

Short communication

Greater seasonal variation in blood and ectoparasite infections in a temperate than a tropical population of House Sparrows *Passer domesticus* in North America

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Parasite pressure has been predicted to be greater on tropical vs. temperate organisms because climatic stability in the tropics is thought to promote the persistence of parasites and their vectors year-round (Janzen 1970, Connell 1971, Leigh 1994). Thus far, only a few studies have compared parasite pressure across latitudes in passerine birds. Contrary to the above prediction, most have found that parasite prevalence (the proportion of infected individuals in a population) is lower in Neotropical than in Nearctic birds (Greiner *et al.* 1975, White *et al.* 1978, Sousa & Herman 1982, Ricklefs 1992; but see Booth & Elliott 2003). Although several explanations for this unexpected pattern have been offered, one important influence may be irresolute methodology. Typically, latitudinal comparisons of parasite load in birds have been conducted at single points in space and time (Deviche *et al.* 2001a). For example in Dark-eyed Juncos *Junco hyemalis*, haematozoan prevalence varied between birds captured just after arrival from migration vs. during active breeding (Deviche *et al.* 2001b). Further, haematozoan infection varied among Greenfinch *Carduelis chloris* populations sampled during the same time of the year, but variation did not follow a systematic geographical pattern (Merilä *et al.* 1995).

A second (but not mutually exclusive) possibility is that tropical birds in general possess stronger anti-parasite defences than temperate birds, which lead to lower parasite infections on tropical individuals even though parasite pressure may actually be greater near the equator (Ricklefs 1992). Recently, we found that aspects of immune defence vary seasonally and latitudinally in one species of passerine,

the House Sparrow *Passer domesticus*. Specifically, birds from New Jersey (hereafter temperate) mounted weak cutaneous immune responses early in the breeding season followed by stronger ones later, whereas birds from Colon, Panama (hereafter tropical), showed no intra-annual variation over the year (Martin *et al.* 2004). Cutaneous immune activity was also suppressed by experimentally elevated glucocorticoids, an avian stress hormone, in the temperate population, but the same treatment had no effect on the same immune response in the tropical population (Martin *et al.* 2005). Finally, tropical Sparrows exhibited greater increases in whole-body energy turnover post immune challenge and favoured more costly types of immune defence than temperate birds (Martin *et al.* 2006), suggesting that, at least in the short term, tropical House Sparrows invest more in immune defence.

Based on these findings, parasite prevalence and intensity were predicted to be low in tropical House Sparrows at all times of the year. In the temperate population, parasite prevalence and intensity were predicted to vary seasonally, and more specifically, seasonality of parasite load was expected to mirror seasonality of (cutaneous) immune function. We tested this hypothesis by comparing ectoparasite and intracellular blood parasite prevalence and intensity (mean number of parasites per bird) during the three periods of the year that are most comparable (in terms of reproductive activity) between populations – early breeding, late breeding and non-breeding (Fig. 1a).

METHODS

House Sparrows were captured via mist-netting in the Zona Libre, Colon, Panama (9°1'N, 80°1'W) or from the Belle Mead Co-op or the Princeton Shopping Center, both near Princeton, New Jersey (40°21'N, 74°40'W; Martin *et al.* 2004). Birds were collected during March–April 2002 or 2003 (early breeding season), July–August 2001 (late breeding season) and October–November 2002 (non-breeding season). These times of year were chosen because they represent the most similar portions of the breeding seasons of both populations (Fig. 1a; data for temperate population (Ithaca, NY) from Weaver 1943). In addition, previous work had indicated that cutaneous immune activity varied across these seasons differently in each population (Martin *et al.* 2004). After capture, ectoparasite and blood samples were taken (see below), and birds were weighed (to 0.1 g) and released. Breeding status of individuals was not determined owing to time constraints. We did, however, account for potential effects of individual age on parasite load by including only adult birds in the study (as identified by plumage characteristics; *sensu* Summers-Smith 1988). Furthermore, individual sex was included in statistical comparisons in case it influenced parasite prevalence or intensity.

Ectoparasite loads were quantified for each bird using the dust-ruffling technique of Poiani *et al.* (2000), which

