



HAEMOSPORIDIAN PARASITE INFECTIONS IN THE DICKCISSEL (*SPIZA AMERICANA*): PREVALENCE AND COSTS

Todd M. Jones¹, Loren Merrill², Steven E. Zachar³, Jeffrey D. Brawn¹, and Michael P. Ward^{1,2}

¹ Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, Illinois 61801.

² Illinois Natural History Survey, Prairie Research Institute, University of Illinois at Urbana-Champaign, 1816 S. Oak St., Champaign, Illinois 61820.

³ College of Veterinary Medicine, University of Illinois at Urbana-Champaign, 2001 S. Lincoln Ave., Urbana, Illinois 61802.

Correspondence should be sent to Todd Jones at: toddmj@illinois.edu

KEY WORDS ABSTRACT

Birds Condition Fitness Infection Haemosporidian Parasites	Haemosporidian parasites are widespread in birds and are commonly used to evaluate ecological, evolutionary, and behavioral consequences of pathogen infection. While haemosporidian parasites may be important agents of selection, information on the incidence and outcomes of infection in many wild birds remains incomplete and warrants further inquiry. We examined the prevalence and costs of haemosporidian parasites in a wild population of dickcissels (<i>Spiza americana</i> ; n = 170) breeding in central Illinois. Over 2 breeding seasons, prevalence of haemosporidian parasites was 41% and was similar between sexes and years. Within each sex, there was no association between proxies of fitness (body condition and number of fledglings produced) and infection status. While we found no evidence that haemosporidian parasites have a direct, negative impact on dickcissels, we discuss how extra-pair paternity and changes across the life cycle of dickcissels and haemosporidian parasites may have masked or prevented detection of negative effects in our study. Ultimately our findings contribute to a growing literature providing improved clarity on how and when parasite infections can negatively impact wild host organisms and can aid in guiding future work examining such associations.
---	--

Haemosporidian blood parasites of the *Plasmodium* and *Haemoproteus* genera are vectored by biting dipterans and are globally distributed (Valkiunas, 2005). Widespread among most terrestrial vertebrate taxa, these parasites have been documented in every avian order and have been used to evaluate ecological, evolutionary, and behavioral consequences of infection (Asghar et al., 2011). Past studies examining the effects of haemosporidian infection on birds have documented negative effects on body condition (Valkiunas et al., 2006), growth (Coon et al., 2016), physiological factors (Arriero et al., 2008), and estimates of survival and reproductive success (Merino et al., 2000; Marzal et al., 2008). Though negative effects have been documented in wild populations (e.g., Christe et al., 2012), the impacts of haemosporidian parasite infection are often ambiguous and/or depend on case-specific conditions (Asghar et al., 2011); the direction of cause and effect can be hard to disentangle (sensu Fleskes et al., 2017; Merrill et al., 2018). Furthermore, our understanding of whether costs of haemosporidian infections are likely to differ between the sexes is limited. Some studies have documented apparently higher costs in males (e.g., Christe et al., 2012), while others have found higher costs of infection in females (e.g., Calero-Riestra and Garcia, 2016). Sex-linked costs of infection are likely influenced by a species' life history and the timing of

sampling relative to the period of peak reproductive investment. Consequently, we still have a rudimentary understanding of the underlying sources of variation and outcomes of infection in wild birds.

One of the most commonly described patterns of infection is that of sex-linked variation in parasite prevalence, in which males of many species exhibit higher rates of infection than females (Poulin, 1996). Theory indicates that this difference should be most pronounced in species with high levels of sexual dimorphism (Hamilton and Zuk, 1982; Zuk and McKean, 1996; Moore and Wilson, 2002), although the underlying causes of variation by sex remain unclear. More case studies are needed to determine the generality of this pattern and to help determine the potential causes of sex-linked biases.

Here we present findings from our study examining prevalence and costs of haemosporidian parasite infection in a wild population of dickcissels (*Spiza americana*) breeding in central Illinois. One of the most common and emblematic birds of the North American grasslands (Temple, 2002), dickcissels are a mid-sized songbird of the order Passeriformes and family Cardinalidae (Cardinals and allies, "large finches"). Additionally, dickcissels are a polygynous, sexually dimorphic, migratory species—congregating in large flocks in Central America and northern

South America during the non-breeding season. Dickcissels commonly feed on grassland seeds and invertebrates and nest just off the ground in grassland vegetation. Thus, life history traits of the dickcissel make it a suitable species for examining sex-linked variation and costs in parasite prevalence.

