

Leukocyte Profiles in Wild House Finches with and without Mycoplasmal Conjunctivitis, a Recently Emerged Bacterial Disease

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Abstract: Leukocyte profiles (relative numbers of white blood cell types) have been used by a growing number of ecological studies to assess immune function and stress in wild birds. House Finches (*Carpodacus mexicanus*) in eastern North America are susceptible to an eye disease caused by the bacterium *Mycoplasma gallisepticum*, providing the opportunity to examine whether leukocyte profiles are associated with infection status and other host characteristics. In this study, we compared blood smears from 297 wild House Finches with and without conjunctivitis to examine whether leukocyte profiles were associated with the presence and severity of mycoplasmal conjunctivitis. We also evaluated the influence of age, sex, and molt on leukocyte profiles in both diseased and nondiseased birds. Of 243 House Finches of known age and sex sampled, 30% showed clinical signs of mycoplasmal conjunctivitis. House Finches with conjunctivitis had significantly higher heterophil to lymphocyte (H/L) ratios and harbored greater numbers and proportions of heterophils and monocytes than nondiseased birds. Leukocyte profiles of noninfected birds did not differ significantly with respect to sex, but young birds had significantly greater numbers of lymphocytes and total white blood cells than adults. Molting birds had significantly more eosinophils than nonmolting birds. Finally, House Finches with the most severe outward signs of conjunctivitis showed the most dramatic leukocyte changes relative to noninfected individuals, and increasing H/L ratios and monocytes in diseased birds were paralleled in a subset of birds that were recaptured during the study period. These results are consistent with patterns observed in domestic poultry and suggest that understanding patterns of leukocyte differentials in this host-pathogen system could improve our understanding of innate immunity and infectious disease risk in other wild passerines.

Key words: *Mycoplasma gallisepticum*, *Carpodacus mexicanus*, white blood cells, innate immunity, heterophils, lymphocytes, monocytes

INTRODUCTION

Among birds and other vertebrates, white blood cells (WBCs) or leukocytes associated with the innate immune

system represent one of the first lines of defense against infectious diseases (Roitt et al., 2001). Leukocytes such as monocytes, heterophils, and some lymphocytes can therefore offer an important measure of nonspecific host immune function and health status (Wakelin, 1996; Harmon, 1998; Norris and Evans, 2000; Roitt et al., 2001). Circulating leukocytes can increase rapidly in number following

infection by bacteria, blood protozoa, and larger macroparasites, and particular types of leukocytes can target broad pathogen groups (Roitt et al., 2001). For example, avian heterophils, like neutrophils in mammals, are the primary line of innate defense for bacterial infections, especially for microbial pathogens in the respiratory tract where few macrophages exist (Harmon, 1998). Monocytes are long-lived macrophages that have also been shown to be important in responding to infections by bacteria and other pathogens (Qureshi, 1998). The relative proportion of each type of leukocyte in an animal's circulating bloodstream is referred to as its leukocyte profile (also called the leukocyte differential), and assessing leukocyte profiles can allow for estimates of infection status and provide indices of stress at the time of sampling (e.g., Vleck et al., 2000; Ruiz et al., 2002).

Most conclusions concerning the function of avian leukocytes in innate immunity are based on research conducted with domestic poultry (e.g., Branton et al., 1997; Qureshi, 1998; Zulkifli et al., 1999; Campo et al., 2000; Elston et al., 2000), but a growing number of experimental and descriptive studies have examined leukocytes in wild passerines. Leukocyte profiles have been used in recent empirical studies to assess general stress and immunocompetence in wild birds (e.g., Svensson and Skarstein, 1997; Figuerola et al., 1999; Bortolotti et al., 2002). Other ecological studies have suggested that the ratio between two avian leukocytes, the heterophil/lymphocyte (H/L) ratio, provides an indicator of overall stress levels of individual birds (e.g., Gross and Siegel, 1983; Vleck et al., 2000; Ruiz et al., 2002). Finally, leukocyte differentials and H/L ratios have been used in recent studies to demonstrate links between stress and territory quality (Mazerolle and Hobson, 2002) and trade-offs between reproductive effort and immune function (Norris and Evans, 2000).

Despite their recent increase in use in avian ecology, predicting directional changes in leukocyte differentials is challenging, because higher proportions of any leukocyte type could reflect greater host immunocompetence, or could be caused by a host's response to current infections. For example, if a bird that appears otherwise healthy harbors high levels of circulating heterophils or monocytes, this might indicate either strong baseline defenses or the presence of an active infection. Because heterophils are the major component of the widely-used H/L ratio, understanding processes that underlie variation in heterophil or lymphocyte numbers is crucial to interpreting this measure in studies of wild and captive birds.

House Finches in eastern North America are susceptible to a recently emerged eye disease caused by the bacterium *Mycoplasma gallisepticum* (MG). This novel strain of a domestic poultry disease has caused dramatic reductions in House Finch abundance following its emergence in 1993–1994 (Ley et al., 1996; Dhondt et al., 1998; Hochachka and Dhondt, 2000). Infected House Finches usually develop eye infections within 7–10 days after exposure, and clinical signs, including swelling and discharge from conjunctival tissues, can persist for many weeks (Luttrell et al., 1998; Roberts et al., 2001a). The obvious physical manifestations of MG infections, combined with the fact that wild House Finches are easy to observe, capture, and handle, make this a model system for understanding the role of infectious diseases in wild avian systems (e.g., Dhondt et al., 1998; Nolan et al., 1998; Hochachka and Dhondt, 2000; Hartup et al., 2001a, b). This host-pathogen system also provides a unique opportunity to examine how avian leukocyte differentials are associated with infections in wild birds, and to compare the differentials from wild birds and poultry infected with a similar pathogen. For example, infections with MG in commercial hens have been shown to cause significant increases in the proportions of circulating heterophils and monocytes (Branton et al., 1997). However, it is not known if the innate immune system of House Finches responds similarly to MG infection.

