Reference Class: 2001			
Species: California Quail	1.416 ± 0.486	2.913	**0.004**
Species: Hybrid Quail	0.942 ± 0.469	2.010	**0.044**
Year: 1998	0.770 ± 0.827	0.932	0.351
Year: 1999	1.089 ± 0.811	1.343	0.179
Year: 2000	0.682 ± 0.805	0.848	0.397
Sex: Male	-0.244 ± 0.356	-0.686	0.493

732The results presented in each subsection of this table represent the same model with different
733years coded as the reference class. Presenting the same model with each year coded as the
734reference class allows for a comparison between each pair of years in *H. lophortyx* infection
735intensity. The reference class for species is Gambel's quail, and the reference class for sex is
736female.

737**Table S4.** Summary of the two mutually exclusive explanations for our observed results and how
738each scenario is expected to affect species barrier dynamics, as well as the potential range
739expansion of *H. lophortyx*.

Scenario	Species		Species Barrier Maintenance or Breakdown	Hybrid zone acts as a bridge for *H. lophortyx* to expand its range?
	Gambel's Quail	California/Hybrid Quail		
1	*Host behavior/vector preference leads to higher exposure to *H. lophortyx*, which leads to higher infection prevalence *Have higher resistance to *H. lophortyx*, due to longer coevolution with the parasite, which leads to lower intensities of infection (i.e., they are better at fighting off infection once infected)	*Host behavior/vector preference leads to lower exposure to *H. lophortyx*, which leads to lower infection prevalence *Have lower resistance to *H. lophortyx*, due to shorter coevolution with the parasite, which leads to higher intensities of infection (i.e., they are worse at fighting off infection once infected)	Species barrier maintenance is expected if the higher intensity of infection in hybrid quail leads them to have lower fitness, compared to Gambel's quail	If Gambel's quail have a longer co-evolution with *H. lophortyx* than California quail, hybrid zones may act as a bridge for the parasite to colonize California quail
2	*Have similar rates of exposure to *H. lophortyx* as California/ hybrid quail, but have lower resistance, and are therefore more likely to succumb to initial infection and are less able to completely clear infections, once infected *This leads to a high prevalence of low-intensity, chronic infections	*Have similar rates of exposure to *H. lophortyx* as Gambel's quail, but have higher resistance, and are therefore better at resisting initial infection and clearing an infection, once infected *This leads to a low prevalence of high intensity infections, given that infection intensities are highest during the initial stages of infection	Species barrier breakdown is expected if chronic infection in Gambel's quail leads them to have lower fitness, compared to hybrid quail	n/a because the ecology of each quail species leads to similar rates of exposure across species

Table S5. Summary of winter precipitation across our four study years and how infection prevalence and intensity differed across years, relative to one another. We found that significantly fewer individuals were infected in 1999 than in 1998 or 2000, and, once infected, individuals expressed higher infection intensities in 1999 compared to 2000. Because 1) there was a significant difference in the proportion of infected individuals between the first and second and between the first and forth driest years, but not between the first and third driest years and 2) the only significant difference in infection intensity occurred between 1999 and 2000, which were the two study years with the lowest levels of precipitation, it is unlikely that interannual variation in precipitation drove the differences in infection status and intensity seen between years in our study. Furthermore, given that we had a very limited sample size in 1999 and 2001, for the analysis examining the intensity of infection, both the results and any interpretations of the underlying mechanisms must be taken with caution.

Year	Precipitation (mm)	Infection Prevalence	Infection Intensity
1998	149.2	High	-
1999	28.3	Low	High
2000	59.3	High	Low
2001	81.1	-	-

We calculated winter precipitation from December 1 (of the preceding year) – February 28 for each year of our study using data acquired from the University of California, Boyd Deep Canyon Desert Research Center (http://deepcanyon.ucnrs.org).

Table S6. Effects of species (i.e., California quail, Gambel's quail, California × Gambel's quail hybrid), year (1998, 1999, 2000, 2001), and sex on *Haemoproteus lophortyx* infection status, when infection was determined by examining at least 10,000 erythrocytes (N = 189).

