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ORIGINAL ARTICLE

Widespread lineage diversity of *leucocytozoon* blood parasites in distinct populations of western Red-tailed Hawks

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Abstract This study examines the relationship between genetically distinct populations of Red-tailed Hawks (*Buteo jamaicensis*) with hemosporidian parasite phylogenetic data to examine geographic structuring of parasite lineages and to test for impacts of parasitic infection on host migration timing. We screened 296 hatch-year Red-tailed Hawks for infection with hemospridian parasites of the genus *Leucocytozoon* at a raptor migration site in the Marin Headlands, California, just north of San Francisco. Phylogenetic analysis based on cytochrome *b* sequences revealed a high diversity of closely related *Leucocytozoon buteonis* lineages (11 distinct haplotypes recorded) infecting the sampled hawk populations. Previous microsatellite analyses of breeding and migratory populations of Red-tailed Hawks revealed that the Marin Headlands migrants originate from two genetically distinct breeding populations from Central California and the Intermountain West. Early hawk arrivals to the study site (15 August–30 September 2004) are primarily non-migrant juveniles dispersing from Central California, while later arrivals (1 October–30 December 2004) are a mix of both California dispersals and migratory individuals from the Intermountain West population. We observed no correlation between the occurrence of parasitic infection and hawk migration timing in either hawk population. However, geographic structuring of *Leucocytozoon* parasite lineages was documented with one dominant lineage more prevalent within the Central California hawk population than the Intermountain West population. Future studies of the effects of *Leucocytozoon* infection on migrating Red-tailed Hawks should take into consideration the region of origin because birds from different geographical areas may be exposed to distinct parasite lineages.

Keywords *Leucocytozoon* · Raptor · Migration · Prevalence · Phylogenetics

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Zusammenfassung

Weit verbreitete Diversität in der Abstammung von *Leucocytozoon* Blutparasiten in verschiedenen Populationen von westlichen Rotschwanzbussarden

Diese Studie untersucht sie Beziehung zwischen genetisch verschiedenen Populationen von Rotschwanzbussarden mit phylogenetischen Daten von Parasiten der Ordnung hemosporida, um geographische Strukturen von Parasiten-Abstammungslinien zu bestimmen und um die Einflüsse parasitischer Infektionen auf die zeitliche Koordinierung des Zuges der Wirte zu testen. Wir untersuchten 296 diesjährige Rotschwanzbussarde (*Buteo jamaicensis*) auf Infektionen mit Haemasporida-Parasiten der Gattung *Leucocytozoon* an einem Greifvogel-Zugort in Marin

Headlands, Kalifornien, nördlich von San Francisco. Phylogenetische Analysen basierend auf Cytocrome b Sequenzen zeigten eine hohe Diversität an eng verwandten *Leucocytozoon buteonis* Abstammungslinien (11 verschiedene Haplotypen erfasst), mit der die beprobten Bussardpopulationen infiziert waren. Vorausgegangene Mikrosatellitenanalysen von Brut- und Zugpopulationen des Rotschwanzbussards zeigten, dass die Zugvögel in den Marin Headlands aus zwei genetisch unterschiedlichen Brutpopulationen aus Zentralkalifornien und der Region Intermountain West stammen. Früh ankommende Bussarde im Untersuchungsgebiet (15. August 2004–30. September 2004) sind vornehmlich nicht ziehende Jungvögel, die sich von Zentralkalifornien aus ausbreiten. Dagegen sind späte ankommende Vögel (1. Oktober 2004–30. Dezember 2004) sowohl kalifornische Dispergierer als auch ziehende Individuen der Intermountain West Population. Wir beobachteten keine Korrelation zwischen dem Auftreten von Parasiteninfektionen und dem Timing des Bussardzuges in beiden Populationen. Dennoch konnten geographische Strukturen der Parasiten-Abstammung mit einer dominanten Linie festgestellt werden, die prävalenter war in der zentralkalifornischen Bussardpopulation als in der Intermountain West Population. Zukünftige Studien zu den Auswirkungen von Leucocytozoon Infektionen auf ziehende Rotschwanzbussarde sollten die Ursprungsregion mit einbeziehen, da Vögel aus unterschiedlichen geographischen Gebieten verschiedenen Parasiten-Linien ausgesetzt sein könnten.