Recent analyses of continent-wide, citizen science data on the prevalence of conjunctivitis in House Finches has documented annual fluctuations in prevalence characterized by predictable fall–winter epidemics and a consistent summer decline (Hartup et al., 2001a; Altizer et al., 2004a). Seasonal changes in House Finch life history and behavior, including flocking behavior, feeder use, and the recruitment of large numbers of juvenile birds may initiate annual outbreaks of this pathogen (Altizer et al., 2004b). Variation in host immunocompetence as influenced by molting stress, reproductive effort, or environmental stress could also contribute to annual variation in House Finch susceptibility. Thus, an important question, and the primary goal of this study, is whether House Finch characteristics, including age, sex, or molting status, are associated with innate immune status (as measured by leukocyte profiles) and the occurrence of MG.

We compared leukocyte differentials among House Finches with and without physical signs of mycoplasmal conjunctivitis during two successive fall–winter epidemics in a region where MG prevalence is typically high (in the

southeastern US; Altizer et al., 2004b). We predicted that leukocyte differentials in House Finches would covary with the interactive effects of host age, sex, molt, and infection status. Moreover, based on observations made from poultry infections with MG, we expected that heterophils and monocytes in particular would increase with the severity of mycoplasmal conjunctivitis. Finally, because infection with MG could induce physiological stress in House Finches, we predicted that H/L ratios would be positively correlated with the severity of clinical signs.

METHODS

We trapped House Finches at five locations within 10 km of the Emory University campus in Atlanta, GA between August 15, 2001 and January 15, 2003 using a combination of mist nets and wire mesh cages placed around tube-style bird feeders following Hill (2002). Upon capture, all birds were given a unique combination of one numbered aluminum leg band and three colored leg bands. Trapping methods and field protocols were approved by the Emory University Animal Care and Use Committee (#189-2002), and were conducted under Federal Bird Marking permit #23141, and GA Scientific Collecting Permit #29-WMB-02-176. All birds were aged and sexed according to Pyle (1997), and we recorded basic morphological characteristics (e.g., wing chord, tarsus length, molting status) of each bird. Adult House Finches undergo one (prebasic) molt each year between July and October (Michener and Michener, 1940; Hill, 1993), whereas the prebasic molt of juvenile House Finches is usually incomplete (Stangel, 1985). Because the annual prebasic molt begins and ends with the replacement of primary flight feathers (Michener and Michener, 1940), we recorded molting status of each bird (as yes/no) based on whether or not any primaries were being replaced at the time of capture.

We scored the clinical signs of mycoplasmal conjunctivitis in each eye using a four-point scale after Roberts et al. (2001a) as follows: 0=no signs of conjunctivitis; 1=eyes with minor swelling; 2 = eyes with moderate swelling and discharge; and 3 = extreme swelling or crusting to the point of near blindness. Past work has shown that clinical sign of conjunctivitis in House Finches closely correspond with the presence of the *M. gallisepticum* (Ley et al., 1996; Luttrell et al., 1996, 1998; Hartup et al., 2001a), so that clinical signs provide a reliable measure of infection status. Furthermore, a subset of birds captured in our study were tested for the

presence of the MG bacterium via culture and polymerase chain reaction (PCR) from eye swabs. Of 87 House Finches that showed no clinical signs of conjunctivitis between August 2001 and September 2002, 97.7% tested negative for the bacterium [D. Ley, unpublished data]. Similarly, over 85% of a subset of birds observed with clinical signs of conjunctivitis also tested positive for *M. gallisepticum* ($N = 75$). In the present article, we therefore assumed that birds showing clinical signs were indeed infected with MG, and we further assumed that birds not showing clinical signs represented uninfected individuals and hereafter are referred to as such.

To collect blood samples, we punctured the brachial vein of each finch with a 25-gauge needle and collected 15–20 μ l of blood in a heparinized microhematocrit tube. Blood smears were made using the two-slide wedge technique (Messonnier, 2000; Walberg, 2001), and slides were air-dried, fixed with methanol, and stained with Giemsa. We examined each smear under 1000 \times and counted all heterophils, lymphocytes, eosinophils, monocytes, and basophils following Campbell (1995). We examined a minimum of 100 white blood cells on each slide and calculated the proportions of each WBC type, in addition to H/L ratios. We further estimated total numbers of all leukocytes, and of each leukocyte type, per 10,000 red blood cells based on the numbers of cells of each type per field of view. All blood smears were divided approximately evenly (with respect to date and infection status) among two observers for examination. Observers were blind to the infection status during slide examination.

Repeatability of Leukocyte Differentials

We evaluated the repeatability of our leukocyte differentials in two ways. First, duplicate blood smears from 10 uninfected birds were examined by a single observer, and we used Pearson's correlation to evaluate the similarity between WBC differentials for these two sets of smears for each bird. We found that correlation coefficients for all arcsine square-root transformed proportions (heterophils, lymphocytes, basophils, and eosinophils) except monocytes exceeded 0.74, and these correlations were significant at the 0.05 level or higher. For monocytes, proportions recorded for all birds were less than 0.05, but repeated observations within the same bird were not significantly positively correlated. Second, 15 identical blood smears were evaluated by the same two examiners that recorded all WBC differentials used in this study. We used Pearson's correlation

and repeated measures analysis of variance (ANOVA) to examine the consistency between both examiners in reporting counts for the same set of birds. As in the earlier test, Pearson correlation coefficients based on transformed proportions for all values except monocytes exceeded 0.73 and were significant at the 0.01 level or greater. For monocytes, all reported proportions were less than 0.07, but values were not significantly correlated between the two examiners. Repeated measures ANOVAs based on transformed proportions of heterophils, lymphocytes, and monocytes showed a significant effect of examiner at the 0.05 level or greater (e.g., for the proportion of heterophils, examiner effect was associated with mean square [MS] = 0.092, $F = 12.98$, $P = 0.003$). The effect of examiner was not significant in repeated measures ANOVAs based on the proportions of eosinophils and basophils. Thus, despite the fact that our WBC differentials showed a high degree of similarity between observers for nearly all measures, we could not rule out an effect of examiner, and therefore included this as an independent variable in analyses described below.