Predictor	Est ± SE	Z	p
Reference Class: California Quail			
Species: Hybrid Quail	0.009 ± 0.369	0.024	0.981
Species: Gambel's Quail	1.370 ± 0.546	2.509	**0.012**
Year: 1999	-2.702 ± 0.824	-3.278	**0.001**
Year: 2000	-0.272 ± 0.391	-0.696	0.486
Year: 2001	-1.044 ± 0.767	-1.362	0.173
Sex: Male	0.085 ± 0.356	0.239	0.811
Reference Class: Hybrid Quail			
Species: California Quail	-0.009 ± 0.369	-0.024	0.981
Species: Gambel's Quail	1.361 ± 0.523	-2.602	**0.009**
Year: 1999	-2.702 ± 0.824	-3.278	**0.001**
Year: 2000	-0.272 ± 0.391	-0.696	0.486
Year: 2001	-1.044 ± 0.767	-1.362	0.173
Sex: Male	0.085 ± 0.356	0.239	0.811
Reference Class: Gambel's Quail			
Species: California Quail	-1.370 ± 0.546	-2.509	**0.012**
Species: Hybrid Quail	-1.361 ± 0.523	-2.602	**0.009**
Year: 1999	-2.702 ± 0.824	-3.278	**0.001**
Year: 2000	-0.272 ± 0.391	-0.696	0.486
Year: 2001	-1.044 ± 0.767	-1.362	0.173
Sex: Male	0.085 ± 0.356	0.239	0.811

The results presented in each subsection of this table represent the same model with different species coded as the reference class. Presenting the same model with each species coded as the reference class allows for a comparison in *H. lophortyx* infection status between each pair of species. For year, the reference class is 1998 (see Table S8 for a comparison between years). For sex, the reference class is female.

Table S7. Effects of species (i.e., California quail, Gambel's quail, California × Gambel's quail hybrid), year (1998, 1999, 2000, 2001), and sex on the intensity of *Haemoproteus lophortyx* infection (i.e., proportion of infected erythrocytes), when infection was determined by examining at least 10,000 erythrocytes (N = 63). Fixed effects that differ in significance between models using data where at least 10,000 erythrocytes were examined and models using data where at least 30 fields of view at 100x magnification were examined are shaded in grey.

Predictor	Est ± SE	Z	p
Reference Class: California Quail			
Species: Hybrid Quail	-0.494 ± 0.407	-1.216	0.224
Species: Gambel's Quail	-1.482 ± 0.537	-2.762	**0.006**
Year: 1999	0.370 ± 0.319	1.160	0.246
Year: 2000	-0.037 ± 0.304	-0.123	0.903
Year: 2001	-0.753 ± 0.857	-0.879	0.380
Sex: Male	-0.399 ± 0.391	-1.020	0.308
Reference Class: Hybrid Quail			
Species: California Quail	0.494 ± 0.407	1.216	0.224
Species: Gambel's Quail	-0.988 ± 0.504	-1.959	0.050
Year: 1999	0.370 ± 0.319	1.160	0.246
Year: 2000	-0.037 ± 0.304	-0.123	0.903
Year: 2001	-0.753 ± 0.857	-0.879	0.380
Sex: Male	-0.399 ± 0.391	-1.020	0.308
Reference Class: Gambel's Quail			
Species: California Quail	1.482 ± 0.537	2.762	**0.006**
Species: Hybrid Quail	0.988 ± 0.504	1.959	**0.050**
Year: 1999	0.370 ± 0.319	1.160	0.246
Year: 2000	-0.037 ± 0.304	-0.123	0.903
Year: 2001	-0.753 ± 0.857	-0.879	0.380
Sex: Male	-0.399 ± 0.391	-1.020	0.308

The results presented in each subsection of this table represent the same model with different species coded as the reference class. Presenting the same model with each species coded as the reference class allows for a comparison in *H. lophortyx* infection intensity between each pair of species. For year, the reference class is 1998 (see Table S9 for a comparison between years). For sex, the reference class is female.

Table S8. Effects of species (i.e., California quail, Gambel's quail, California × Gambel's quail hybrid), year (1998, 1999, 2000, 2001), and sex on *Haemoproteus lophortyx* infection status, when infection was determined by examining at least 10,000 erythrocytes (N = 189).

