

## SUSCEPTIBILITY OF A NAÏVE POPULATION OF HOUSE FINCHES TO *MYCOPLASMA GALLISEPTICUM*

Kristy L. Farmer,<sup>1,2</sup> Geoffrey E. Hill,<sup>1</sup> and Sharon R. Roberts<sup>1</sup>

<sup>1</sup> Department of Biological Sciences, Auburn University, Auburn, Alabama 36849, USA

<sup>2</sup> Corresponding author (e-mail: farmekr@auburn.edu)

**ABSTRACT:** Since 1994 an epidemic of mycoplasmal conjunctivitis has spread throughout the eastern house finch (*Carpodacus mexicanus*) population leading to a significant decline in this population. The infection has not yet been reported from house finch populations west of the Great Plains. We hypothesized that the western population, like the eastern population, is susceptible to infection, and we tested this hypothesis by experimentally infecting house finches from Missoula, Montana (USA) with the house finch strain of *Mycoplasma gallisepticum* (MG). We compared the response of finches from Montana infected with MG to that of finches from Auburn, Alabama (USA) (October 1999–February 2000). Fifteen house finches from Montana were shipped to Auburn and quarantined for 6 wk at the Auburn University aviary. All birds were negative for MG antibodies when tested by serum plate agglutination assay and MG could not be detected in any bird by polymerase chain reaction. We tested two methods of inoculation, ocular inoculation and contact exposure to an infected finch. Seven house finches from Montana and four house finches from Alabama were infected by bilateral ocular inoculation with 20  $\mu$ l of a culture containing  $1 \times 10^6$  color changing units of the house finch strain of MG. The remaining eight house finches from Montana were co-housed with a house finch from Alabama exhibiting mycoplasmal conjunctivitis. After exposure to the pathogen, all house finches became infected, regardless of origin or method of exposure, and all developed conjunctivitis. All birds seroconverted, and evidence of infection could be detected in every bird at some point during the course of disease. Our results suggest that house finches from the western United States are highly susceptible to infection with the house finch strain of MG.

**Key words:** *Carpodacus mexicanus*, conjunctivitis, house finch, *Mycoplasma gallisepticum*, western house finch.

### INTRODUCTION

In February 1994 an outbreak of conjunctivitis was observed in house finches (*Carpodacus mexicanus*) in suburban Washington D.C. (USA; Ley et al., 1996; Luttrell et al., 1996). Mycoplasmas were isolated from lesions of affected birds and subsequently identified as a unique strain of *Mycoplasma gallisepticum* (MG), a pathogen not previously associated with clinical disease in passerines from North America (Ley et al., 1996). From the point of initial detection the disease spread through the entire eastern population of house finches (Fischer et al., 1997; Dhondt et al., 1998). In early years of the epidemic, prevalence of clinical disease and mortality were high (Sauer et al., 1997; Nolan et al., 1998). In recent years, mortality and prevalence of the disease has declined (Hartup et al., 2000; Roberts et al., 2001b).

House finches are not native to eastern

North America. House finches originating from coastal California were introduced to Long Island, New York in 1940 (Elliot and Aribib, 1953), and from there the birds spread throughout the eastern United States and into Canada (Hill, 1993). It is speculated that 50 or more individuals founded the eastern population (Cant, 1962; Munding, 1975; Hill, 1993). Although the eastern population originated from a relatively small number of birds, it appears that most of the genetic diversity of the parent population has been retained (Vazquez-Phillips, 1992). Populations in the Great Plains, which divides the native western and introduced eastern populations, are sparse due to lack of suitable habitat. Gene flow and disease transmission between eastern and western populations of house finches appears to be low (Hill, 1993) and there has been no published report of mycoplasmal conjunctivitis west of the 100th meridian.

Many basic questions regarding this new host-pathogen relationship remain to be answered. The objective of this study was to test susceptibility of western house finches to the house finch MG strain. We infected western house finches from Missoula, Montana (USA) with an isolate of the house finch MG strain and compared their response to that of eastern house finches from Auburn, Alabama (USA). We also compared two routes of infection: bilateral ocular inoculation and direct exposure to an infected finch.