Specifically, we examined (1) if infections were costly by investigating possible associations of infection status (presence/absence of haemosporidian) on body condition and reproductive success of breeding adults and (2) general rates of haemosporidian infection with respect to sex. We predicted that the probability of infection would be higher in males, that infections would be negatively associated with body condition and reproductive success across sexes, but that there would be a stronger effect of infection on male condition and fitness due to their polygynous breeding system and presumed elevated investment in current reproduction.

MATERIALS AND METHODS

From May through August of 2014 and 2015, we sampled breeding dickcissels at 2 grasslands located in central Illinois. We captured adult dickcissels with mist nets by luring males in with conspecific playbacks and flushing females off their nests. We banded captured birds with a U.S. Geological Survey bird band and a unique combination of plastic color bands, took morphometric measurements (un-flattened wing length [± 0.5 mm], tail length [± 0.5 mm], tarsus length [± 0.01 mm], and mass [± 0.01 g]), and drew a blood sample (see below for details). No birds captured in 2014 were recaptured in 2015.

To estimate reproductive success (the number of fledglings produced annually), we searched for dickcissel nests (May–August 2014 and 2015) by observing behavioral cues of adults and systematically searching vegetation. We monitored active nests every 1 to 6 days until nests either failed or fledged. We identified social pairs/harems (male dickcissels can be polygynous and have multiple females/nests) and associated adults with nests using behavioral observations (mate guarding, copulating, incubation, provisioning, etc.) and identifying them with their color band combinations. This study was part of a larger project examining the post-fledging ecology of dickcissels. Thus, we banded all nestlings at the onset of fledging and were able to document the exact number of fledglings fledged from each nest (Jones et al., 2017). Additionally, we kept track of territories where we had difficulty finding nests to ensure we did not miss any fledging events (i.e., while searching for nests in focal territories every 1–3 days, we observed adults to make sure they were not feeding nestlings/fledglings on each visit). These data then allowed us to calculate the total number of fledglings produced in a given year (from all associated nests) for each male and female.

We were unable to determine the paternity of all fledged young, thus our estimate of reproductive success for males does not account for extrapair paternity (EPP) within their social nest(s) and their extrapair young in the surrounding male territories. To our knowledge, only 1 study has examined EPP in the dickcissel, with an estimated 20–40% of young being extrapair (Sousa and Westneat, 2013). Such rates could make our reproductive estimates inaccurate; however, rates of extrapair young per male are positively associated with harem size (Sousa and Westneat, 2013). Additionally, our population is unusual in that most males (>95%) are socially monogamous; we would, therefore, predict

extrapair paternity to be low and similar across all males. Thus, the number of fledglings produced should act as a suitable proxy for male reproductive success in our system.

Screening for parasites

To determine haemosporidian infection status, we took a small (ca. 10 μ l) blood sample from the brachial vein of each bird and stored the blood in Longmire's lysis buffer (Longmire et al., 1997). For DNA extraction, we added 5 μ l of 20 mg/ml proteinase K solution (MidSci catalog no. IB05406) to each sample, then incubated them overnight at 60 C in a hot water bath. We extracted genomic DNA from the samples using a standard ammonium acetate-isopropanol protocol (Svensson and Ricklefs, 2009). Among birds, the most common haemosporidian blood parasites (order Haemosporida) are in the genera *Plasmodium* and *Haemoproteus*, and we used a PCR protocol designed to amplify a 154 bp fragment of rRNA-coding parasite mitochondrial DNA to detect the presence of parasites from these 2 genera (Fallon et al., 2003). For details on PCR technique, see Fallon et al. (2003) and Fecchio et al. (2013). Samples were run out on a gel and imaged to obtain infection status. Once results were obtained, we classified each individual as either parasitized or unparasitized (Weatherhead and Boag, 1995).