Statistical Analysis

Because our primary objective was to examine the association between leukocyte differentials and infection with *M. gallisepticum*, we used samples from 297 birds captured during two seasonal periods of high prevalence: August 2001–January 2002, and August 2002–January 2003. Consistent with previous reports of highly seasonal epidemics of this bacterial disease (e.g., Hartup et al., 2001a; Altizer et al., 2004a), over 97% of blood samples from birds with clinical signs of MG were collected during these two fall–winter periods. The prevalence of House Finches with clinical signs of MG during these intervals ranged from 6% (August 2002) to 62% (October 2001), and prevalence during both years peaked during October (Altizer et al., 2004b).

With the data outlined above, we used ANOVA to examine associations between leukocyte parameters and House Finch age, sex, molt, and MG infection status (SPSS, 2002). Each of the following leukocyte parameters were treated as separate dependent variables: H/L ratios, and estimated numbers (per 10,000 red blood cells) of heterophils, lymphocytes, basophils, monocytes, eosinophils, and total WBCs. Moreover, we repeated these same analyses of each leukocyte type using proportions as well as total counts. All proportions were arcsine square-root transformed, and counts were log-transformed (e.g., Log_{10}

[Count + 1]) prior to analysis to normalize the error variance. Our initial statistical model included the main effects of each variable of interest, two-way interactions between infection status and age, sex, and molt, the three-way interaction infection status * age * sex, and the main effects of examiner and year. Because no two- or three-way interaction effects were statistically significant for any dependent variable tested, we removed these from the final analysis to include only main effects as predictor variables. Because of the large number of both dependent and independent variables, we reported only results for variables with significant test results (e.g., $P < 0.05$) or marginally significant trends (e.g., $P < 0.1$).

In a separate analysis of variance, we examined the associations between infection *severity* and each dependent leukocyte parameter. We averaged the infection score across both eyes per individual bird, and collapsed these averages into four severity categories: 0 (not infected), 1 (mean = 0.5), 2 ($1.0 \leq \text{mean} \leq 1.5$), and 3 ($2.0 \leq \text{mean} \leq 3.0$). Our statistical model was again based on arcsine square-root transformed proportions or log-transformed counts, and included the main effects of severity, year, and examiner (i.e., effects of age, sex, and molt were not included). We used Tukey's test at the 0.05 level to compare means and determine homogeneous subsets for levels of infection severity.

Finally, for a subset of birds that were captured more than once within each observation period, we examined how leukocyte differentials changed as a function of capture events and infection status. We used data from 37 individual birds that were captured between August 1–December 1 of 2001 or 2002 and were recaptured between 7–60 days later in either the same or a different state of infection with mycoplasmal conjunctivitis. A total of 15 birds were captured twice with no clinical signs (transition category 0–0), 7 birds were captured twice with clinical signs both times (transition category 1–1), and 15 birds were captured initially without clinical signs, but upon recapture showed signs of MG (transition category 0–1). We used repeated measures ANOVA to test the effects of capture event (first vs. second) and transition category on transformed values of each leukocyte count and proportion and H/L ratios.

RESULTS

Of the 243 birds of known age captured during two annual periods of high prevalence, 124 were aged as AHY (adults),

Table 1. Numbers of House Finches of Known Age, Sex, and Molting Status Trapped during Two Successive Periods of High Prevalence of Mycoplasmal Conjunctivitis (August 2001–January 2002 and August 2002–January 2003)^a

Infection status ^b	Leukocyte parameter	AHY			HY total ^d	Total
		No molt	Molt	Total		
0	<i>n</i>	65	28	93	74	167
	H/L ratio	0.07	0.08	0.08	0.08	0.08
	Heterophils	0.05	0.06	0.05	0.06	0.06
	Lymphocytes	0.79	0.74	0.77	0.81	0.79
	Eosinophils	0.07	0.15	0.09	0.08	0.08
	Monocytes	0.02	0.01	0.02	0.01	0.02
	Basophils	0.08	0.04	0.07	0.05	0.06
	Total WBCs ^c	79.1	86.1	74.9	90.1	81.6
1	<i>n</i>	19	12	31	45	76
	H/L ratio	0.18	0.14	0.16	0.13	0.15
	Heterophils	0.11	0.10	0.10	0.09	0.09
	Lymphocytes	0.77	0.75	0.76	0.75	0.75
	Eosinophils	0.05	0.07	0.06	0.07	0.06
	Monocytes	0.03	0.04	0.03	0.03	0.03
	Basophils	0.05	0.04	0.05	0.06	0.06
	Total WBCs ^c	83.5	73.9	79.8	98.5	90.7

AHY, adults; H/L, heterophil/lymphocyte; HY, juveniles; MG, *Mycoplasma gallisepticum*; RBC, red blood cells; WBC, white blood cells.

^aThe mean proportion of each WBC type and H/L ratios are shown together with sample sizes for each category.

^b0 indicates no clinical signs of MG; 1 indicates birds showing visible signs of mycoplasmal conjunctivitis.

^cEstimated number of WBCs per 10,000 RBCs.

and 119 were aged as HY (juveniles). Sex was assigned to 284 birds, of which 200 were male and 84 were female. A total of 56 birds were reported as actively molting, and most of these (78.6%) were adults. Almost 30% of the samples examined (89/297) were from birds with visible signs of mycoplasmal conjunctivitis.

Leukocytes in Noninfected, Nonmolting Birds

Among nonmolting, noninfected birds of known age ($n = 133$), proportions of lymphocytes ranged from 0.39–0.99 (mean = 0.79). The mean proportions of heterophils, eosinophils, monocytes, and basophils were much lower (Table 1). Among individual birds, heterophils accounted for between 0.0–0.32 of all WBCs, and eosinophils and basophils ranged from 0.0–0.40. Monocytes were the least common WBC type, with proportions ranging from 0.0–0.15 among individual birds (Table 1). H/L ratios in uninfected, nonmolting House Finches averaged 0.08, with a range from 0.0–0.48 among nondiseased individuals. Our estimates of total WBCs per 10,000 red blood cells ranged

from 21 to 202, with a mean of 81.6 leukocytes per non-diseased bird.