#### MATERIALS AND METHODS

House finches were captured in Missoula, Montana (46°52'N, 114°00'W) in October 1999 and transported to the Auburn University campus (32°35'N, 85°28'W). The birds were trapped using wire-mesh basket traps under permits from Montana Department of Fish, Wildlife, and Parks (Helena, Montana; No. 1456) and a federal collecting permit (MB784373-2). All procedures involving live animals were reviewed and approved by the Auburn University Institutional Animal Care and Use Committee (PRN No. 0303R2249). Upon arrival the house finches were banded and divided into three flocks of seven or eight birds. Each flock was housed separately in an indoor, temperature-controlled room (1.6 × 2.3 × 2.6 m) with natural light and maintained on a diet of sunflower seed, red and white millet, and water ad libitum with sufficient grit. The water was supplemented with high potency multivitamins (Premium Multi-Drops, 8 in 1 Pet Products, Inc., Hauppauge, New York, USA.). Four house finches from Auburn, Alabama were caught and housed in a similar manner to the Montana house finches under a permit from the Alabama Department of Conservation (Montgomery, Alabama; No. 12). All finches used in this study were identified as 1999 hatch-year birds based on plumage.

To prevent transmission of infection between flocks a quarantine area was established around the door to each room. Investigators wore gloves and disposable booties when entering the rooms or handling the birds. All dishes were soaked in a 10% bleach solution, and separate nets were used to capture each flock. All four flocks were quarantined for 6 wk prior to infection to monitor for diseases. At the end of the quarantine period, blood was collected from all 27 birds for serology as previously described (Roberts et al., 2001b). Blood was tested for antibodies to MG with a commercial se-

rum plate agglutination (SPA) assay (Luttrell et al., 1996) (Intervet Inc., Millsboro, Delaware, USA). After 2 min the extent of agglutination was scored on a scale of 0–4, with a score  $\geq 2$  considered positive. Birds also were tested for MG by polymerase chain reaction (PCR). Samples for PCR analysis were obtained by gently swabbing the choanal cleft using a microtip swab (Becton Dickinson and Co., Sparks, Maryland, USA). DNA extraction and PCR amplification of a 185-bp fragment using MG-specific primers (LTI, Gaithersburg, Maryland) was performed as described previously (Lauerman, 1998; Roberts et al., 2001b).

House finches were exposed to MG by one of two methods. One group of 11 house finches, including the four from Alabama, were inoculated with a 72-hr broth culture of house finch MG, grown in SP4 broth, via a bilateral ocular route for a total dose of 20  $\mu$ l/bird. Serial dilution of the broth culture determined each dose contained  $1 \times 10^6$  color changing units per ml of MG (generously provided by P. Luttrell, Southeastern Cooperative Wildlife Disease Study, The University of Georgia, Athens, Georgia, USA). The isolate was obtained in Clark County, Georgia in November 1995 and had undergone five passages before being introduced into the birds. A second group of eight house finches from Montana was exposed to MG by co-housing them in a room with a house finch, caught in Auburn, Alabama, exhibiting mycoplasmal conjunctivitis. *Mycoplasma gallisepticum* was confirmed in the Alabama house finch by SPA, culture (see below), and PCR. The remaining eight house finches from Montana were inoculated with sterile SP4 broth and served as a negative control.

Finches were monitored daily for signs of disease. Once each week for 12 wk the finches were captured and scored for conjunctivitis in each eye using a scale from 0–4 with one being minimal signs of the disease and four complete blindness due to swelling of the conjunctiva. During weekly capture birds were bled for serology and swabbed for PCR analysis. Three attempts at isolation of the MG were made during the study. The infected finch from Alabama introduced into the flock of birds from Montana, one of the finches from Montana in that flock, and one finch from Montana inoculated with the isolate from 1995 were swabbed as described above and the swab was placed into a SP4 broth tube pre-warmed to 37 C. A blind 1:10 passage was made 24 hr following initial culture. Broth cultures were incubated at 37 C for 5 wk or until a phenol-red-indicated color change occurred at which time the culture was tested for the presence of MG by PCR.

Results for finches are given in average days  $\pm$  1 SD. Differences in incubation period and duration of illness between house finches from Montana and house finches from Alabama directly inoculated with an MG isolate were evaluated with a two-tailed Mann-Whitney *U*-test. This same test was also used to evaluate the difference between house finches from Montana inoculated directly and those exposed to an infected bird. Differences in mortality between these flocks were evaluated with a Chi-squared ( $\chi^2$ ) test. All statistics were done using Statsview 5.0.

### RESULTS

Prior to infection, all birds were healthy. No house finch had antibodies to MG and MG was not detected by PCR. After exposure to MG all birds became infected and developed conjunctivitis, regardless of method of exposure or geographic origin. All birds seroconverted and MG was detected by PCR in every bird at least once during the course of the disease. In three attempts at isolation of MG from infected finches there was a color change in the cultures indicating growth and MG was confirmed by PCR. The eight control finches never exhibited signs of disease and MG infection was not detected by serology or PCR at any time during the study in these birds.