Introduction

Parasites of the genus *Leucocytozoon* in the family Leucocytozoidae are commonly found in California raptors (Sehgal et al. 2006; Valkiūnas 2010). Previous taxonomy had classified *Leucocytozoon todii* as the sole valid species of leucocytozoids infecting falconiform birds in North America (Valkiūnas 2010). However, recent work has provided evidence that *L. todii* is in fact a species cluster including at least *L. mathisi, L. buteonis* and *L. todii* infecting falconiform birds (Sehgal et al. 2006; Valkiūnas 2010). *Leucocytozoon* species are transmitted to host birds by blood-sucking dipteran insects of the black fly family Simuliidae (Valkiūnas 2005; Forrester and Greiner 2008). Here, we examined Red-tailed Hawks (*Buteo jamaicensis*) and their parasites, *Leucocytozoon buteonis*, in northern California.

Avian blood parasites have been shown to have serious consequences for their hosts during periods of high-energy demand such as migration and reproduction (Dawson and Bortolotti 2000; Garvin and Greiner 2003; Hunter et al. 1997; Nordling et al. 1998; Remple 2004), and thus could affect the evolution of migratory bird species (Møller and Szép 2011). Negative fitness impacts previously observed in infected birds include reduction in clutch sizes, nest defense behavior, and hatchling and fledgling success (Korpimäki et al. 1993; Marzal et al. 2005). Several studies have concluded that migratory bird populations are more likely to be parasitized and harbor more intense infections than non-migratory ones (Jenkins et al. 2011; Møller and Erritzøe 1998; Valkiūnas 2005). This is most often attributed to the increased likelihood of initial infection due to the hosts' exposure to vectors within numerous, varied habitats during migration (Møller and Erritzøe 1998; Smith et al. 2004; Valkiūnas 2005).

The exact impact of *Leucocytozoon* spp. infection on Red-tailed Hawks is not well known. In most cases, it is understood that avian hemosporidian infections (in particular species of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*), especially at low parasitemia, have negligible effects on host fitness (Valkiūnas 2005). However, some species of *Leucocytozoon* can cause severe pathologies in their hosts, associated with the development of megalomeronts in various tissues (Valkiūnas 2005). For example, *Leucocytozoon* parasites have recently been implicated in the deaths of Yellow-eyed Penguins (*Megadyptes antipodes*) (Argilla et al. 2013). In addition, several studies have documented negative fitness effects on different avian species caused by infection with *Plasmodium* and *Haemoproteus* spp., which are related hemosporidians (Korpimäki et al. 1993; Hakkarainen et al. 1998; Marzal et al. 2005). However, relatively little is known about how infections can affect bird migration. One study of Sharp-shinned hawks (*Accipiter striatus*) found no relationship between distance travelled and the prevalence of *Haemoproteus* infection (Smith et al. 2004), but did not use molecular techniques to identify parasite lineages. The impact of these parasites on their hosts' migration timing thus requires further examination, as does the biogeography of the parasites themselves.

Red-tailed Hawks are a polytypic, widespread raptor species, with populations inhabiting all North American habitats except tundra (Preston and Beane 1993). Recent molecular genetic analysis of breeding and migratory populations of Red-tailed hawks determined that Red-tailed Hawks encountered in the Marin Headlands, California, during the fall migration season originate primarily from two breeding populations; a Central California breeding populations and a breeding population from the Intermountain West which includes Idaho, Nevada, and Utah (Hull et al. 2008, 2009). Long term banding and count data revealed distinct patterns of migration by Red-tailed Hawks, with two waves of arrivals to the study station each fall season: an early arrival period between 15 August and

1 October with the peak of arrivals occurring around 15 September, and a later arrival period between 1 October and 30 November with a peak of arrivals occurring around the second week of November (Hull et al. 2009). Microsatellite analysis grouped the individuals from the first period with the Central California breeding population, and individuals of the second period with both the Central California and the Intermountain West breeding populations (Hull et al. 2008, 2009). Band encounter and telemetry data from these studies also revealed that movement patterns of early arrivals (Central California) were more suggestive of regional juvenile dispersals, while later arrivals (Central California and Intermountain West) indicated both juvenile dispersal as well as strong southerly movements more consistent with directed migration (Hull et al. 2009).