Molt, Age, Sex, and Mycoplasmal Conjunctivitis

Analysis of variance using transformed leukocyte counts and proportions showed that heterophils, monocytes, and H/L ratios were significantly higher among birds with visible signs of MG (when measured as a 0–1 variable; Tables 1 and 2). Molting birds had more eosinophils than nonmolting birds (Table 1), and this association was highly significant (Table 2). WBC differentials appeared similar for both HY and AHY birds (Table 1), but analysis of variance revealed that HY birds had significantly more lymphocytes and total WBCs than AHY birds (Table 2). Sex was not significantly associated with any leukocyte parameter. Finally, main effects of examiner and year were statistically significant for a subset of leukocyte parameters (Table 2). Because results were qualitatively similar when analyses were performed on log-transformed counts of each leukocyte type versus arcsine square-root transformed

Table 2. Analysis of Variance Results for Associations between Leukocyte Parameters and Age, Sex, Molt, and the Presence of Mycoplasma Conjunctivitis^a

Effect	Predictor	<i>df</i>	MS	<i>F</i>	Sig
Inf	H/L ratio	1	0.24	9.90	0.002
	Heterophils	1	1.02	8.28	0.004
	Basophils	1	0.54	3.63	0.062
	Monocytes	1	3.07	39.06	0.000
	Total WBCs	1	0.149	2.75	0.099
Age	Heterophils	1	0.34	2.79	0.096
	Lymphocytes	1	0.57	9.27	0.003
	Eosinophils	1	0.44	3.11	0.079
	Total WBCs	1	0.52	9.63	0.002
Molt	Eosinophils	1	4.46	31.48	0.000
	Total WBCs	1	2.55	4.68	0.032
Examiner	Monocytes	1	1.17	21.44	0.000
	Lymphocytes	1	0.45	7.19	0.008
	Basophils	1	1.56	10.17	0.002
	Total WBCs	1	0.55	10.14	0.002
Year	H/L ratio	1	0.082	3.40	0.066
	Heterophils	1	0.81	6.54	0.011
	Eosinophils	1	1.66	11.89	0.001
	Basophils	1	1.93	12.59	0.000
	Total WBCs	1	0.15	2.83	0.094
Error	H/L ratio	223	0.02		
	Heterophils	223	0.12		
	Lymphocytes	223	0.06		
	Eosinophils	223	0.14		
	Monocytes	218	0.15		
	Basophils	218	0.02		
	Total WBCs	223	0.05		

MS, mean square; Sig, significance.

^aModel: dependent variable = Infection (Inf) + Age + Sex + Molt + Examiner + Year. Dependent variables tested for each model effect were H/L ratios and estimated numbers of heterophils, lymphocytes, eosinophils, monocytes, basophils, and total WBCs per 10,000 RBCs. H/L ratios were arcsine square-root transformed prior to analysis, and all other dependent variables were log-transformed. Only results for combinations of independent and dependent variables with one or more marginal or significant effects ($P < 0.1$) are shown. The final model was run with no two- or three-way interactions because these effects did not approach significance for any dependent variable (as described in Methods).

proportions, only statistical results for count data are reported in Table 2.

Infection Severity and Leukocyte Parameters

Of the 89 visibly infected birds sampled during the study period (including those for which age was unknown), 23 had mild conjunctivitis (score = 1), 45 had moderate outward signs (score = 2), and 21 had severe outward signs (score = 3). Analysis of variance showed a significant effect of severity on H/L ratios and on the numbers of heter-

ophils, monocytes, and total WBCs (Table 3). Heterophils and monocytes increased in birds with moderate and severe infections (Fig. 1), as did estimates of total WBCs and H/L ratios. Comparison of means showed that proportions of heterophils and monocytes were indistinguishable for class 0 and class 1 birds, but birds in classes 2 and 3 had significantly higher proportions of both WBC types (Fig. 1). Comparison of estimated counts of heterophils and monocytes showed similar differences, with one exception: Monocytes in birds with mild, moderate, or severe infections were sharply and significantly higher than nondis-

Table 3. Analysis of Variance Results for Associations between Leukocyte Parameters and Infection Severity of Mycoplasmal Conjunctivitis^a

Variable					
Independent	Dependent	<i>df</i>	MS	<i>F</i>	Sig
Severity	H/L ratio	3	0.19	7.31	0.000
	Heterophils	3	0.65	5.17	0.002
	Monocytes	3	1.43	19.4	0.000
	Lymphocytes	3	0.13	2.12	0.098
	Total WBCs	3	0.16	2.86	0.037
Examiner	H/L ratio	1	0.09	3.47	0.064
	Monocytes	1	1.82	24.61	0.000
	Lymphocytes	1	0.42	6.90	0.009
	Basophils	1	0.91	5.57	0.019
	Total WBCs	1	0.34	6.27	0.013
Year	Heterophils	1	0.96	7.67	0.006
	Eosinophils	1	0.97	6.02	0.015
	Basophils	1	2.58	15.82	0.000
	Total WBCs	1	0.31	5.75	0.017
Error	H/L ratio	289	0.03		
	Heterophils	289	0.13		
	Monocytes	289	0.07		
	Lymphocytes	289	0.06		
	Eosinophils	289	0.16		
	Basophils	289	0.16		
	Total WBCs	289	0.15		

^aModel: dependent variable = Severity class + Examiner + Year. Birds were assigned to one of four severity classes as described in Methods. Dependent variables tested for each model effect were H/L ratios and estimated numbers of heterophils, lymphocytes, eosinophils, monocytes, basophils, and total WBCs per 10,000 RBCs. H/L ratios were arcsine square-root transformed prior to analysis, and all other dependent variables were log-transformed. Only results for combinations of independent and dependent variables with one or more marginal or significant effects ($P < 0.1$) are shown.

eased birds but counts among the 1–3 severity classes were similar to each other. As before, results were qualitatively similar when analyses were performed on counts of each leukocyte type versus proportions, and only statistical results based on count data are reported in Table 3.