Following ocular inoculation with MG all finches rapidly developed conjunctivitis, but the incubation period in finches from Alabama was an average of  $5.25 \pm 1$  days, significantly longer than the incubation period of finches from Montana ( $3.71 \pm 1.1$  days) ( $Z = -1.97$ ,  $P = 0.049$ ; Fig. 1A). All of these finches exhibited moderate to severe unilateral or bilateral conjunctivitis (score of 2–3) with some birds experiencing complete blindness in one or both eyes (score of 4). Although six of the seven finches from Montana and all four finches from Alabama died during the study, the house finches from Alabama survived an average of  $58 \pm 9.9$  days from onset of disease, significantly longer than the house finches from Montana that survived an average of  $35 \pm 11.8$  days after onset of disease ( $Z = -2.15$ ,  $P = 0.03$ ; Fig. 1B).

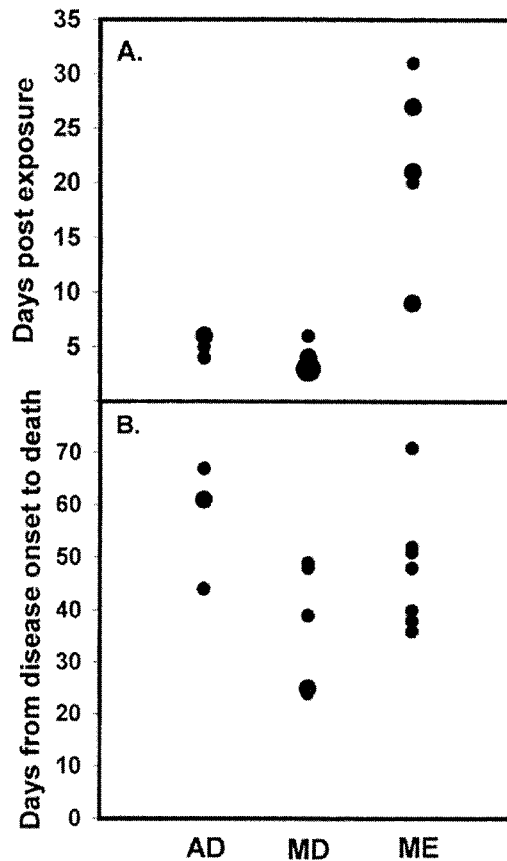


FIGURE 1. Differences in (A) time period until all finches demonstrated Mycoplasma conjunctivitis and (B) duration of illness between three flocks of house finches. AD = finches from Alabama inoculated directly, MD = finches from Montana inoculated directly, and ME = finches from Montana exposed to a naturally infected finch. Size of data point increases with number of birds per point.

When the finches from Montana were exposed to MG by co-housing them with a naturally infected finch from Alabama we observed a significantly longer ( $20.6 \pm 8.1$  days) and more variable time period until all finches in the flock exhibited clinical disease when compared to the finches from Montana exposed to MG by ocular inoculation ( $Z = -3.28$ ,  $P = 0.001$ ; Fig. 1A). Regardless of mode of exposure we observed no differences between these two flocks in severity of disease, duration of illness ( $Z = -0.13$ ,  $P = 0.90$ ; Fig. 1B), or mortality rate ( $\chi^2 = 0.01$ ,  $df = 1$ ,  $P = 0.94$ ).

Results for finches are given in average days  $\pm$  1 SD. Differences in incubation period and duration of illness between house finches from Montana and house finches from Alabama directly inoculated with an MG isolate were evaluated with a two-tailed Mann-Whitney *U*-test. This same test was also used to evaluate the difference between house finches from Montana inoculated directly and those exposed to an infected bird. Differences in mortality between these flocks were evaluated with a Chi-squared ( $\chi^2$ ) test. All statistics were done using Statsview 5.0.

### RESULTS

Prior to infection, all birds were healthy. No house finch had antibodies to MG and MG was not detected by PCR. After exposure to MG all birds became infected and developed conjunctivitis, regardless of method of exposure or geographic origin. All birds seroconverted and MG was detected by PCR in every bird at least once during the course of the disease. In three attempts at isolation of MG from infected finches there was a color change in the cultures indicating growth and MG was confirmed by PCR. The eight control finches never exhibited signs of disease and MG infection was not detected by serology or PCR at any time during the study in these birds.