Data analyses

To estimate body condition for each individual, we first used a principal component analysis (PCA, Proc Factor, SAS Institute, 1990) to reduce wing length, tail length, and tarsus length to a single PC score (ordinating "body size"; see results below). We then regressed mass against the PCA score and used the residuals as a body condition index. We examined variation in body condition and reproductive success (number of fledglings produced) by infection status and variation in prevalence of infection by year and sex using generalized linear mixed models (Proc Glimmix, SAS Institute, 1990). For reproductive success, we used a negative binomial distribution because the number of fledglings produced was not normally distributed, and there was overdispersion with a Poisson distribution. For each model, the site from which an adult was sampled was included as a random factor to account for issues of non-independence within sites.

RESULTS

We captured 186 adult dickcissels (119 in 2014, 67 in 2015; 116 males and 70 females), of which 170 (103 in 2014, 67 in 2015; 100 males and 70 females) were screened for the presence of haemosporidian parasites. For each sex, principal component analysis derived one factor suitable for use as a measure of "body size." The factor explained 62% of the sample variation (eigenvalue = 1.86) in males and 51% (eigenvalue = 1.54) in females. Wing length, tail length, and tarsus length loaded positively for both sexes, suggesting the factor represented overall "body size" (i.e., individuals that are larger have longer wings, tails, and tarsi). As expected, mass was positively related to body size (males $P < 0.001$, $\beta = 0.50 \pm 0.14$ SE, females $P < 0.001$, $\beta = 0.73 \pm 0.14$ SE).

Overall prevalence of haemosporidian parasites in our dickcissel population was 41% ($\pm 4\%$ SE). We found infections in 37 males (0.37 ± 0.05 SE) and 33 females (0.47 ± 0.06 SE), and the

probability of infection did not differ by sex ($F = 1.75$, $P = 0.188$). Within each sex, we found no relationship between infection status and year (males $F = 0.00$, $P = 0.971$, females $F = 1.24$, $P = 0.269$), body condition (males $F = 0.50$, $P = 0.482$, females $F = 0.90$, $P = 0.345$), or reproductive success (males $F = 0.63$, $P = 0.430$, females $F = 0.11$, $P = 0.744$).

DISCUSSION

Haemosporidian parasites are ubiquitous among wild birds, but we have an incomplete understanding of this host-parasite dynamic. We examined rates of haemosporidian parasite infections in males and females of the polygynous dickcissel over 2 breeding seasons, as well as associations between infection status and aspects of condition and fitness to help better understand this coevolutionary relationship. Though prevalence of parasite infection is often male-biased (Zuk and McKean, 1996; Klein, 2004) and predicted to be even more pronounced in sexually dimorphic species (Moore and Wilson, 2002), we found no difference in haemosporidian infection rates between the sexes. Prevalence in the population remained relatively steady between years (41%), and we found no relationships between infection status and proxies of fitness. Below we discuss caveats and potential factors affecting our findings.

Male-biased parasitism can occur from a number of factors, including differences in immunocompetence, in which males invest heavily in current reproduction at the expense of immune function (Zuk and McKean, 1996; Zuk and Stoehr 2002; but see Kelly et al., 2018) and/or differences in exposure to the vectors (Medeiros et al., 2015). We did not measure these parameters for this study, but the degree of polygyny in our study population provides some indication that males and females in our study population may not have differed as much as other populations of dickcissels, or other polygynous-breeding species. Dickcissels generally exhibit moderate to high levels of polygyny (up to 6 females, average 2–3; Temple, 2002), but in our system, many males had only 1 or (rarely) 2 females on their territory. If patterns of sex-linked differences in parasite infection status are driven primarily by sex-linked variation in immune function (sensu Zuk and Stoehr, 2002), the low reproductive skew in our study population may have resulted in reduced differential investment in immune function, thereby leading to similar rates of parasitism. Alternatively, if infection status in dickcissels is primarily driven by exposure to the vectors (sensu Medeiros et al., 2015), the absence of sex-specific differences in infection rate in our study population may be explained by similar patterns of habitat usage across the year (“behavioral hypothesis”; Guerra-Silveira and Abad-Franch, 2013; Fast et al., 2016). In our system, males and females occupy the same breeding territories and forage in similar locations, and both feed and take care of the young (T. Jones, pers. obs.). In addition, on the wintering grounds males and females group together in large flocks, occur in the same fields, and roost in the same areas (Temple, 2002). This should result in similar rates of exposure to the vectors, although individuals may exhibit variation in behavioral defenses against the vectors.