Leukocyte Changes in Recaptured Birds

Repeated measures ANOVAs that treated first and second capture events as within-subject effects showed that H/L ratios depended on both capture event and transition group, with a nearly significant effect of capture event on numbers of heterophils (Table 4). For monocytes, effects of capture event, transition group, and the transition group * capture event interaction were highly significant, so that both proportions and total counts of monocytes were lowest among birds with no clinical signs at each

capture event (0–0), increased following infection for birds that developed conjunctivitis (0–1), and were highest for birds showing conjunctivitis at both capture events (Fig. 2a). Heterophils and H/L ratios were lowest among uninfected birds, and increased upon second capture for birds in both the 0–1 and 1–1 transition groups (Fig. 2b). For the proportions of heterophils and H/L ratios, these effects of capture event and transition group were again statistically significant, although estimated heterophil counts were significantly associated with capture event alone (Table 4). Proportions of lymphocytes varied significantly with transition group and were lower among birds in the 1–1 category, but no effects were significant for estimated lymphocyte counts (Table 4). Finally, no effects of capture event or transition group were significant for the counts or proportions of basophils or eosinophils (Table 4).

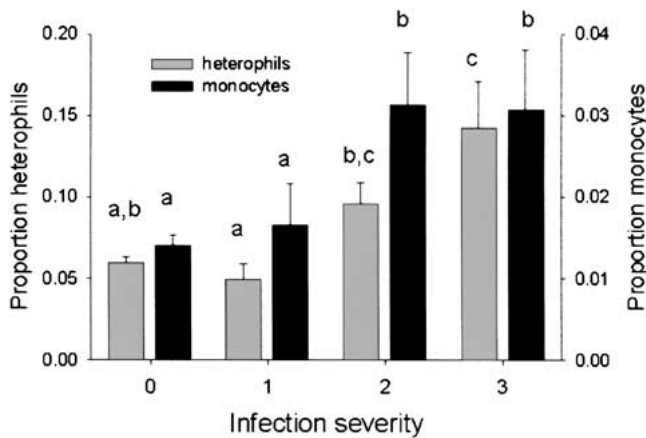


Figure 1. Proportions of monocytes (black bars) and heterophils (gray bars) relative to the severity of clinical conjunctivitis in wild House Finches. Severity categories are explained in the Methods section. Sample sizes for each group were as follows: 0 = 208, 1 = 29, 2 = 45, 3 = 21. Error bars represent standard errors. Letters (a–c) indicate homogeneous subsets based on Tukey's paired comparison of means at the 0.05 level.

DISCUSSION

Leukocytes and Mycoplasmal Conjunctivitis

Consistent with studies of responses of domestic poultry infected with *M. gallisepticum* (Branton et al., 1997), the proportions of heterophils and monocytes in wild House Finches showed the most extreme changes as a function of visible signs of mycoplasmal conjunctivitis. Relative levels of heterophils and monocytes were clearly higher among House Finches with clinical signs of MG, and both leukocyte types increased with infection severity (Fig. 1), as did total WBC counts. Moreover, leukocyte comparisons among recaptured birds showed that both heterophils and monocytes increased upon second capture for birds that developed clinical signs, and for birds that showed conjunctivitis at both first and second capture (Fig. 2). Collectively, these results suggest that heterophils and monocytes play an important role in mitigating infection by this recently emerged bacterial disease in wild House Finches.

Interestingly, comparison of heterophil and monocyte proportions among House Finches with different levels of infection severity showed that proportions were similar for birds with both mild and no clinical signs relative to birds with moderate and severe conjunctivitis (Fig. 1). This could be caused by a lag time in the response of leukocyte differentials to infection with MG, or could be due to the

possibility that some birds with mild clinical signs were in the recovery stage of infection. For example, Kollias et al. (2004) showed that the severity of physical signs of conjunctivitis among captive House Finches peaked at 2–3 weeks following exposure to MG, and gradually declined over the subsequent 4–8 weeks to undetectable levels. Isolation of the bacterium from eye and choanal swabs followed a similar time course, and in some cases clinical signs persisted for 2 or more weeks beyond the point of bacterial shedding. However, it is important to note that estimated counts of monocytes showed a slightly different pattern relative to the proportions shown in Figure 1, with monocyte counts increasing in birds with mild infections and remaining similarly high across all three severity classes.

Ratios of heterophils to lymphocytes are commonly used to measure chronic stress levels in birds (e.g., Gross and Siegel, 1983; Elston et al., 2000; Horak et al., 2002; Ruiz et al., 2002). Not unexpectedly, we found higher H/L ratios in House Finches infected with MG, and these ratios increased among birds with greater levels of disease severity. A recent study of an overlapping set of birds (Altizer et al., 2004b) showed that diseased House Finches were also in poorer condition than nondiseased birds, as measured by a composite index of pectoral muscle condition, subcutaneous fat, and residuals of body mass regressed against tarsus length. Thus, greater stress among House Finches with severe visible signs of MG could be linked with loss of body mass and reduced fat loads resulting from infection. Indeed, comparison of this same composite measure of body condition and leukocyte differentials among infected birds in this study showed a negative relationship between body condition and proportions of heterophils, monocytes, and H/L ratios ($-0.19 < \text{Pearson's } R < -0.17$), although these correlations were not significant at the 0.05 level ($0.08 < P < 0.12$).

