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Age-related decline in humoral immune function in Collared Flycatchers

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Abstract

Although immune function usually declines with age in humans and captive animals, little is known about whether immune function deteriorates with age in natural populations. Here we present evidence for such an age-related deterioration in humoral immune function from a wild population of the Collared Flycatcher (*Ficedula albicollis*). In this study, young (1-year old), mid-age (3-year old) and old (5–6-year old) females were challenged with a nonpathogenic antigen, sheep red blood cells (SRBC), while provisioning their nestlings. The level of antibodies against SRBC was measured thereafter. Old females showed markedly lower humoral immune response and produced fledglings of lower body mass in comparison with the other two age classes. Moreover, the age classes differed in the relationship between immune response and fledgling body mass with mid-age females showing a significant positive relationship while the relationship was negative but nonsignificant among young and old females. The results are discussed in light of existing theories of optimal resource allocation, ageing and the theory of terminal investment.

Introduction

Life history theory assumes that because of resource limitation, life history strategy results from various constraints on resource allocation (Stearns, 1992). It has been postulated that ageing may also result from the life history optimization of resource partitioning, in which an optimal low level of repair of somatic damage (not sufficient to prevent all encountered damage) leads to a gradual deterioration of the organism's physiology (the so-called disposable soma theory; e.g. Kirkwood, 1993). The theory assumes that organisms adjust their investment of resources between self-maintenance and other various functions like growth and reproduction in a way that maximizes fitness. The optimal strategy, as age increases, is to spend fewer and fewer resources on self-maintenance in favour of reproduction and this leads to an acceleration of ageing (Cichoń, 1997, 2001).

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An immune system can be considered as a self-maintenance mechanism as it prevents harmful damage from pathogens. Thus, according to life history theory an age-related decrease in a share of resources devoted to immune function and, as a consequence, deterioration of immunity later in life, should be expected. In fact, together with many other physiological functions, the immune system is known to be affected by ageing. In human beings and the laboratory animals a consistent decline in immune functions is usually observed in many ways (see Nagel *et al.*, 1986 and Miller, 1990, for review). Recently, a decline in immune function has also been reported in invertebrates (Adamo *et al.*, 2001; Kurtz, 2002). The physiological mechanisms underlying the observed decline in immune function has been widely documented. For example, T-cell proliferation, generation of cytotoxic effectors, production of some interleukins by T-cells, T-cells activity and even antigen presentation may become problematic in old individuals (Miller, 1996, 2000).

Although immune function declines with age in humans and captive animals, little is known about whether immune function deteriorates with age in

natural populations. The data from humans come mainly from developed countries with high-standards of medical care, and the data from captive animals are also affected by the fact that the animals, because of care keeping, avoid pathogen-caused mortality and usually live much longer than under natural conditions. Because of much higher rates of selective death among wild animals compared with laboratory animals, any decline in immune function may not in fact be observed in the wild. It is possible that in the wild only individuals equipped with a well functioning immune system can survive to advanced age in the world full of pathogens. In fact, studies of senescence in natural populations do not always show the expected elevation in the rate of intrinsic mortality or a drop in fecundity with age (Promislow, 1991; Holmes & Austad, 1995, but see Ricklefs, 2000). Thus, similarly, under natural conditions deterioration of immune function in old animals may not be detectable.

Here we aim at studying age-related ability to mount a humoral immune response (immunocompetence) evoked by a challenge with a nonpathogenic antigen, sheep red blood cells (SRBC) in female Collared Flycatchers from a free-living population. We address the question of whether a decline in humoral immune function with age can actually be observed in the wild. Although variation in immune function in response to various factors has been investigated in natural populations (see Norris & Evans, 2000; Kurtz *et al*., 2002, for review), to date, the age-related effects on immunocompetence have been rather neglected in such studies. The Collared Flycatcher is a small migratory passerine bird species, which in our population have been observed to live up to 9 years. A decline in reproductive performance at advanced age caused by costs of reproduction has already been shown in this population (Gustafsson & Pärt, 1990). This species has also been involved in a number of immunological studies (Nordling, 1998; Andersson, 2001; Cichoń *et al*., 2001). According to the resource allocation principle, immune function is expected to compete for limited resources with other vital functions like reproduction. Thus, in order to increase the chance of detecting any differences between age classes in terms of immunocompetence and to study a potential trade-off between reproduction and immunity, the humoral immune response was evoked at the time, when the reproductive investment peaked, namely in the second half period of feeding nestlings.