A separate examination of Red-tailed Hawk migration through the Marin Headlands observed a higher prevalence of *Leucocytozoon* infection in hawks arriving later in the fall migration season (Ishak et al. 2010). Additionally, a recent study on host–parasite coevolution among multiple species of *Leucocytozoon* and their hosts reported a greater genetic diversity of parasites in migrant bird hosts than in sedentary host species (Jenkins and Owens 2011). These studies imply possible differences in parasitic diversity associated with migration timing in Red-tailed Hawks moving through Central California.

Information about migratory populations including their origins and impact of parasite load on their migration phenology is essential to species conservation efforts. The studies by Hull et al. (2008, 2009) provided valuable insight into the origins and migratory patterns of migrating Red-tailed Hawks in California. This study takes the next step to determine whether *Leucocytozoon* parasite lineages infecting these hosts exhibit geographic structuring. We also use parasite prevalence data in conjunction with analyses from previous studies on the hawks to examine the relationship between parasitic infection and raptor migration timing.

Methods

Sample collection

Blood samples were collected from ($n = 296$) hatch-year Red-tailed Hawks caught at the Golden Gate Raptor Observatory (at Hawk Hill) in Sausalito, California, within the Marin Headlands (37°40′N, 122°20′W) during the fall migration periods (defined here as 15 August through 31 December) of 2004, 2006, and 2007. Because raptors tend to avoid flying over water bodies of more than 25 km (Bildstein 2006), the Marin peninsula acts as a funnel or "migration bottleneck" for migrating hawks traveling southward along the northern California coast (Fig. 1). Due to the concentrating effect of the Marin peninsula, 25,000–30,000 hawks are counted at the Golden Gate Raptor Observatory from late August through late December each year (Miller 2007). We captured hawks using bow-nets, dho-gazas, or mist nets (Bloom 1987). We fitted each hawk captured with a U.S. Geological Survey leg band, identified the hawk to species and age, and collected blood via medial metatarsal venipuncture (following Hull et al. 2008). We stored blood samples in lysis buffer (10 mM Tris–HCL pH 8.0, 100 mM EDTA, and 2 % dodecyl sulfate), and placed them in a −20 °C freezer at the end of the day, where they remained frozen until DNA extraction (Ishak et al. 2008; Sehgal et al. 2006).

Blood parasite screening

We extracted DNA from all hatch-year Red-tailed Hawk blood samples using DNeasy kits (Qiagen, Valencia, CA, USA) following the animal tissue protocol. To screen for the presence of *Leucocytozoon* species within hawk blood DNA samples, we used a nested PCR reaction to amplify the cytochrome *b* region of *Leucocytozoon* mtDNA. We performed the nested PCR as described by Sehgal et al. (2006). All *Leucocytozoon* screening by PCR included positive and negative controls. As positive controls, we used birds with known infections evident from PCR detection of a previous study and microscopy (Ishak et al. 2008). Our negative controls were purified water instead of DNA template. We ran PCR products on a 1.8 % agarose gel using 1× TBE as buffer, and visualized by ethidium bromide stain under UV light.

DNA sequencing

To determine parasite species and cyt *b* lineages found in each Red-tailed Hawk testing positive for the genus *Leucocytozoon*, we purified PCR product from all positive samples for DNA sequencing using ExoSap-IT following the manufacturer's protocol (USB, Cleveland, OH, USA). We performed bi-directional sequencing with dye-terminator fluorescent labeling in an ABI Prism 3100 automated sequencer (Applied Biosystems, Foster City, CA, USA). We used the same primers from the second PCR reaction (Lcytb F and Lcytb R) in the sequencing reaction. We sequenced 800 base pairs and edited them using Sequencher v.4.8 (GeneCodes, Ann Arbor, MI, USA). We identified all edited sequences to lineage by identifying their exact sequence matches in GenBank via the National Center for Biotechnology Information (NCBI) nucleotide BLAST search. We identified all sequences that did not match another published sequence in Genbank as a new lineage