Effects of Molt, Age, and Sex

Molting has been shown to be a stressful and energetically costly event in the life cycle of passerines, especially during the end of their breeding phase (Hemborg and Lundberg, 1998). However, it remains unclear whether molting is costly in terms of innate immunity, and if molting birds show significant white blood cell changes. To our knowledge, only one study to date has examined associations between white blood cell numbers and molt in passerines (Nava et al., 2001), and showed that captive House Sparrows (*Passer domesticus*) had higher numbers of basophils

Table 4. Repeated Measures Analysis of Variance Results Showing Effects of Capture Event (as a Within-subject Effect) and Transition Group (as a Between-subject Factor) on Leukocyte Parameters^a

Effect	Dependent variable	<i>df</i>	MS	<i>F</i>	Sig
Capture event	H/L ratio	1	0.29	7.24	0.011
	Heterophils	1	0.38	3.77	0.060
	Monocytes	1	0.67	9.25	0.005
Transition group	H/L ratio	2	0.28	5.76	0.007
	Monocytes	2	1.10	9.26	0.001
	Lymphocytes	2	0.18	2.53	0.095
Capture event * transition group	Monocytes	2	0.34	4.65	0.016
Error	H/L ratio	34	0.04		
	Heterophils	34	0.10		
	Monocytes	34	0.07		
	Lymphocytes	34	0.05		
	Eosinophils	34	0.12		
	Basophils	34	0.14		
	Total WBCs	34	0.03		

^aTransition groups based on whether or not birds developed clinical signs of conjunctivitis are explained in Methods (and see Fig. 2). Dependent variables tested for each model effect were H/L ratios and estimated numbers of heterophils, lymphocytes, eosinophils, monocytes, basophils, and total WBCs per 10,000 RBCs. H/L ratios were arcsine square-root transformed prior to analysis, and all other dependent variables were log-transformed. Only results for combinations of independent and dependent variables with one or more marginal or significant effects ($P < 0.1$) are shown.

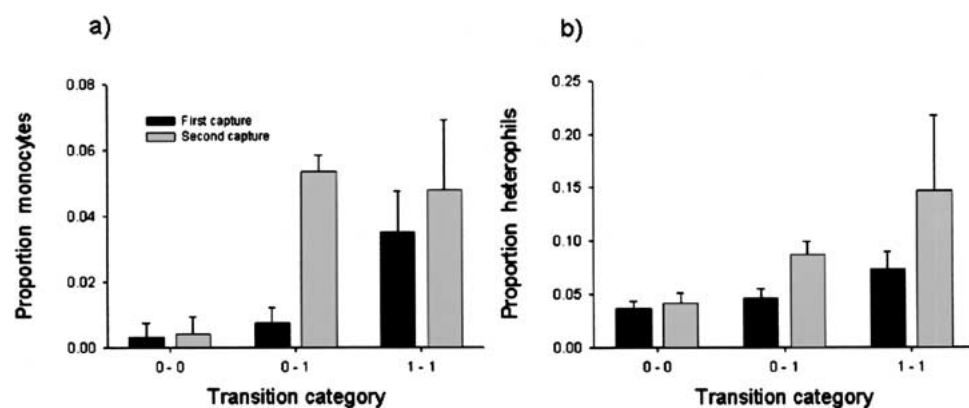


Figure 2. Proportions of monocytes (a) and heterophils (b) relative to capture event and transition group among 37 House Finches that were captured twice within the same study period. Transition categories are as follows: 0-0 = bird captured twice with no clinical signs ($n = 15$), 0-1 = birds initially captured with no clinical signs

but later recaptured with conjunctivitis ($n = 15$), and 1-1 = birds with conjunctivitis upon both capture events ($n = 7$). Gray bars indicate first capture events, and black bars indicate second capture. Error bars represent standard errors for each group.

and monocytes after their molt had ended (relative to prior to molting). In our study, we found that nondiseased, actively molting, adult House Finches had sharply elevated eosinophils (but not basophils or monocytes; Table 1) relative to nonmolting birds. Possible explanations for an association between eosinophil production and molt in wild House Finches could relate to the timing of exposure to other parasites (e.g., in mammals, eosinophils are in-

involved in attacking macroparasites and release agents that reduce inflammation at the site of invasion; Roitt et al., 2001). We also found that molting adult House Finches with conjunctivitis had lower proportions of eosinophils and basophils than noninfected, molting birds (Table 1). One possible explanation might be that MG impedes or interferes with eosinophil production during the molting process. In support of an interaction between molting and

Table 5. Previously Published and Nonpublished Leukocyte Parameters from Wild Passerines, Including Proportions of Heterophils, Lymphocytes, and H/L Ratios^a

Species	Latin name	<i>n</i>	Heterophil	Lymphocyte	H/L	Source
Cirl Bunting	<i>Emberiza cirlus</i>	17	0.37	0.56	0.66	Figuerola et al., 1999
Zebra Finch (wild)	<i>Taeniopygia guttata</i>	34	0.27	0.47	0.58	Ewenson et al., 2001
Great Tit	<i>Parus major</i>	81	0.20	0.69	0.29	Hauptmanova et al., 2002
Rufous-collared Sparrow	<i>Zonotrichia capensis</i>	75	0.20	0.71	0.30	Ruiz et al., 2002
Ovenbird	<i>Seiurus aurocapillus</i>	65	0.14	0.66	0.22	Mazerolle and Hobson, 2002
Pied Flycatcher	<i>Ficedula hypoleuca</i>	18	0.17	0.59	0.29	J. Moreno, J. Morales, personal communication
Song Sparrow	<i>Melospiza melodia</i>	9	0.12	0.83	0.16	A. Davis, unpublished data
Common Grackle	<i>Quiscalus quiscula</i>	5	0.15	0.73	0.22	A. Davis, unpublished data
American Robin	<i>Turdus migratorius</i>	5	0.12	0.78	0.18	A. Davis, unpublished data
House Finch	<i>Carpodacus mexicanus</i>	65	0.06	0.79	0.08	This article

^aValues shown for House Finches represent nonmolting adult birds without physical signs of mycoplasmal conjunctivitis.

mycoplasmal conjunctivitis, Brawner et al. (2000) found that captive male House Finches that were infected with MG while completing their prebasic molt did not develop their normal plumage coloration.

