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Host inflammatory response governs fitness in an avian ectoparasite, the northern fowl mite (*Ornithonyssus sylviarum*)

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ABSTRACT

Vertebrate immune responses to ectoparasites influence pathogen transmission and host fitness costs. Few studies have characterized natural immune responses to ectoparasites and resultant fitness effects on the ectoparasite. These are critical gaps in understanding vertebrate–ectoparasite interaction, disease ecology and host-parasite co-adaptation. This study focused on an ectoparasite of birds—the northern fowl mite (NFM) (*Ornithonyssus sylviarum*). Based on prior evidence that chickens develop resistance to NFM, these experiments tested two hypotheses: (i) skin inflammation blocks mite access to blood, impairing development, reproduction and survival; and (ii) host immunogenetic variation influences the inflammatory response and subsequent effects on the ectoparasite. On infested hosts, histology of skin inflammation revealed increased epidermal cell number and size, immigration of leukocytes and deposition of serous exudates on the skin surface. Survival of adult mites and their offspring decreased as the area of skin inflammation increased during an infestation. Inflammation increased the distance to blood vessels beyond the length of mite mouthparts (100–160 μm) and prevented protonymphs and adults from reaching a blood source. Consequently, protonymphs could not complete development, evidenced by a significant inverse relationship between inflammation and protonymph feeding success, as well as an increasing protonymph/adult ratio. Adult females were unable to feed and reproduce, indicated by an inverse relationship between inflammation and egg production, and decreasing female/juvenile ratio. These combined impacts of host inflammation reversed NFM population growth. Intensity of inflammation was influenced by the genotype of the major histocompatibility complex (MHC), supporting previous research that linked these immunological loci with NFM resistance. Overall, these data provide a model for a mechanism of avian resistance to an ectoparasitic arthropod and the fitness costs to the parasite of that host defense.

1. Introduction

Trager (1939) first demonstrated that vertebrates are capable of acquiring immune-mediated resistance (adaptive immunity) to blood-feeding arthropods. This work stimulated considerable research elucidating pathogen transmission relative to host-ectoparasite interactions (Wikel, 1996; Ribeiro and Francischetti, 2003; Peters et al., 2008). Although vertebrate hosts acquire resistance to ectoparasitic arthropods and up-regulate immune effectors in response to infestation (DeVaney and Ziprin, 1980; Bany et al., 1995; Heeb et al., 1998), little is known about the effects of these events on the ectoparasite. There are few comprehensive studies demonstrating a specific immune response to an ectoparasite and the resultant effect(s) on fitness parameters of the arthropod (e.g. parasite survival, development and reproduction). This gap in our knowledge of vertebrate–ectoparasite interaction constrains studies of coevolution using vertebrate–ectoparasite models and impairs a thorough understanding of the ecology of arthropod-transmitted disease.

The northern fowl mite (NFM), *Ornithonyssus sylviarum,* is a cosmopolitan ectoparasite of birds with a broad host range that includes wild and domesticated species (Knee and Proctor, 2007). The NFM is a significant economic pest of production poultry in North America (Hinkle and Hickle, 1999; Mullens et al., 2009). The life cycle of NFM is completed entirely on-host in galliform birds (chickens and turkeys), wherein the mites reside on the feathers and migrate to the skin surface to blood-feed (Owen and Mullens, 2004). There are five stages of development (egg, larva, protonymph, deutonymph, adult), with two blood-feeding stages (protonymph and adult), and a generation interval of 5–7 days (Combs and Lancaster, 1965). The domestic chicken (*Gallus gallus*

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domesticus) develops resistance to NFM, which manifests as restricted population growth through time and smaller populations on previously infested birds (Matthysse et al., 1974; DeVaney and Ziprin, 1980). The chicken develops NFM-specific antibody titers that increase with higher intensities of NFM infestation (Burg et al., 1989; Wikel et al., 1989; Minnifield et al., 1993). However, host antibodies alone do not affect NFM fitness (Minnifield et al., 1993) and the exact immunological mechanism(s) responsible for development of host resistance to NFM remains unclear.

Host genetic background appears to influence variation in NFM resistance with heritability estimates of 0.26 (Eklund et al., 1980), resistance differences between breeds (Burg et al., 1988) and infestation as well as resistance differences between Rous sarcoma virus-selected lines (DeVaney et al., 1982). The latter study established major histocompatibility complex (MHC) haplotype variation among the chicken lines. The MHC is a tightly linked set of vertebrate genes responsible for multiple components of the innate and adaptive immune system, including antigen presentation, complement proteins and cytokines (cell signaling) (Parslow et al., 2001). The MHC of the domestic chicken is reduced and compact with low levels of recombination, causing the genes to be inherited as a discrete haplotype (Kaufman, 2008). Class I and class II loci are inherited as one linkage group. Variation in the MHC is associated with variation in resistance to both endoparasites and ectoparasites (Untalan et al., 2007; Westerdahl, 2007; Owen et al., 2008). However, the influence of MHC variation on host resistance to ectoparasites in particular has not been determined in the context of the immune response.