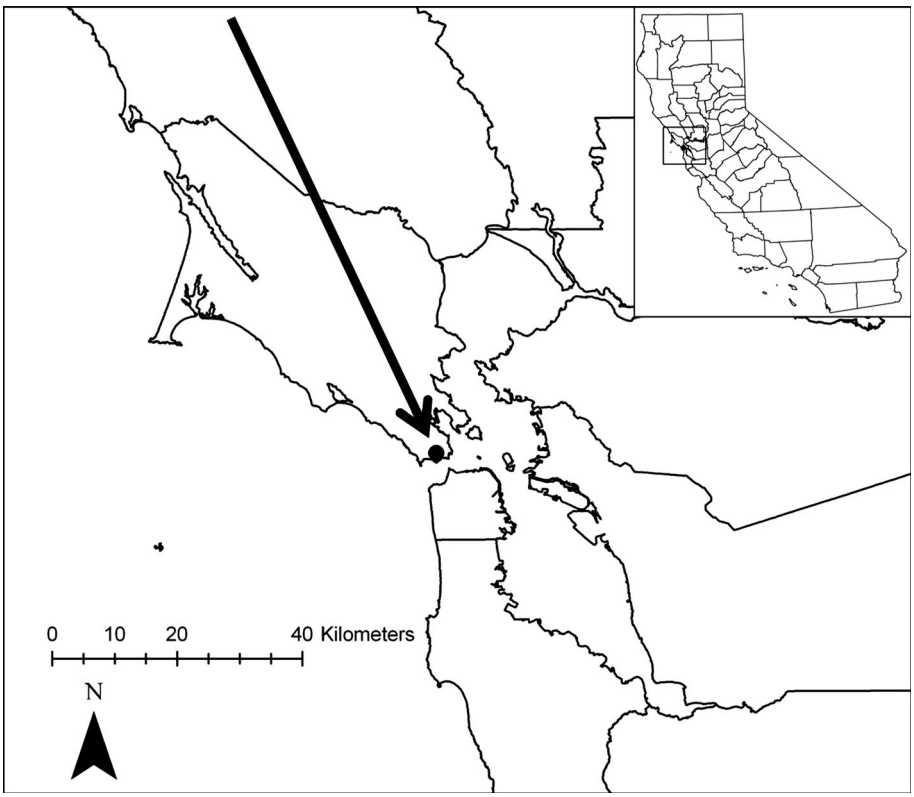

Fig. 1 Study Site: Map of the location of Golden Gate Raptor Observatory (at Hawk Hill; *black* circle) along the Pacific Flyway. *Arrow* represents the typical migration route of raptors along the Pacific Flyway

and labeled it using the nomenclature LC2, LC3, etc. We considered sequences that differed by one or more nucleotides to be separate distinct lineages when verified by repeated sequencing (Ricklefs and Fallon 2002). Lineages from coinfections, as identified by double peaks in the chromatograms, were delineated visually by comparing the position of double peaks with previously identified lineages.

Phylogenetic analysis

We implemented phylogenetic analyses using maximum likelihood (ML) techniques and computed sequence divergence algorithms using PAUP* 4.0 (Swofford 2002). We constructed parsimony and neighbor-joining trees and verified that they had similar topologies as the maximum likelihood trees, and that *Plasmodium relictum* was an appropriate outgroup to use to root the trees. We performed a maximum likelihood heuristic search using a TBR branch-swapping algorithm with a neighbor-joining tree as the starting tree and *Plasmodium relictum* as the outgroup. We performed a bootstrap analysis with 1,000 replicates using stepwise addition under the likelihood settings. We estimated genetic differences using the HKY85 distance setting as determined by MrModeltest, which accounts for unseen variation due to multiple base pair hits. We determined bootstrap values using the GTR+G model as determined by MrModeltest.

In addition, we used Bayesian analyses to generate a phylogeny of cyt *b* lineages (Fig. 2). We analyzed sequence data using MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) and implemented the model (GTR+G) obtained from MrModeltest. We ran two Markov chains simultaneously for 2.5 million generations with sampling every 100 generations for a total of 25,000 trees each, sampled from the posterior distribution. Those trees sampled prior to the runs reaching a split deviation frequency of 0.01 were discarded from the sample as the "burn-in" period that accounted for 25 % of the trees. We used the remaining trees to calculate the posterior probabilities of the individual clades.