Counter to our predictions, we found few significant main effects or interactions involving age and sex on leukocyte parameters of wild House Finches, with the exception that HY birds had significantly higher counts of lymphocytes and total WBCs relative to adult birds (Tables 1 and 2). These results overall suggest that the innate immune systems of adult and juvenile finches were similar both in their normally circulating levels of most leukocytes and in their response to a bacterial infection. Recent studies have suggested that juvenile House Finches were more likely to contract mycoplasmal conjunctivitis than adults (Roberts et al., 2001b; Altizer et al., 2004b), but our study suggests that that differential susceptibility between juveniles and adults is probably not related to differences in the proportions of any type of circulating white blood cell. Moreover, the response to infection was similar across all categories of age and sex, indicating that relative increases in heterophils and monocytes occurred across multiple classes of infected birds.

Leukocyte Parameters in Noninfected Birds

One striking pattern to emerge from the House Finch leukocyte parameters we observed was that the differentials of uninfected, nonmolting House Finches deviated substantially from previously published values for other passerine species, especially with respect to the proportions of heter-

ophils and H/L ratios (assembled for comparison in Table 5). That we found a lower proportion of circulating heterophils in uninfected House Finches relative to other passerine species is of particular interest, as heterophils are known to actively counter bacterial infections in birds (Harmon, 1998). If it is indeed true that House Finches have lower proportions or numbers of circulating heterophils, further comparisons of leukocyte differentials among House Finches and other songbird species would be warranted, as one factor underlying the extreme susceptibility of House Finches to *M. gallisepticum* could relate to these low baseline levels of circulating heterophils. However, the possibility exists that the low proportion of heterophils we observed in House Finches was an artifact of the smear preparation method we used. For example, Fudge (1994) pointed out that the two-slide-wedge technique we employed can result in artificially low numbers of granulocytic leukocytes in pet birds (which often have baseline levels of heterophils above 50%). To address this possibility, we captured and examined blood smears from three other common passerine species at our study sites for comparison (Table 5). We examined blood smears from 5–10 individuals of each species, using the same methods for smear preparation and leukocyte counting as with House Finches, and examinations were performed by one of the observers who performed the House Finch counts. Average heterophil proportions in these other species were consistent with values reported from the majority of other passerine species and were approximately twice as high as heterophil proportions observed in wild House Finches (Table 5).

Leukocytes in Ecological Research

Ornithologists and ecologists have increasingly studied relationships between host immunity, stress, and ecology, contributing to a growing field of “ecological immunology” (Svensson and Skarstein, 1997). Recent studies have demonstrated that leukocyte differentials offer reliable and meaningful measures of avian stress and condition (e.g., Vleck et al., 2000; Horak et al., 2002). Cross-species comparisons of leukocyte counts in mammals and birds have been used to ask whether features of host behavior or ecology are associated with risk of parasite infection and host immune defenses (e.g., Møller et al., 1998; Nunn et al., 2000; Nunn, 2002). A critical task that remains is to determine which leukocyte parameters in wild birds are linked with greater baseline immune defenses, and which cell types play significant roles in responding to infectious diseases. This is difficult in part due to a lack of information on the function of specific leukocytes in birds, and to differences in the responses to stress or infection among different bird species.

Our results concerning the effects of infection on leukocyte differentials in wild House Finches might be useful for interpreting leukocyte differentials in other bird species. For example, we found a close correspondence between H/L ratios and heterophil proportions, suggesting that variation in H/L ratios reflected underlying changes in the numbers and proportions of heterophils. In other words, we found that higher H/L ratios in wild House Finches were driven primarily by elevated heterophils as opposed to reduced numbers of lymphocytes (Table 1), and that H/L ratios did not provide additional information beyond patterns derived from the proportion of heterophils. In addition, further studies that follow the fates of large numbers of marked House Finches for which blood parameters are known might identify blood characteristics associated with future susceptibility to infection. Such comparisons would provide information regarding the role of specific leukocytes in protecting hosts against bacterial disease, which would in turn provide a more definitive assessment of immune defenses relative to leukocyte differentials.

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REFERENCES

- Altizer S, Hochachka WM, Dhondt AA (2004a) Seasonal dynamics of mycoplasmal conjunctivitis in eastern North American house finches. *Journal of Animal Ecology* 73:309–322
- Altizer S, Davis AK, Cook KC, Cherry JJ (2004b) Age, sex, and season affect the risk of mycoplasmal conjunctivitis in a southeastern house finch population. *Canadian Journal of Zoology* 82:755–763
- Bortolotti GR, Dawson RD, Murza GL (2002) Stress during feather development predicts fitness potential. *Journal of Animal Ecology* 71:333–342
- Branton SL, May JD, Lott BD, Maslin WR (1997) Various blood parameters in commercial hens acutely and chronically infected with *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. *Avian Diseases* 41:540–547
- Brawner WR, Hill GE, Sundermann CA (2000) Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. *Auk* 117:952–963
- Campbell TW (1995) *Avian hematology and cytology, 2nd ed.*, Ames, IA: Iowa State University Press
- Campo JL, Gil MG, Torres O, Davila SG (2000) Association between plumage condition and fear and stress levels in five breeds of chickens. *Poultry Science* 80:549–552
- Dhondt AA, Tessaglia DL, Slothower RL (1998) Epidemic mycoplasmal conjunctivitis in house finches from eastern North America. *Journal of Wildlife Diseases* 34:265–280
- Elston JJ, Beck M, Alodan MA, Vega-Murillo V (2000) Laying hen behavior 2. Cage type preference and heterophil to lymphocyte ratios. *Poultry Science* 79:477–482
- Ewenson EL, Zann RA, Flannery GR (2001) Body condition and immune response in wild Zebra Finches: effects of capture, confinement and captive-rearing. *Journal of Comparative Physiology* 88:391–394
- Figuerola J, Munoz E, Gutierrez R, Ferrer D (1999) Blood parasites, leucocytes and plumage brightness in the ciril bunting, *Emberiza cirius*. *Functional Ecology* 13:594–601
- Fudge AM (1994) Blood testing artifacts: interpretation and prevention. *Seminars in Avian and Exotic Pet Medicine* 3:2–4
- Gross WB, Siegel HS (1983) Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Diseases* 27:972–979
- Harmon BG (1998) Avian heterophils in inflammation and disease resistance. *Poultry Science* 77:972–977
- Hartup BK, Bickal JM, Dhondt AA, Ley DH, Kollias GV (2001a) Dynamics of conjunctivitis and *Mycoplasma gallisepticum* infections in house finches. *Auk* 118:327–333
- Hartup BK, Dhondt AA, Sydenstricker KV, Hochachka WM, Kollias GV (2001b) Host range and dynamics of mycoplasmal