While patterns of male-biased parasitism seem to be relatively robust, the generality of male-biased infections for haemosporidian parasites is unclear. Some studies do document male-biased patterns of infection (e.g., Guerra-Silveira and Abad-Franch,

2013; Calero-Riestra and García, 2016), whereas others do not (e.g., Fecchio et al., 2015; Stewart and Merrill, 2015). The study by Stewart and Merrill (2015) on Red-winged Blackbirds (*Agelaius phoeniceus*) is particularly relevant because Red-winged Blackbirds exhibit similar reproductive and ecological traits to the dickcissel; they are both polygynous species, in which males are larger and exhibit sexually dimorphic plumage, and they often breed in grasslands throughout North America. It is also worth noting that a recent meta-analysis examining the generality of male-biased reductions in immunocompetence across host taxa found that when controlling for phylogeny, there was no significant difference between the sexes (Kelly et al., 2018). Such work highlights the need for further case studies to determine the generality of sex-biased parasitism across parasite taxa.

Our finding of no associations between infection status and proxies of fitness for either male or female dickcissels suggests that both sexes can tolerate infections without incurring high costs (e.g., Davidar and Morton, 1993; Bensch et al., 2006), although there are several caveats to our findings. First, without accounting for extra-pair paternity we may have under- or overestimated the reproductive success of each male dickcissel, which may have masked potential associations with infections. Second, in wild populations, detecting relationships among infections and parameters of fitness is often difficult owing to changes in infection stage and seasonality (Asghar et al., 2011; Fast et al., 2016)—which we were unable to account for—and in difficulties assessing long-term aspects of fitness (e.g., realized fitness, number of offspring reaching adulthood). For instance, following infection with haemosporidian parasites, birds typically undergo an acute infection stage, during which parasitemias in the blood reach high levels. High parasitemias (and potentially the resulting immune response) can result in morbidity and mortality in the bird (Valkiunas, 2005; Ellis et al., 2015), and this phase of the infection may act as a powerful selective filter (e.g., Marzal et al., 2008; Asghar et al., 2011), making it less likely for lower-quality individuals to survive into the breeding season. Moreover, we sampled only breeding birds, which may have skewed our study population to those higher-quality individuals that were uninfected, cleared their infections, or were able to tolerate a chronic infection with minimal effect on condition. In such cases, costs of infection on dickcissel fitness would have been masked or undetectable under our sampling methods. Finally, because we screened only to the level of order (Haemosporida), we may have missed differences in costs of infection (or sex-linked differences in prevalence) associated with either *Plasmodium* or *Haemoproteus* infections. However, given the overlap between male and female dickcissels in habitat usage and general activity patterns throughout the year, we suspect that exposure to the 2 types of vectors that carry these parasites (*Plasmodium* parasites are vectored by mosquitoes, whereas *Haemoproteus* parasites are vectored by *Culicoides* midges) (Valkiunas, 2005) should be roughly comparable for the sexes. Despite presumed similar exposure risk across the genera, there is good evidence that within the genera there are more virulent species/lineages that have higher costs (see references in Valkiunas and Iezhova, 2018). Thus, it is entirely possible that higher resolution data on infection status would have uncovered sex-linked differences in infection probability or costs of infection.

Our findings did not support our predictions but provide important information regarding haemosporidian parasite prev-

alence and associations with traditional measures of condition and fitness. Additionally, they contribute to a growing body of literature providing improved clarity on how and when parasite infections can negatively impact wild host organisms and can help guide future work examining these associations.

ACKNOWLEDGMENTS

This research was generously funded through a Pittman-Robertson grant of the Illinois Department of Natural Resources (W-154-R), and research grants from the Illinois Ornithological Society, Association of Field Ornithologists, Wilson Ornithological Society, and American Ornithologists Union. This work would not be possible without the dedicated efforts of U. Agada, K. Heffernan, R. Leeson, T. Lyons, K. Ripple, and E. Ospina. We are grateful to the Illinois Department of Natural Resources for access to study sites and help in the field.