Statistical analyses

We tested for correlations between parasite prevalence and migration timing for hawk arrivals to the study site during both the 2004 and 2006 seasons, and between parasite prevalence and population of origin for the 2004 hawk arrivals. Due to low sample numbers, the individuals from 2007 were excluded from statistical analyses regarding parasite prevalence and migration timing. We analyzed the same correlations for each identified *Leucocytozoon* parasite lineage from hawks sampled during the 2004 and 2006 fall migration seasons. For all analyses on effects of infection on migration timing, we used the 1 October cutoff

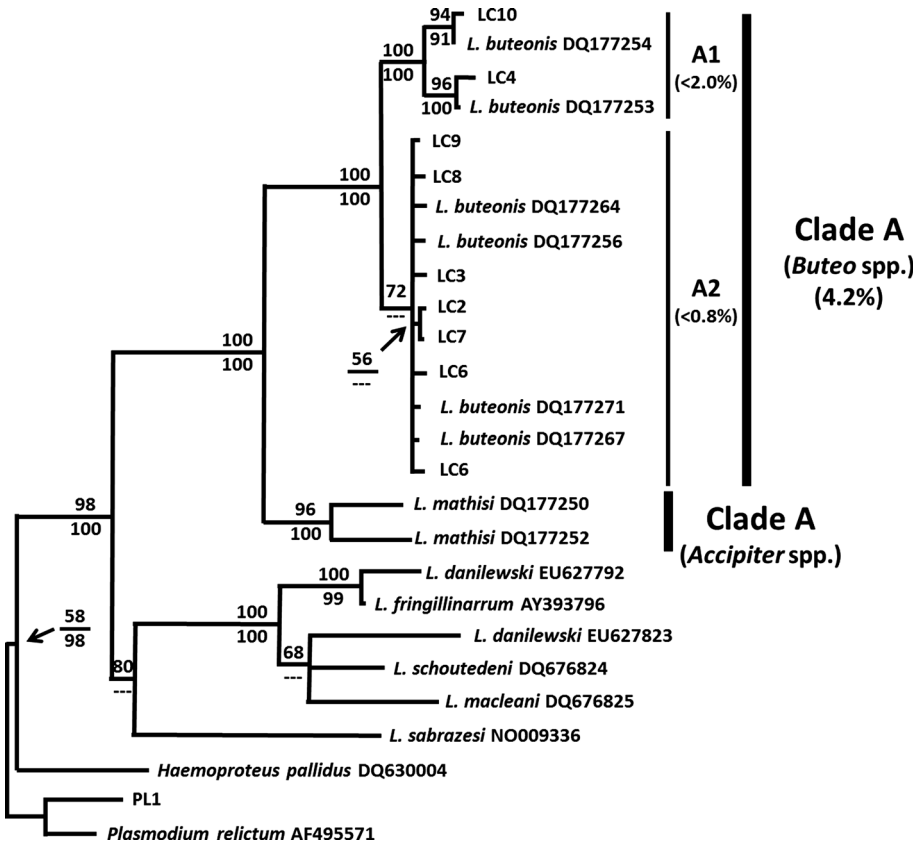

Fig. 2 Maximum likelihood bootstrap phylogram of cyt *b Leucocytozoon* lineages identified in this study presented with other previously identified cyt *b* lineages for comparison. Newly identified lineages from this study are labeled LC2 up to LC10. The single new *Plasmodium* lineage identified is labeled as PL1. *Plasmodium relictum* was used as the outgroup. Previously identified lineages are represented by species with Genbank accession numbers. Numbers located on the top of the branches indicate maximum likelihood (ML) bootstrap support (1000 replications, only values above 50 % are shown) and below are from Bayesian (BY) probability values. The *brackets* indicate the two clades of *Leucocytozoon* lineages isolated from Buteo and Accipiter hawks species

date from Hull et al. (2009) to separate early and late hawk arrivals to our study site. Early arrivals are defined as hawks sampled between 15 August and 30 September for both years, while late arrivals are those sampled between 1 October and 31 December. We defined the fall migration seasons of 2004 and 2006 as 15 August–31 December. Hawks sampled in this study are the same individuals as those examined by Hull et al. (2009).