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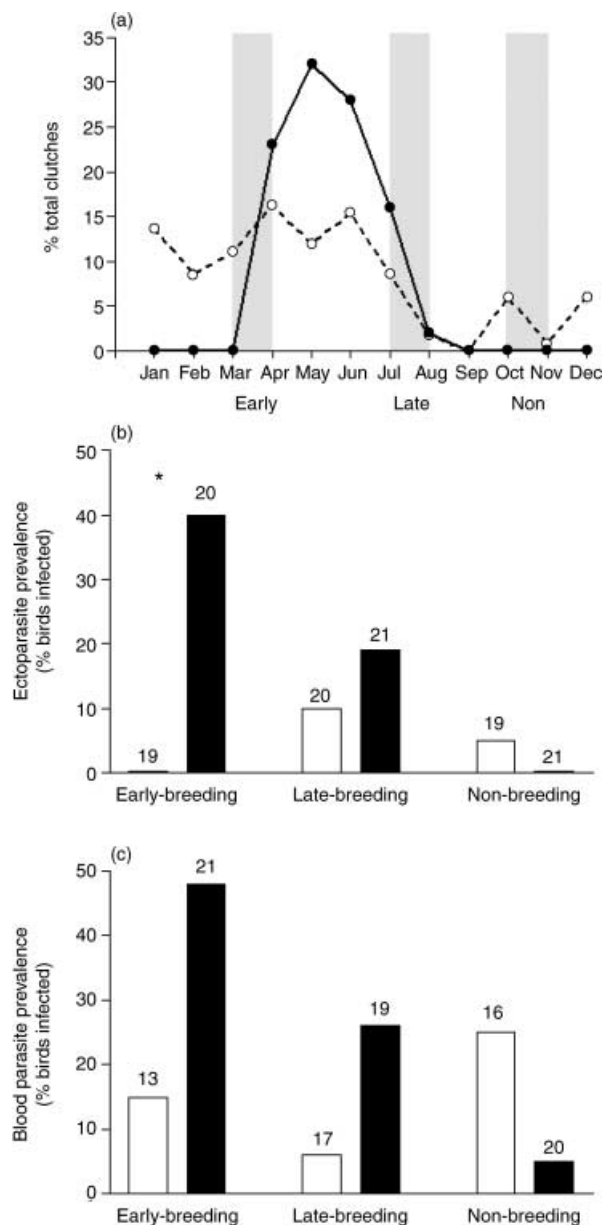


Figure 1. (a) Breeding phenology, (b) ectoparasite prevalence and (c) blood parasite prevalence in tropical and temperate House Sparrow populations. Grey-shaded bars and labels below the ordinal in (a) represent the timing of parasite samplings in the present study. Data for the temperate population in (a) taken from Weaver (1943) based on a House Sparrow population in Ithaca, NY, USA; the tropical population is from Colon, Panama. Bars in (b) and (c) indicate percentage of birds infected, and numbers above bars are total birds sampled. Open bars represent the tropical population and solid bars represent the temperate population. Asterisk indicates $P < 0.05$ (chi-squared test).

provides an accurate assessment of the total number of chewing lice (Clayton & Drown 2001). Birds were dusted with 0.1% pyrethrin powder (Sergeant's Pet Care Products, Omaha, NE, USA) over a plastic box lined with coloured construction paper, held in a plastic sandwich bag for 10 min to allow the powder to take effect, and then inspected by ruffling the feathers while holding the bird over the construction paper. Any parasite found during a 5-min visual examination of the construction paper, the plastic bag or the bird was preserved in 70% ethanol for later identification. Upon return to the lab, specimens were identified to the family level and quantified using a dissecting microscope (Bausch and Lomb, Rochester, NY, USA) under 30 \times magnification (Bland & Jaques 1978, McDaniel 1979).

For blood parasite quantification, approximately 10 μ L of blood was collected into a heparinized microcapillary tube from the brachial vein of each bird. Blood smears were then immediately made on glass microscope slides, air-dried for 12–24 h, fixed for 10 min in 100% methanol and stained with Hema 3 (Fisher, Fairlawn, NJ, USA) 1 week later (Bennett 1970). For each blood smear, one investigator (M.I.P.), unaware of the identity of the slide, examined 10 000 erythrocytes per slide by using a Nikon Photoshoot microscope at 1000 \times magnification under oil-immersion. The presence of *Plasmodium* spp. or *Haemoproteus* spp. and the intensity of infections (number of parasites per 10 000 erythrocytes examined) was noted based on descriptions by Garnham (1966).

Prevalence of blood and ectoparasites was calculated as the proportion of individuals in each population/season infected with parasites. Intensity of infection with ectoparasites refers to the number of parasites counted per infected bird, irrespective of the parasite species. These aggregate calculations were used because of the overall low species diversity in either population (New Jersey: $n = 2$; Panama: $n = 1$). Intensity of infection with blood parasites refers to the number of infected cells per 10 000 uninfected red blood cells in an individual bird. All blood parasites were *Plasmodium* spp., except one parasite in one bird from Panama. Mean intensity for both blood and ectoparasites in a population was defined as the average intensity among all infected birds. Unlike abundance, infection intensity excludes uninfected birds.