LITERATURE CITED

- ARRIERO, E., J. MORENO, S. MERINO, AND J. MARTINEZ. 2008. Habitat effects on physiological stress response in nestling Blue Tits are mediated through parasitism. *Physiological and Biochemical Zoology* 81: 195–203.
- ASGHAR, M., D. HASSELQUIST, AND S. BENSCH. 2011. Are chronic avian haemosporidian infections costly in wild birds? *Journal of Avian Biology* 42: 530–537.
- BENSCH, S., J. WALDENSTROM, N. JONZEN, H. WESTERDAHL, B. HANSSON, D. SEJBERG, AND D. HASSELQUIST. 2006. Temporal dynamics and diversity of avian malaria parasites in a single host species. *Journal of Animal Ecology* 76: 112–122.
- CALERO-RIESTRA, M., AND J. T. GARCÍA. 2016. Sex-dependent differences in avian malaria prevalence and consequences of infections on nestling growth and adult condition in the Tawny pipit, *Anthus campestris*. *Malaria Journal* 15: 178. doi:10.1186/s12936-016-1220-y.
- CHRISTE, P., O. GLAIZOT, N. STREPPARAVA, G. DEVEVEY, AND L. FUMAGALLI. 2012. Twofold cost of reproduction: An increase in parental effort leads to higher malarial parasitaemia and to a decrease in resistance to oxidative stress. *Proceedings of the Royal Society B: Biological Sciences* 279: 1142–1149.
- COON, C. A. C., L. GARCIA-LONGORIA, L. B. MARTIN, S. MAGALLANES, F. DE LOPE, AND A. MARZAL. 2016. Malaria infection negatively affects feather growth rate in the house sparrow *Passer domesticus*. *Journal of Avian Biology* 47: 779–787.
- DAVIDAR, P., AND E. S. MORTON. 1993. Living with parasites: Prevalence of a blood parasite and its effect on survivorship in the Purple Martin. *Auk* 110: 109–116.
- ELLIS, V. A., S. CORNET, L. MERRILL, M. R. KUNKEL, T. TSUNEKAGE, AND R. E. RICKLEFS. 2015. Host immune responses to experimental infection of *Plasmodium relictum* (lineage SGS1) in domestic canaries (*Serinus canaria*). *Parasitology Research* 114: 3627–3636.
- FALLON, S. M., R. E. RICKLEFS, B. L. SWANSON, AND E. BIRMINGHAM. 2003. Detecting avian malaria: An improved polymerase chain reaction diagnostic. *Journal of Parasitology* 85: 1044–1047.
- FAST, K. M., V. W. WALSTROM, AND D. C. OUTLAW. 2016. Haemosporidian prevalence and parasitemia in the Tufted Titmouse (*Baeolophus bicolor*). *Journal of Parasitology* 102: 636–642.
- FECCHIO A., M. R. LIMA, P. SILVEIRA, A. C. A. RIBAS, R. CAPARROZ, AND M. A. MARINI. 2015. Age, but not sex and seasonality, influence Haemosporida prevalence in White-banded Tanagers (*Neothraupis fasciata*) from central Brazil. *Canadian Journal of Zoology* 93: 71–77.
- FECCHIO, A., M. R. LIMA, M. SVENSSON-COELHO, M. A. MARINI, AND R. W. RICKLEFS. 2013. Structure and organization of an avian haemosporidian assemblage in a Neotropical savanna in Brazil. *Parasitology* 140: 181–192.
- FLESKES, J. P., A. M. RAMEY, A. B. REEVES, AND J. L. YEE. 2017. Body mass, wing length, and condition of wintering ducks relative to hematozoa infection. *Journal of Fish and Wildlife Management* 8: 89–100.
- GUERRA-SILVEIRA, F., AND F. ABAD-FRANCH. 2013. Sex bias in infectious disease epidemiology: Patterns and processes. *PLoS ONE* 8: e62390. doi:10.1371/journal.pone.0062390.
- HAMILTON, W. D., AND M. ZUK. 1982. Heritable true fitness and bright birds: A role for parasites? *Science* 218: 384–387.
- JONES, T. M., M. P. WARD, T. J. BENSON, AND J. D. BRAWN. 2017. Variation in nestling body condition and wing development predict cause-specific mortality in fledgling Dickcissels. *Journal of Avian Biology* 48: 439–447.
- KELLY C. D., A. M. STOEHR, C. NUNN, K. N. SMYTH, AND Z. M. PROKOP. 2018. Sexual dimorphism in immunity across animals: A meta-analysis. *Ecology Letters* 21: 1885–1894.
- KLEIN, S. L. 2004. Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunology* 26: 247–264.
- LONGMIRE, J. L., M. MALTBIE, AND R. J. BAKER. 1997. Use of “lysis buffer” in DNA isolation and its implication for museum collections. *Occasional Papers Museum of Texas Tech University* 163: 1–4.
- MARZAL, A., S. BENSCH, M. REVIRIEGO, J. BALBONTIN, AND F. DE LOPE. 2008. Effects of malaria double infection in birds: One plus one is not two. *Journal of Evolutionary Biology* 21: 979–987.
- MEDIROS, M. C., R. E. RICKLES, J. D. BRAWN, AND G. L. HAMER. 2015. Plasmodium prevalence across avian host species is positively associated with exposure to mosquito vectors. *Parasitology* 142: 1612–1620.
- MERINO, S., J. MORENO, J. J. SANZ, AND E. ARRIERO. 2000. Are avian blood parasites pathogenic in the wild? A medication experiment in Blue Tits (*Parus caeruleus*). *Proceedings of the Royal Society of London B* 267: 2507–2510.
- MERRILL, L., J. LEVENGOOD, J. C. ENGLAND, J. OSBORN, AND H. M. HAGY. 2018. Blood parasite infection linked to condition of spring-migrating Lesser Scaup (*Aythya affinis*). *Canadian Journal of Zoology* 96: 1145–1152.
- MOORE, S. L., AND K. WILSON. 2002. Parasites as a viability cost of sexual selection in natural populations of mammals. *Science* 297: 2015–2018.
- POULIN, R. 1996. Sexual inequalities in helminth infections: A cost of being a male? *American Naturalist* 147: 287–295.
- SAS INSTITUTE. 1990. SASSTAT user's guide. SAS Institute, Cary, North Carolina.
- SOSA, B. F., AND D. F. WESTNEAT. 2013. Positive association between social and extra-pair mating in a polygynous