We tested the association between the infection status (dependent, binary variable, infected vs. not infected), the arrival date (before 1 October vs. after 1 October), the population (Central California vs. Intermountain West), and the year (2004 vs. 2006) using generalized linear models (Proc GENMOD with binomial distribution of errors and logit link function, SAS Institute, 1999). In a second step, we performed additional analyses for 2 of the 11 identified parasite lineages, both of which were previously identified in an earlier study by Sehgal et al. (2006): *L. buteonis* DQ177256 ($n = 30$) and *L. buteonis* DQ177267 ($n = 28$) using the same explanatory variables. These lineages were chosen because they were the most common lineages infecting our sample of study hawks. In each case, we selected the best model by starting from a full model with the two explanatory variables and their interactions, and sequentially removing variables according to the Akaike information criterion (AIC). We conducted all analysis using the SAS software 9.1 (SAS 1999).

Results

Analysis of population of origin

Due to the fact that we sampled from the same individuals, we grouped hawks into Central California and Intermountain West populations of origin as determined by Hull et al. (2009). Briefly, using 17 microsatellite loci, Hull et al. (2008) examined the multilocus genotypes of 1,083 Red-tailed Hawks collected during the breeding and migration seasons from 2003 through 2006 from 18 breeding and migration study sites. The relationships among these 18 sites were examined using (1) multilocus clustering methods as implemented in Structure (Pritchard et al. 2000) to probabilistically cluster related individuals, (2) analysis of molecular variation to test for differentiation among sites, and (3) Monmonier's maximum distance algorithm (Manni et al. 2004) to identify barriers to gene flow between breeding populations. These analyses indicated that Red-tailed Hawk breeding populations in western North America can be grouped into two distinct populations: a Central California breeding population and

an Intermountain West population. The genetic results from Hull et al. (2008) were applied by Hull et al. (2009), in combination with long-term banding and count data, in an examination of the migration timing and direction of genetically distinct breeding populations through the Marin Headlands study area.

Parasite prevalence and lineage diversity

We screened 296 hatch-year Red-tailed Hawks sampled during the 2004 and 2006 fall migration seasons for parasite infection with *Leucocytozoon* species by PCR. Forty-two of 138 hawks (30 %) from 2004 tested positive for *Leucocytozoon* infection, while 25 of 158 (16 %) tested positive from the 2006 season. We found a significant effect of the interaction between the date of arrival (early vs. late season) and the year on the infection status ($c^2 = 9.30$, $P = 0.002$) indicating that the date of arrival had opposing effects on parasite prevalence depending on the year sampled. For the 2004 season, individuals arriving before 1 October had a lower prevalence of infection (13 %, i.e. 4 infected of 30 sampled) than individuals arriving after 1 October (35 %, i.e. 38 infected of 108 sampled; $c^2 = 5.21$, $P = 0.02$). The opposite effect was observed during the 2006 fall season: early arrivals had higher parasite prevalence (29 %, i.e. 8 infected out of 28 sampled) than late arrivals (13 %, i.e. 17 infected out of 130 sampled; $c^2 = 3.66$, $P = 0.05$).

We found nine distinct *Leucocytozoon* and one *Plasmodium* mitochondrial cyt *b* lineages (LC2-LC10, PL1 in Fig. 2; Genbank accession numbers HM142915–HM142923) that produced no matches from NCBI nucleotide BLAST searches. We did not specifically test for *Haemoproteus* or *Plasmodium* infections, but the PCR primers for *Leucocytozoon* did detect one *Plasmodium* lineage (PL1). This type of cross-reactivity for primer sets has been documented before (Szöllsi et al. 2008). The two dominant *Leucocytozoon* lineages infecting our hawk samples were both previously identified lineages classified as *L. buteonis* (Sehgal et al. 2006; Valkiunas 2010; Genbank accession #DQ177267 and #DQ177256). Of all infected hawks successfully sequenced ($n = 57$), 30 were infected with *L. buteonis* DQ177256, while 28 were infected with *L. buteonis* DQ177267. Table 1 shows the numbers of individuals infected with each lineage as well as the number of individuals from the 2004, 2006, and 2007 fall seasons that were infected with multiple lineages. Hawks from the Central California population were infected with 5 of the 11 lineages, while 6 of the 11 *Leucocytozoon* lineages infected individuals from the Intermountain West population.