Predictor	Est ± SE	Z	p
Reference Class: 1998			
Species: California Quail	-1.370 ± 0.546	-2.509	**0.012**
Species: Hybrid Quail	-1.361 ± 0.523	-2.602	**0.009**
Year: 1999	-2.702 ± 0.824	-3.278	**0.001**
Year: 2000	-0.272 ± 0.391	-0.696	0.486
Year: 2001	-1.044 ± 0.767	-1.362	0.173
Sex: Male	0.085 ± 0.356	0.239	0.811
Reference Class: 1999			
Species: California Quail	-1.370 ± 0.546	-2.509	**0.012**
Species: Hybrid Quail	-1.361 ± 0.523	-2.602	**0.009**
Year: 1998	2.702 ± 0.824	3.278	**0.001**
Year: 2000	2.430 ± 0.807	3.012	**0.003**
Year: 2001	1.658 ± 1.044	1.587	0.112
Sex: Male	0.085 ± 0.356	0.239	0.811
Reference Class: 2000			
Species: California Quail	-1.370 ± 0.546	-2.509	**0.012**
Species: Hybrid Quail	-1.361 ± 0.523	-2.602	**0.009**
Year: 1998	0.272 ± 0.391	0.696	0.486
Year: 1999	-2.430 ± 0.807	-3.012	**0.003**
Year: 2001	-0.772 ± 0.755	-1.023	0.306
Sex: Male	0.085 ± 0.356	0.239	0.811
Reference Class: 2001			
Species: California Quail	-1.370 ± 0.546	-2.509	**0.012**
Species: Hybrid Quail	-1.361 ± 0.523	-2.602	**0.009**
Year: 1998	1.044 ± 0.767	1.362	0.173
Year: 1999	-1.658 ± 1.044	-1.587	0.112
Year: 2000	0.772 ± 0.755	1.023	0.306
Sex: Male	0.085 ± 0.356	0.239	0.811

The results presented in each subsection of this table represent the same model with different years coded as the reference class. Presenting the same model with each year coded as the reference class allows for a comparison between each pair of years in *H. lophortyx* infection status. The reference class for species is Gambel's quail, and the reference class for sex is female.

Table S9. Effects of species (i.e., California quail, Gambel's quail, California × Gambel's quail hybrid), year (1998, 1999, 2000, 2001), and sex on the intensity of *Haemoproteus lophortyx* infection (i.e., proportion of infected erythrocytes), when infection was determined by examining at least 10,000 erythrocytes (N = 63). Fixed effects that differ in significance between models using data where at least 10,000 erythrocytes were examined and models using data where at least 30 fields of view at 100x magnification were examined are shaded in grey.

Predictor	Est ± SE	Z	p
Reference Class: 1998			
Species: California Quail	1.482 ± 0.537	2.762	**0.006**
Species: Hybrid Quail	0.988 ± 0.504	1.959	0.050
Year: 1999	0.370 ± 0.319	1.160	0.246
Year: 2000	-0.037 ± 0.304	-0.123	0.903
Year: 2001	-0.753 ± 0.857	-0.879	0.380
Sex: Male	-0.399 ± 0.391	-1.020	0.308
Reference Class: 1999			
Species: California Quail	1.482 ± 0.537	2.762	**0.006**
Species: Hybrid Quail	0.988 ± 0.504	1.959	0.050
Year: 1998	-0.370 ± 0.319	-1.160	0.246
Year: 2000	-0.407 ± 0.101	-4.050	**<0.001**
Year: 2001	-1.123 ± 0.837	-1.342	0.180
Sex: Male	-0.399 ± 0.391	-1.020	0.308
Reference Class: 2000			
Species: California Quail	1.482 ± 0.537	2.762	**0.006**
Species: Hybrid Quail	0.988 ± 0.504	1.959	0.050
Year: 1998	0.037 ± 0.304	0.123	0.903
Year: 1999	0.407 ± 0.101	4.050	**<0.001**
Year: 2001	-0.716 ± 0.831	-0.862	0.389
Sex: Male	-0.399 ± 0.391	-1.020	0.308
Reference Class: 2001			
Species: California Quail	1.482 ± 0.537	2.762	**0.006**
Species: Hybrid Quail	0.988 ± 0.504	1.959	0.050
Year: 1998	0.753 ± 0.857	0.879	0.380
Year: 1999	1.123 ± 0.837	1.342	0.180
Year: 2000	0.716 ± 0.831	0.862	0.389
Sex: Male	-0.399 ± 0.391	-1.020	0.308

The results presented in each subsection of this table represent the same model with different years coded as the reference class. Presenting the same model with each year coded as the reference class allows for a comparison between each pair of years in *H. lophortyx* infection intensity. The reference class for species is Gambel's quail, and the reference class for sex is female.