- conjunctivitis among birds in North America. *Journal of Wildlife Diseases* 37:72–81
- Hauptmanova K, Literak I, Bartova E (2002) Haematology and leukocytozoonosis of great tits (*Parus major* L.) during winter. *Acta Veterinaria Brno* 71:199–204
- Hemborg C, Lundberg A (1998) Costs of overlapping reproduction and moult in passerine birds: an experiment with the pied flycatcher. *Behavioral Ecology and Sociobiology* 43:19–23
- Hill GE (1993) House finch (*Carpodacus mexicanus*). In: *Birds of North America*, No. 46, Poole A, Gill F (editors), Philadelphia: Academy of Natural Sciences, and Washington, DC: American Ornithologists' Union
- Hill GE (2002) *A Red Bird in a Brown Bag. The Function and Evolution of Colorful Plumage in the House Finch*, New York: Oxford University Press
- Hochachka WM, Dhondt AA (2000) Density-dependent decline of host abundance resulting from a new infectious disease. *Proceedings of the National Academy of Sciences* 97:5303–5306
- Horak P, Saks L, Ots I, Kollist H (2002) Repeatability of condition indices in captive greenfinches (*Carduelis chloris*). *Canadian Journal of Zoology* 80:636–643
- Kollias GV, Sydenstricker KV, Kollias HW, Ley DH, Hosseini PR, Connolly V, et al. (2004) Experimental infection of individually caged house finches with *Mycoplasma gallisepticum*. *Journal of Wildlife Diseases* 40:79–86
- Ley DH, Berkhoff JE, McLaren JM (1996) *Mycoplasma gallisepticum* isolated from house finches (*Carpodacus mexicanus*) with conjunctivitis. *Avian Diseases* 40:480–483
- Luttrell MP, Fischer JR, Stallknecht DE, Kleven SH (1996) Field investigation of *Mycoplasma gallisepticum* infections in house finches (*Carpodacus mexicanus*) from Maryland and Georgia. *Avian Diseases* 40:335–341
- Luttrell MP, Stallknecht DE, Fischer JR, Sewell CT, Kleven SH (1998) Natural *Mycoplasma gallisepticum* infection in a captive flock of house finches. *Journal of Wildlife Diseases* 34:289–296
- Mazerolle DF, Hobson KA (2002) Physiological ramifications of habitat selection in territorial male ovenbirds: consequences of landscape fragmentation. *Oecologia* 130:356–363
- Messonnier S (2000) Counting white blood cells. *Exotic Pet Practice* 5:2
- Michener H, Michener JR (1940) The molt of house finches of the Pasadena region, California. *Condor* 42:140–153
- Møller AP, Dufva R, Erritzoe J (1998) Host immune function and sexual selection in birds. *Journal of Evolutionary Biology* 11:703–719
- Nava MP, Veiga JP, Puerta ML (2001) White blood cell counts in house sparrows (*Passer domesticus*) before and after moult and after testosterone treatment. *Canadian Journal of Zoology* 79:145–148
- Nolan PM, Hill GE, Stoehr AM (1998) Sex, size, and plumage redness predict house finch survival in an epidemic. *Proceedings of the Royal Society of London. Series B* 265:961–965
- Norris K, Evans MR (2000) Ecological immunology: life history trade-offs and immune defense in birds. *Behavioral Ecology* 11:19–26
- Nunn CL (2002) A comparative study of leukocyte counts and disease risk in primates. *Evolution* 56:177–190
- Nunn CL, Gittleman JL, Antonovics J (2000) Promiscuity and the primate immune system. *Science* 290:1168–1170
- Pyle P (1997) *Identification Guide to North American Birds. Part 1*, Bolinas, CA: Slate Creek Press
- Qureshi MA (1998) Role of macrophages in avian health and disease. *Poultry Science* 77:978–982
- Roberts SR, Nolan PM, Hill GE (2001a) Characterization of *Mycoplasma gallisepticum* infection in captive house finches (*Carpodacus mexicanus*) in 1998. *Avian Diseases* 45:70–75
- Roberts SR, Nolan PM, Lauerman LH, Li L-Q, Hill GE (2001b) Characterization of the mycoplasmal conjunctivitis epizootic in a house finch population in the southeastern USA. *Journal of Wildlife Diseases* 37:82–88
- Roitt I, Brostoff J, Male D (2001) *Immunology, 6th ed.*, New York: Harcourt
- Ruiz G, Rosenmann M, Novoa FF, Sabat P (2002) Hematological parameters and stress index in rufous-collared sparrows dwelling in the urban environments. *Condor* 104:162–166
- SPSS (2002) Version 11.5.1, Chicago: SPSS, Inc
- Stangel PW (1985) Incomplete first prebasic molt of Massachusetts house finches. *Journal of Field Ornithology* 56:1–8
- Svensson E, Skarstein F (1997) The meeting of two cultures: bridging the gap between ecology and immunology. *Trends in Ecology and Evolution* 12:92–93
- Vleck CM, Vortalino N, Vleck D, Bucher TL (2000) Stress, corticosterone, and heterophil to lymphocyte ratios in free-living Adelle penguins. *Condor* 102:392–400
- Wakelin D (1996) *Immunity to Parasites: How Parasitic Infections Are Controlled*, Cambridge, UK: Cambridge University Press
- Walberg J (2001) White blood cell counting techniques in birds. *Seminars in Avian and Exotic Pet Medicine* 10:72–76
- Zulkifli I, Norma MTC, Chong CH, Loh TC (1999) Heterophil to lymphocyte ratio and tonic immobility reactions to preslaughter handling in broiler chickens treated with ascorbic acid. *Poultry Science* 79:402–406