Following ocular inoculation with MG all finches rapidly developed conjunctivitis, but the incubation period in finches from Alabama was an average of  $5.25 \pm 1$  days, significantly longer than the incubation period of finches from Montana ( $3.71 \pm 1.1$  days) ( $Z = -1.97$ ,  $P = 0.049$ ; Fig. 1A). All of these finches exhibited moderate to severe unilateral or bilateral conjunctivitis (score of 2–3) with some birds experiencing complete blindness in one or both eyes (score of 4). Although six of the seven finches from Montana and all four finches from Alabama died during the study, the house finches from Alabama survived an average of  $58 \pm 9.9$  days from onset of disease, significantly longer than the house finches from Montana that survived an average of  $35 \pm 11.8$  days after onset of disease ( $Z = -2.15$ ,  $P = 0.03$ ; Fig. 1B).

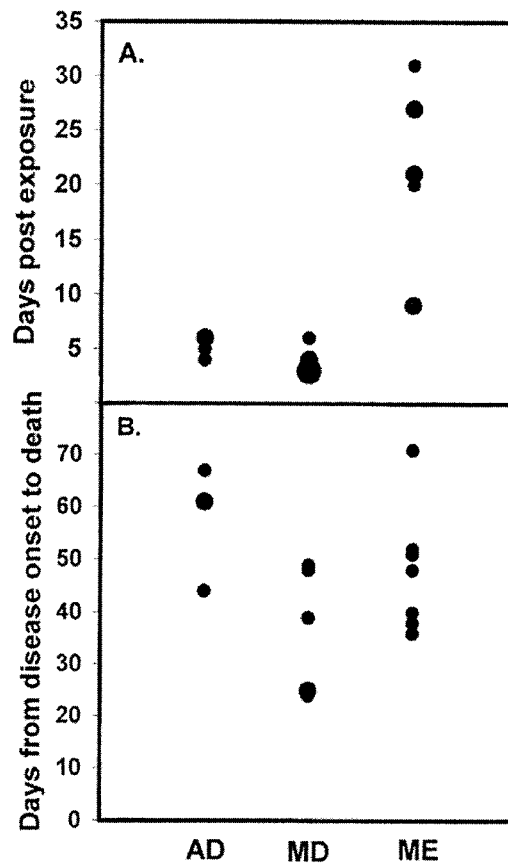


FIGURE 1. Differences in (A) time period until all finches demonstrated Mycoplasma conjunctivitis and (B) duration of illness between three flocks of house finches. AD = finches from Alabama inoculated directly, MD = finches from Montana inoculated directly, and ME = finches from Montana exposed to a naturally infected finch. Size of data point increases with number of birds per point.

When the finches from Montana were exposed to MG by co-housing them with a naturally infected finch from Alabama we observed a significantly longer ( $20.6 \pm 8.1$  days) and more variable time period until all finches in the flock exhibited clinical disease when compared to the finches from Montana exposed to MG by ocular inoculation ( $Z = -3.28$ ,  $P = 0.001$ ; Fig. 1A). Regardless of mode of exposure we observed no differences between these two flocks in severity of disease, duration of illness ( $Z = -0.13$ ,  $P = 0.90$ ; Fig. 1B), or mortality rate ( $\chi^2 = 0.01$ ,  $df = 1$ ,  $P = 0.94$ ).

### DISCUSSION

Our results indicate that finches from Montana, part of the western population of house finches, are highly susceptible to infection with the house finch MG strain. All 15 house finches captured in Montana and experimentally infected with MG by direct exposure to MG or by being housed with an infected finch developed clinical disease. We detected MG by PCR and antibody production in serum by SPA. Our observations suggest that the large western population of house finches may be susceptible to the house finch MG strain, and if MG were introduced into the western population, it would spread rapidly causing an epidemic similar to the eastern population killing millions of house finches.

We also observed differences in response to infection between finches from Montana and finches from Alabama. Previous published reports of both wild and captive finches indicate a changing relationship between MG and the house finch (Luttrell et al., 1996; Nolan et al., 1998; Roberts et al., 2001a, b). One reason for this change may be an increased resistance in house finches to MG. Although all four finches from Alabama died during the course of the study they all survived significantly longer than finches from Montana. This may suggest an increase in resistance to the pathogen in the exposed population when compared to the response of the naïve western population.

We also wanted to compare the response in birds to different modes of exposure. This study demonstrated that finches are susceptible to infection with MG by either co-housing the birds with an infected finch or by direct ocular inoculation and their response to infection is the same regardless of the method used.

