

Comparison of Immune Structure and Function of Nestling and Adult Tree Swallows

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Introduction

Immature individuals are generally more susceptible to infection and disease than adults. Study of humans and domesticated animals suggest that the immune system mediates this age-related pattern. Despite the rapid growth of ecological immunology in the last few decades, we still know very little about age-related patterns of immunity of organisms in their natural environments. In this study we compared several aspects of immune structure and function between free-living nestling and adult tree swallows (*Tachycineta bicolor*). We measured several components of both the innate and acquired arms of the immune system and predicted that nestlings would have reduced levels when compared with adults.

Materials and Methods

Study population and field sampling methods. We conducted this study in the tree swallow nest-box population at the Horticulture Research Station. During the 2005 breeding season, we sampled nestlings 11 to 14 days-old (n=12) and 1 year-old adults (n=8), which have a fully developed immune system. For all birds we collected blood (~140 μ l) for the immune assays. A drop of blood was used to prepare a thin blood smear. We estimated body condition of each bird as the residual from the linear regression of body mass against head-bill length (a measure of structural size).

Measures of immune structure. We assessed immune structure by estimating the total white blood cell (WBC) count using a hemocytometer

and the differential WBC count on blood smears. For the latter we counted 100 leukocytes under 1000x magnification determining the percentage of lymphocytes, heterophils, eosinophils, basophils, and monocytes. Lymphocytes are the main leukocytes involved in acquired immunity whereas all other leukocyte types play major roles in innate immunity.

Measures of immune function. We assessed acquired immune function by measuring the ability of lymphocytes to proliferate *in vitro* upon mitogenic stimulation. We used two T-cell mitogens, phytohaemagglutinin (PHA), and concanavalin A (ConA), to stimulate T-lymphocytes; and the B-cell mitogen lipopolysaccharide (LPS) to stimulate B-lymphocytes. The proliferative response of lymphocytes was expressed as a stimulation index (SI). Finally, we assessed innate immune function by determining the levels of natural antibodies (NAb) in blood and their ability to cause complement-mediated cell lysis. We used a hemolysis-hemagglutination assay and titers were expressed as the \log_2 of the reciprocal of the highest dilution of plasma that showed the corresponding response.

Results and Discussion

Body condition did not explain variation in immune parameters within each age group (all $P > 0.05$). No difference was detected between nestlings and adults in percentage heterophils, percentage basophils, and percentage monocytes (all $P > 0.1$). However, nestlings had lower percentage lymphocytes and total white blood cell counts, but higher percentage eosinophils than young adults (Figure. 1).

Regarding immune function, no difference between age groups was detected in

proliferation of T-lymphocytes in response to ConA ($t=0.83$, $P=0.43$) or in proliferation of B-lymphocytes in response to LPS ($t=0.13$, $P=0.90$). On the other hand, nestlings showed lower titers of natural antibodies (NAb titer) and of complement mediated-cell lysis (Lysis titer), and lower proliferation of T-lymphocytes in response to PHA (PHA SI) than young adults (Figure 2).

In summary, our study in free-living tree swallows demonstrates that important aspects of the immune system are poorly developed in nestlings compared with adults. This supports the idea that immature individuals face a period

of increased vulnerability to pathogens and disease before their immune system reaches adult levels of structure and function. The increased susceptibility of young to infections might be an important factor contributing to the reduced annual survival rates of juveniles compared with those of adults that are commonly documented in ecological studies.

Acknowledgments

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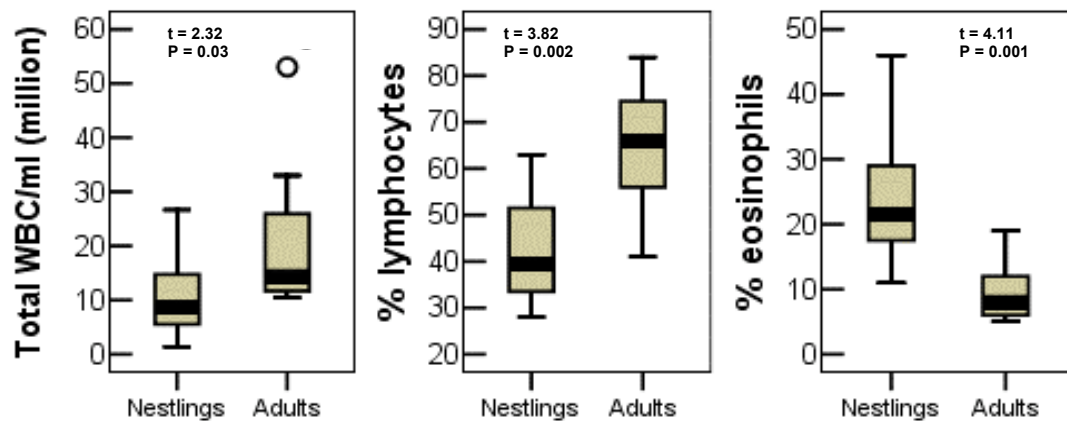


Figure 1. Differences in immune structure between nestling and young adult tree swallows. Box-plots depict the median, interquartile range, extreme values, and outliers for each variable.

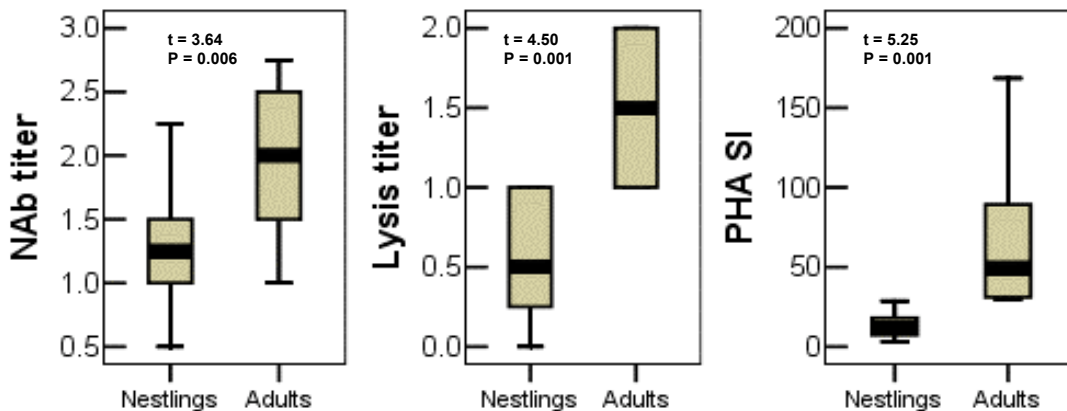


Figure 2. Differences in immune function between nestling and young adult tree swallows. Box-plots depict the median, interquartile range, extreme values, and outliers for each variable.