Matthysse et al. (1974) demonstrated that chickens exposed to NFM antigens in the skin developed immediate hypersensitivity responses (inflammation) if previously infested. The intensity of the inflammatory response was negatively correlated with NFM population density and it was proposed that the inflammatory response could control the level of NFM infestation by altering mite access to blood. To further our understanding of these observations, we examined the hypothesis that host inflammatory responses in the skin impair the ability of NFM to feed, reproduce and survive, resulting in an overall reduction in population growth rate. Further, based on recent studies linking chicken MHC variation to NFM resistance (Owen et al., 2008), we examined the hypothesis that MHC haplotypes influence the intensity of inflammation and the impact on NFM population size. Based on the results of these experiments, we constructed a model for a mechanism of NFM population regulation by the avian immune response.

2. Materials and methods

2.1. Experimental animals and general techniques

2.1.1. Host birds

Commercial egg-laying birds (Hy-Line W-36, white leghorn females) were used as hosts and were obtained from a commercial producer at 18 weeks of age. The hens were beak-trimmed to match standard industry practices (Glatz, 2000). Hens were housed individually in 774 cm^2 cages with food and water ad libitum. The birds were kept in open-sided, screened housing with internal lighting (16 h light/8 h dark). All birds were inspected for mite infestations to ensure that they had no prior infestations. The birds were vaccinated according to industry practices (Newcastle Disease Virus, Infectious Bursal Disease Virus, Infectious Bronchitis Virus, Laryngotracheitis and Fowl Pox Virus) and were not treated with any steroids or growth hormones.

2.1.2. Mites and infestations

Mite maintenance, collection and dispersal were as described in Owen et al. (2008).

It is worth noting that adult females were expected to be the predominant sex in the founding population of each hen. Animal care was monitored by the University of California, Riverside office of the campus veterinarian and followed IACUC-approved protocols.

2.1.3. Visual scoring system

NFM population sizes were estimated visually using a scoring system modified from Arthur and Axtell (1983) and Mullens et al. (2000). Briefly, this process required holding the hen and carefully examining the feathers in the $\sim 4 \times 6$ cm area anterior to the vent. This area contains the vast majority of mites on domestic hens (Matthysse et al., 1974). The original scoring system was 0 = no mites, 1 = 1–10, 2 = 11–50, 3 = 51–100, 4 = 101–500, 5 = 501–1000, 6 = 1001–10,000 and 7 = >10,000. A "low" and "high" qualification for the lower and upper 20 percentiles of each range increased the resolution of the scoring. The accuracy of the visual scoring system was tested by comparing visual scores to counts of extracted mite populations (see below). All scoring was done by a single observer (J.P. Owen) for consistency. Histological data and MHC genotype were not obtained until after the end of the experiment. Therefore, no information was available to potentially bias scoring with regard to host genotype or immune response.

2.1.4. MHC genotyping

MHC haplotypes were determined using PCR amplification of a microsatellite (LEI0258) (Fulton et al., 2006) as described in Owen et al. (2008). The W-36 population segregates MHC genotypes B2, B15 and B21, and the hens were known to be heterozygous (B2/B15 and B2/B21) based on the genotypes of the parent populations used to create the W-36 line (Owen et al., 2008). The two genotypes were discriminated by microsatellite amplicon size: 261 bp (B2/B15) or 261 and 357 bp (B2/B21).

2.2. Surface inflammation and mite survival experiment

2.2.1. Area of inflammation

In the mite survival experiment (Experiment 1), the area (cm^2) of inflammation of the skin on each bird was measured using digital calipers (Fisherbrand Traceable) to quantify maximum anterior–posterior and lateral transects of inflamed skin (red coloration, serous exudates, scab formation). Inflamed skin was easily distinguished from normal (pale-pink/white) skin (Fig. 1). The condition of the skin was categorized as (1) actively inflamed (red/raised skin, serous exudates, intact scabs) or (2) recovering (cracked and lifted scabs, clear white/pink skin). These values were used in modeling effects of inflammation on mite population development.

2.2.2. NFM survival assay

For the survival experiment (Experiment 1) adult mites and eggs were isolated and incubated in vitro to assess off-host survival, hatching success and larval/protonymph viability. The isolated adult mites had no access to blood and could only rely on stored energy for survival. The hatched larvae can molt to the protonymph stage without a blood meal, whereas the protonymphs cannot molt to the deutonymph stage without blood and were similarly reliant on stored energy for survival. For each assay 100–200 mites were aspirated into a glass Pasteur pipette from a population on the host. The pipette of mites was then placed in an incubator for 24 h (75% relative humidity, 30 °C and 12:12 light:dark cycle). Gravid, adult female mites oviposited in the pipette during that period. Following the initial incubation, the new eggs were carefully removed from the pipette using a fine paintbrush and 30 eggs were placed into 10 straight-sided ½ dram vials in groups of three. The vial caps were punctured to allow airflow and the hole was blocked with tissue paper to prevent mite escape. Thirty live, adult mites were then aspirated into a new glass pipette, which was plugged with cotton. The