We found a significant population effect on the prevalence of *L. buteonis* DQ177267 ($c^2 = 5.46$, $P = 0.02$). Indeed, the Central California population had a higher prevalence of this lineage (75 %) than the Intermountain West population (33 %; $c^2 = 5.46$, $P = 0.02$). However, we did not detect a population effect on the prevalence of *L. buteonis* DQ177256. We also found no significant effect of temporal group (early vs. late) on the prevalence of any of the lineages identified. We did not perform analyses of the remaining lineages due to the small sample size (<5 individuals infected with each lineage). We found a higher prevalence of *Leucocytozoon* infection within the Intermountain West population (34 %) than the Central California population (26 %), but the difference in parasite prevalence between the two populations was not statistically significant.

The phylogeny of *Leucocytozoon* lineages identified in this study (Fig. 2) reveals that all identified lineages grouped into two subclades (A1 and A2 in Fig. 2) and that each included previously identified *L. buteonis* lineages. The sequence divergence between lineages within subclade A1 range from 0.2 to 2 % while divergence between lineages within subclade A2 range from 0.1 to 0.8 %. The sequence divergences between the two subclades ranged

Table 1 Number of Red-tailed Hawk individuals infected with *Leucocytozoon* cyt *b* lineages identified during the fall seasons of 2004–2007. If coinfections were detected, they are indicated in the Coinfection column

Leucocytozoon lineages		Year			
Lineage	Coinfection	2004	2006	2007	All years
L. buteonis DQ177267	None	10	4		14
L. buteonis DQ177267	*L. buteonis* DQ177256	9	4	1	14
L. buteonis DQ177256	None	9	3	3	15
	LC2	1			1
LC2	None			1	1
	LC7	1			1
LC3	None	1			1
	LC6	1			1
LC4	None	1			1
LC5	None	1			1
LC6	LC3	1			1
LC7	LC2	1			1
LC8	None		1		1
LC9	LC6	1			
LC10	None		1		1
PL1	None		2		2
Total infected individuals		35	16	5	56
Total L. buteonis DQ177267 infections		19	8		27
Total *L. buteonis* DQ177256 infections		19	7	4	30
Total multiple lineage coinfections		22	4		26

from 2.7 to 4.2 % with an average distance of 3.4 %. The average sequence divergence between the two subclades (A1 and A2) and the *L. mathisi* clade is 10 %.

Discussion

Parasite lineage diversity

Sequence analysis of *Leucocytozoon* species infecting Red-tailed Hawks revealed a high diversity of *L. buteonis* lineages. We found 11 *Leucocytozoon* lineages (nine newly identified lineages) in total that all fell within two closely related monophyletic subclades (A1 and A2 in Fig. 2) with an average sequence divergence of 3.4 % among them. Each subclade includes previously identified *L. buteonis* lineages that differ by <2 % (Sehgal et al. 2006; Valkiūnas 2010). The study by Valkiūnas (2010) identified three distinct lineages infecting different *Buteo* species with an almost identical range of sequence divergence of 1.5–3.4 % compared to a range of 2.7–4.2 % in this study. The lineages isolated from *Buteo* host species (*L. buteonis* spp.; Clade A in Fig. 2) differ from those isolated from *Accipiter* species (*L. mathisi* spp.; Clade B in Fig. 2) by 10 %. Thus, our data support the conclusion that *L. buteonis* and *L. mathisi* are distinct species (Valkiūnas 2010).

With the 11 distinct haplotypes isolated from Red-tailed Hawks identified here, combined with lineages isolated by Sehgal et al. (2006) at the same study site, a total of 21 distinct *Leucocytozoon* lineages have now been identified in California raptors of the genus *Buteo*. It is likely that the high diversity of mitochondrial cyt *b* *Leucocytozoon* lineages found in this study actually reflects only intraspecific variation within this species' cyt *b* gene. Similar to our study, several other *Leucocytozoon* studies observed a high diversity of hemosporidian lineages infecting raptors (Ishak et al. 2008; Valkiūnas 2010; Sehgal et al. 2006; Outlaw and Ricklefs 2009) as well as songbirds (Sato et al. 2009; Martinsen et al. 2008; Ricklefs and Fallon 2002; Bensch et al. 2000; Jenkins and Owens 2011). Further parasite screening of different *Buteo* and *Accipiter* species in North America would be necessary to determine whether the degree of lineage diversity observed in Red-tailed Hawks extends to other raptor species in North America.