Forward logistic regression, with a probability of variable entry into model set at $P = 0.05$, was used to identify factors predictive of ectoparasite and blood parasite infection status (i.e. prevalence), expressed as 1 (infected) or 0 (uninfected) per individual bird sampled. Latitude (Panama or New Jersey), season (early, late or non-breeding), sex, all interactions between these variables and body mass were included as predictor variables. When significant interactions were identified, chi-squared analyses were used to examine relationships separately by latitude, season or sex as appropriate. To determine which factors influenced parasite intensity, General Linear Models (GLMs) were

used as intensity was approximately normally distributed. In these models, latitude, season, sex and all interactions were included as fixed factors and body mass was included as a covariate. For all analyses, we used SPSS v.10 and set $\alpha = 0.05$.

RESULTS

Temperate House Sparrows carried only two morpho-species of ectoparasites, which were identified as feather-chewing lice (Philopteridae) and mites (Dermanyssidae). Among tropical sparrows, only one morphospecies of ectoparasite was found, a louse fly (Hippoboscidae). Because of this low species diversity and low abundance of individuals within taxa, all ectoparasites were analysed collectively. According to the forward logistic regression procedure, the only significant variable was the interaction between latitude and season ($\chi^2_2 = 14.4$, $P < 0.001$), suggesting that prevalence was higher in temperate than in tropical sparrows at some times of the year. Chi-squared analyses of prevalence between latitudes within season indicated greater infection likelihood in the temperate population only in the early breeding season ($\chi^2_2 = 9.6$, $P = 0.002$; Fig. 1b). Chi-squared analyses of prevalence among seasons within latitude indicated that ectoparasite prevalence varied seasonally in the temperate population ($\chi^2_2 = 10.5$, $P = 0.005$) but not in the tropical population. Ectoparasite intensity was not affected by latitude, season, sex, body mass or any interaction (GLM: full model $F_{6,15} = 1.2$, $P = 0.43$).

All blood parasites but one were *Plasmodium* spp.; one tropical bird was infected by one *Haemoproteus* spp. Subsequently, all parasites were analysed collectively regardless of species identity. In common with parasite intensity, the only significant variable selected in the forward logistic regression model was the interaction between latitude and season ($\chi^2_2 = 10.2$, $P < 0.01$), suggesting that blood parasite prevalence was higher in temperate than in tropical birds in the early but not the late or non-breeding seasons (Fig. 1c). Chi-squared analyses of prevalence between latitudes within seasons indicated that prevalence tended to be higher in the temperate than in the tropical population in the early breeding season ($\chi^2_2 = 3.7$, $P = 0.06$), but not the late or non-breeding seasons. Chi-squared analyses of prevalence among seasons within latitudes indicated seasonality of blood parasite prevalence in the temperate population ($\chi^2_2 = 9.5$, $P = 0.009$) but not in the tropical population. Blood parasite intensity was not affected by latitude, season, sex, body mass or any interactions (GLM: full model $F_{8,21} = 0.4$, $P = 0.92$).

DISCUSSION

In descriptive studies of free-living birds, it is impossible to identify the specific factors that drive temporal and spatial changes in parasitization. The goal of this study was to

characterize blood and ectoparasite infections in two House Sparrow populations, then determine whether variation in parasitization complemented previously detected differences in immune activity (Martin *et al.* 2004, 2005, 2006). Broadly, this expectation was supported. Temperate House Sparrows had the highest ectoparasite prevalence during the early breeding season (Foster 1969) but were void of ectoparasites in the non-breeding season. Tropical House Sparrows harboured no ectoparasites in the early breeding season and had low levels in the late breeding and non-breeding seasons. Although blood parasite prevalence did not vary significantly between populations at any time of year (except a nearly significant difference during early breeding), seasonality was detected in the temperate population with prevalence being greatest in the early breeding season then declining over the year. In the tropical population, infections were detected year-round. In terms of immune function, in the early breeding season in temperate birds, cutaneous immune responses are small, but during the late and non-breeding seasons, they increase (Martin *et al.* 2004). In tropical birds, immune responses are similar over the year and are stronger than in temperate birds only during the early breeding season.

Taken together, complementary variation in immune function and parasite prevalence over time and space supports latitudinal (and seasonal) differences in parasitization as being driven by differential immune investment. In the tropical population, similar levels of investment in immune defence in both the breeding and the non-breeding season probably promote resistance to infections year-round. In the temperate population, modest investment in immune defence during the height of the breeding season (perhaps because of the demands of reproduction) may increase likelihood of infection at that time of year. As the year progresses and reproductive demands decrease, immune investments may be augmented, which diminish infection likelihood.