- songbird, the Dickcissel (*Spiza americana*). *Behavioral Ecology and Sociobiology* 67: 243–255.
- STEWART, T. E., AND L. MERRILL. 2015. Host sex and parasitism in Red-winged Blackbirds (*Agelaius phoeniceus*): Examining potential causes of infection biases in a sexually dimorphic species. *Canadian Journal of Zoology* 93: 21–29.
- SVENSSON, L. M. E., AND R. E. RICKLEFS. 2009. Low diversity and high intra-island variation in prevalence of avian Haemoproteus parasites on Barbados, Lesser Antilles. *Parasitology* 136: 1121–1131.
- TEMPLE, S. A. 2002. Dickcissel (*Spiza americana*). In *The birds of North America*, A. F. Poole and F. B. Gill (eds.). Cornell Lab of Ornithology, Ithaca, New York. doi:10.2173/bna.703.
- VALKIUNAS, G. 2005. *Avian malaria parasites and other haemosporidia*. CRC Press, Boca Raton, Florida, 946 p.
- VALKIUNAS, G., AND T. A. IEZHOVA. 2018. Keys to the avian malaria parasites. *Malaria Journal* 17: 212.
- VALKIUNAS, G., T. ZICKUC, A. P. SHAPOVAL, AND T. A. IEZHOVA. 2006. Effect of *Haemoproteius belopolskyi* (Haemosporida: Haemoproteidae) on body mass of the Blackcap *Sylvia atricapilla*. *Journal of Parasitology* 92: 1123–1125.
- WEATHERHEAD, P. J., AND P. T. BOAG. 1995. Pair and extra-pair mating success relative to male quality in Red-winged Blackbirds. *Behavioral Ecology and Sociobiology* 37: 81–91.
- ZUK, M., AND K. A. MCKEAN. 1996. Sex differences in parasite infections: Patterns and processes. *International Journal of Parasitology* 10: 1009–1023.
- ZUK, M., AND A. M. STOEHR. 2002. Immune defense and host life history. *American Naturalist* 160: S9–S22.