Our analysis of the two dominant *Leucocytozoon* lineages revealed that the *L. buteonis* DQ177267 lineage is significantly more prevalent within the central California hawk population. A number of studies have documented differential prevalence of parasite lineages over a limited geographical range in species of the hemosporidian genera *Plasmodium* and *Haemoproteus* (Bensch and Åkesson 2003; Kimura et al. 2006; Durrant et al. 2008). However, Fallon et al. (2006) concluded that *Haemoproteus* and *Plasmodium* are not good geographical indicators of host origins. Data from this study suggest that *Leucocytozoon*, in part due to its high lineage diversity, may exhibit more geographical structuring. In support of this, Hellgren et al. (2007) found geographic structuring in a single *Leucocytozoon* lineage infecting a passerine host species.

Although *Leucocytozoon* lineages frequently have broad distributions (Valkiūnas 2005; Fallon et al. 2006; Valkiūnas 2010), there are potential factors that could contribute to the localization of individual lineages within specific host populations. If a particular *Leucocytozoon* parasite lineage was specific to a single vector species found only in California, then that parasite lineage would be transmitted to the host population breeding in California significantly more than the Intermountain West population. The Sierra Nevada mountain range could serve as a significant physical barrier separating different black fly species in western North America. However, the extent to which this speculation is true requires further research, as very few studies have focused on vector ecology and distribution, especially within Central California and the Intermountain West (Adler et al. 2004).

Differential prevalence between year and date of arrival

Parasite prevalence varied with year and date of arrival of hawks to our study site. However, opposing effects on parasite prevalence were observed within the 2004 and 2006 fall seasons. That is, in the 2004 fall season, early hawk arrivals to the study site had lower prevalence of *Leucocytozoon* parasite species than later hawk arrivals. During the 2006 fall season, early hawk arrivals had higher prevalence of infection than later hawk arrivals. This would seem to imply that parasite infection has no measurable impact on migration timing. However, the microsatellite studies by Hull et al. (2008, 2009) discussed above showed that early hawk arrivals to the study site are primarily from the Central California population while the later arrivals are comprised of both a Central California population and a population from the Intermountain West. Therefore, we are unable to isolate whether the opposing trends in *Leucocytozoon* parasite prevalence between early and late arrivals during the 2004 and 2006 fall seasons are due to an impact of infection on migration timing or to other factors that vary between the two different hawk populations.

Differences in meteorological conditions affecting the abundance of black fly populations within the home ranges of the two host populations is a potential explanation that could account for these opposing trends in parasite prevalence within early and late hawk arrivals during the 2004 and 2006 fall seasons. Other studies have documented differences in parasite prevalence between early and late migrants (Rintamäki et al. 1998, Phalen et al. 1995). A

previous study on the same sample set of Red-tailed Hawks (Ishak et al. 2010) found an increase in *Haemoproteus* prevalence during the second half of the 2004 fall season. That study also observed opposing trends of *Haemoproteus* and *Leucocytozoon* prevalence within early and late Cooper's Hawk (*Accipiter cooperii*) arrivals during the 2004 and 2005 fall seasons. Analysis of meteorological data within ranges of our two host populations is beyond the scope of this study. Further examination into the influence of climatic conditions on vector communities is essential to understanding seasonal differences in parasite prevalence.

Conclusions and suggestions for further research

Our findings on parasite prevalence and sequence data revealed that geographic genetic structuring of host populations is also reflected in the parasites they harbor. Though this study mirrored others in documenting a wide diversity of *Leucocytozoon* lineages infecting raptors, no clear impact of infection with these parasites on Red-tailed Hawk migration timing was observed. Rather, differences in parasite prevalence between early and late arrivals can be attributed to differential movement patterns of distinct host populations and probable seasonal differences in the vector communities infecting them. Further research into the distribution of different vector species within western North America and the role of seasonal climatic conditions on their abundance is needed to help explain interannual variation in parasite prevalence in populations of migratory raptors.

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Conflict of interest The authors declare that they have no conflict of interest.

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