### ACKNOWLEDGEMENTS

We thank the Roberts Laboratory including K. Klenk, S. Singh, and J. C. Rodenberry for help with serology and advice and the Hill Laboratory including A. Dervan and R. Duckworth for help with capture of the finches from Alabama. Special acknowledgements are given to

A. Badyaev for capture and shipment of the finches from Montana and P. Luttrell at the Southeastern Cooperative Wildlife Disease Study for the house finch MG isolate. Finally, we would like to thank the C. S. Roberts Veterinary Diagnostic Laboratory including F. Hoerr for their advice and support. Support for this work was provided by the National Science Foundation (Grant # DEB-0077804 to G.E.H and S.R.R.), the College of Science and Mathematics at Auburn University, the Alabama Agricultural Experiment Station, and the Department of Biological Sciences at Auburn University.

### LITERATURE CITED

- CANT, G. 1962. The house finch in New York State. *Kingbird* 12: 68–72.
- DHONDT, A. A., D. L. TESSAGLIA, AND L. SLOTHOWER. 1998. Epidemic mycoplasmal conjunctivitis in house finches from eastern North America. *Journal of Wildlife Diseases* 34: 265–280.
- ELLIOT, J. J., AND R. S. J. ARIBIB. 1953. Origin and status of the house finch in the eastern United States. *Auk* 70: 31–37.
- FISCHER, J. R., D. E. STALLKNECHT, M. P. LUTTRELL, A. A. DHONDT, AND K. A. CONVERSE. 1997. Mycoplasmal conjunctivitis in wild songbirds: The spread of a new contagious disease in a mobile host population. *Emerging Infectious Diseases* 3: 69–72.
- HARTUP, B. K., G. V. KOLLIAS, AND D. H. LEY. 2000. Mycoplasmal conjunctivitis in songbirds from New York. *Journal of Wildlife Diseases* 36: 257–264.
- HILL, G. E. 1993. House finch (*Carpodacus mexicanus*). In *The birds of North America*. A. Poole and F. Gill (eds.). The American Ornithologist's Union, Washington, D.C., pp. 1–24.
- LAUERMAN, L. H. 1998. Mycoplasma PCR assays. In *Nucleic acid amplification assays for diagnosis of animal diseases*. L. H. Lauerman (ed.). American Association of Veterinary Laboratory Diagnosticians, Turlock, California, pp. 41–43.
- LEY, D. H., J. E. BERKHOFF, AND J. M. MCLAREN. 1996. *Mycoplasma gallisepticum* isolated from house finches (*Carpodacus mexicanus*) with conjunctivitis. *Avian Diseases* 40: 480–483.
- LUTTRELL, M. P., J. R. FISCHER, D. E. STALLKNECHT, AND S. H. KLEVEN. 1996. Field investigation of *Mycoplasma gallisepticum* infections in house finches (*Carpodacus mexicanus*) from Maryland and Georgia. *Avian Diseases* 40: 335–341.
- MUNDINGER, P. C. 1975. Song dialects and colonization in the house finch. *Condor* 77: 407–422.
- NOLAN, P. M., G. E. HILL, AND A. M. STOEHR. 1998. Sex, size and plumage redness predict house finch survival in an epidemic. *Proceedings of the*

- Royal Society of London Series B: Biological Sciences 265: 961–965.
- ROBERTS, S. R., P. M. NOLAN, AND G. E. HILL. 2001a. Characterization of mycoplasmal conjunctivitis in captive house finches (*Carpodacus mexicanus*) in 1998. *Avian Diseases* 45: 70–75.
- , ———, L. H. LAUERMAN, L.-Q. LI, AND G. E. HILL. 2001b. Characterization of the mycoplasmal conjunctivitis epidemic in a house finch population in the southeastern USA. *Journal of Wildlife Diseases* 37: 82–88.
- SAUER, J. R., J. E. HINES, G. GOUGIL, I. THOMAS, AND B. G. PETERJOHN. 1997. The North American breeding bird survey results and analysis. Patuxent Wildlife Research Center, Laurel, Maryland. Version 96.4. <http://www.mbr-pwrc.usgs.gov/bbs/bbs96.html>.
- VAZQUEZ-PHILLIPS, M. A. 1992. Population differentiation of the house finch (*Carpodacus mexicanus*) in North America and the Hawaiian Islands. MS Thesis, University of Toronto, Toronto, Canada, pp. 14–37.

*Received for publication 5 April 2001.*