These results suggest a direct connection between parasite prevalence and immune defence, but a causal link remains to be made. Other factors may produce these differences between populations. Climatic factors in particular are probably influential to levels of parasitization in each locale. The average monthly temperature in New Jersey from December to March (non-breeding season) is much lower than in Panama (Paton 1999). Because ectoparasites and blood parasite vectors require certain minimum temperatures at critical points in their life cycles, low temperatures in temperate winters may reduce pest populations (Hopp & Foley 2001); an absence of low temperatures in the tropics may promote parasitization year-round. Similarly, average monthly humidity is higher in Panama than in New Jersey throughout the year (Paton 1999). Very low humidity, characteristic of high-latitude winters, may also reduce parasite pressure, giving birds the opportunity to eliminate parasites when moisture levels in the air decrease (Moyer *et al.* 2002).

Endogenous differences among individuals from each population may also mediate parasitization variation. First, it is plausible that the general patterns of immunity and parasitism between populations are due to the effects of parasites or increased activity (and hence exposure to parasites) on general condition, which may lead to compromised immune activity in some situations (Martin *et al.* 2004). Alternatively, stronger immune responses and lower relative parasitism in Panama may indicate that parasitism does not reduce condition there, which might allow tropical Sparrows to mount stronger responses during the early breeding season. Secondly, parasite intensity was generally low at all times of the year in both populations and was not significantly different between populations. This outcome indicates that House Sparrows may be able to control ecto- and blood parasite infections fairly well regardless of locale or season. Thirdly, differences in the proportion of breeding vs. non-breeding phenotypes included in each sample from each population may have affected immunological variation or likelihood of infection, but breeding status was not assessed. Fourthly, immunomodulatory hormones, such as corticosteroids, vary across latitudes and seasons in passerines (Silverin & Wingfield 1998). As corticosterone can induce relapses of malarial infections in temperate House Sparrows (Applegate & Beaudoin 1970), higher baseline and stress-induced levels in temperate vs. tropical Sparrows may partially explain differential parasitization (Martin *et al.* 2005). Likewise, androgens and oestrogens may also vary between populations and influence disposition towards infection, but this possibility is as yet untested (Goymann *et al.* 2004, Rödl *et al.* 2004). Finally, length of residency in an area, particularly for invasive or expanding populations, may contribute to variable parasite loads (Lee & Klasing 2004). Many species harbour fewer parasites in their introduced vs. native ranges (Torchin *et al.* 2003), so Panamanian birds, which only arrived there in the last 25 years (Summers-Smith 1988), may be free of many parasites that infect resident species.

Further methodological issues may also obscure true differences in parasitization between populations. First, microscopic evaluation of blood parasitaemia, as we performed, is vulnerable to false-negative sample categorization. Blood parasites can persist for long periods in the liver, spleen and brain then re-appear in the bloodstream (Cranfield *et al.* 1994). Secondly, dust-ruffling for ectoparasites does not adequately dislodge mite species that reside in feather quills (Clayton & Drown 2001). Further intra- or inter-population differences may exist in terms of ectoparasitization, but they may not have been revealed using the dust-ruffling technique. Finally, grouping of multiple parasite taxa into broad categories prevents identification of the factors (ecological vs. immunological) that drive parasitization patterns between or within populations. Further studies involving larger sample sizes (and hence more parasites) in conjunction with controlled

experiments (e.g. manipulation of ambient temperature and/or humidity) could provide reconciliation.

In summary, our results indicate that blood and ectoparasite prevalence varies between these two House Sparrow populations with the main difference being seasonality of infection in the temperate but not in the tropical population. We postulate that differential persistence of infection threat partially explains why tropical and temperate House Sparrows engage different immune defence strategies (Martin *et al.* 2004, 2005, 2006). Future studies should attempt to ascertain whether similar patterns hold for other tropical and temperate populations of this species and others; a two-population comparison alone may not fairly represent all House Sparrow populations (Garland & Adolph 1994). In a broader context, however, this study highlights the necessity of incorporating seasonality into latitudinal comparisons of parasitism and immune activity. Indeed, our work indicates that the persistence of threat, not necessarily the intensity, may be the more consistent change with latitude for passerines.

For help with fieldwork, we thank J. Gilliam, J. Lewittes and J. Svoboda. M. Hau, D. Westneat and one anonymous reviewer provided helpful criticism on earlier drafts, and D. Clayton provided advice on data collection and analysis. We thank the administration of the Zona Libre de Colon and the management staff and owners of the Princeton Shopping Center and the Belle Mead Co-op for allowing us to conduct our study on their property, and we thank the Smithsonian Tropical Research Institute and Princeton University for logistical support. Funding for this work comes from grants to L.B.M. from the EPA-STAR Fellowship programme, to M.I.P. from the Arnold and Mabel Beckman Foundation, the Round Table Fund, and the John Tyler Bonner Senior Thesis Fund, and to M.C.W. from NSF-IRCEB #0212587. All procedures were approved by Princeton University Animal Care and Use Committee and comply with current US laws.

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Received 21 January 2006; revision accepted 20 December 2006.