Methods

The study was conducted in a nest-box breeding population of Collared Flycatchers on the island of Gotland, Sweden (for details about the study area see Gustafsson, 1989). The Collared Flycatcher is a small (13 g) migratory passerine bird species breeding mainly in eastern and central Europe and wintering in southern and central Africa. It nests in natural tree cavities, but prefers nest boxes when provided. The Gotland population is characterized by fairly restricted dispersal of adults and young (Pärt & Gustafsson, 1989). Thus, it is possible to follow individuals throughout their lifetime. Many individuals are ringed as nestlings or yearlings and therefore are of known age.

In the present study, nest boxes were inspected every 5 days to look for new nests. In order to determine their age, females were captured on the seventh day of incubation. Age was determined on the basis of ring number, when the female was already ringed, or classified as yearling or older for unringed females, according to the shape of the primary coverts and the colour of the upper mandible (Karlsson *et al*., 1986).

Three groups of females were involved, (1) young (1-year old), (2) mid-age (3-year old), and (3) old females (5–6-year old) (the maximal longevity of females recorded in this population was 8 years, but individuals aged 7–8 are very rare). In order to assess whether immune function differs between these age classes a humoral immune response was evoked with a challenge of a nonpathogenic antigen, SRBC. SRBC is a standard complex antigen commonly used in immunological studies (Hay & Hudson, 1989). Females were caught at the fifth day after hatching while feeding young and were injected intraperitoneally with 100 μL of sterile SRBC (obtained from Hatunaholm, Stockholm) suspended in phosphate buffered saline (PBS) and adjusted to 5×10^7 cells in 100 μL PBS. Immunized females were recaptured 6 days later when the chicks were 12 days old. At the same time nestlings were weighed with a Pesola spring balance (to the nearest 0.1 g) and their tarsus length was measured with a calliper (to the nearest 0.1 mm). One hundred microlitres of blood was taken from a brachial vein into an ethylenediaminetetraacetic acid-coated capillary, and transported to the lab in a cool-box. Afterwards, blood was centrifuged at 1000 rpm for 10 min, and then at 2000 rpm for 20 min, after which the plasma was separated and stored in a freezer at 20 °C until analysed.

A standard haemaglutination test was employed to quantify the specific antibody concentration against SRBC (Hay & Hudson, 1989). The presence of antibodies is associated with agglutination of erythrocytes. The numbers of titres showing positive haemaglutination represents the antibody concentration based on a ^{2}log scale (Hay & Hudson, 1989). The test was performed twice for each bird and the average titre number was used in the subsequent analyses.

In total 79 females were involved in the study (29 young, 20 mid-age and 30 old females). Because of nest desertions or whole brood death, we failed to recapture the number of females after immunization, so finally the total number of 56 females was used in later analyses. Probability that we failed to recapture a female was not related to age ($\chi^2_2 = 0.03$; $P = 0.87$).

In the statistical analyses we used one-way AN(C)OVA or two-way nested ANOVA followed by Tukey *post hoc* tests. Analysis of differences in nestling body mass was based on individual nestlings. Thus to account for within-nest variation, two-way ANOVA with female ID nested in the age factor was employed. The relationship between female humoral immunocompetence and nestling body mass was assessed in an ANOVA in which nest-averaged body mass of nestlings was the dependent variable, female age the independent factor and female immune response a covariate.

Results

Young, mid-age and old females differed significantly in terms of immune response to SRBC. Among old females only eight of 22 (36%) showed detectable response, while all 21 young females and 12 of 13 mid-aged females (92%) responded to the antigen ($\chi^2_2 = 29.84$, $P < 0.001$). There was also a significant difference in the strength of the humoral immune response between age classes (one-way ANOVA; $F_{2,53} = 6.26$, $P = 0.004$, Fig. 1). The Tukey *post hoc* test showed significant differences only between yearlings and old females ($P = 0.004$).

Females of different age classes did not differ in number of fledglings they raised (oneway ANOVA; $F_{2,53} = 0.35$, $P = 0.71$) or in fledgling success (one-way ANOVA; $F_{2,53} = 0.33$, $P = 0.72$). Old females raised chicks of lower body mass (two-way nested ANOVA; $F_{2,236} = 3.90$, $P = 0.02$, followed by Tukey *post hoc* test, Fig. 2) in comparison with females from the other age classes. There was no relationship between female humoral immune response and nestling body mass (one-way ANCOVA; $\beta = -0.01$, $F_{,150} = 0.006$, $P = 0.94$). However, the interaction between the level of immune response and female age was close to statistical significance ($F_{2,48} = 2.96$, $P = 0.06$, Fig. 3), which means that the relationship between immune response and nestling body mass differed between age classes. Among mid-age females the relationship was significantly positive ($r = 0.67$, $P = 0.02$) and it was negative, but not significant, in old females ($r = -0.25$, $P = 0.27$).

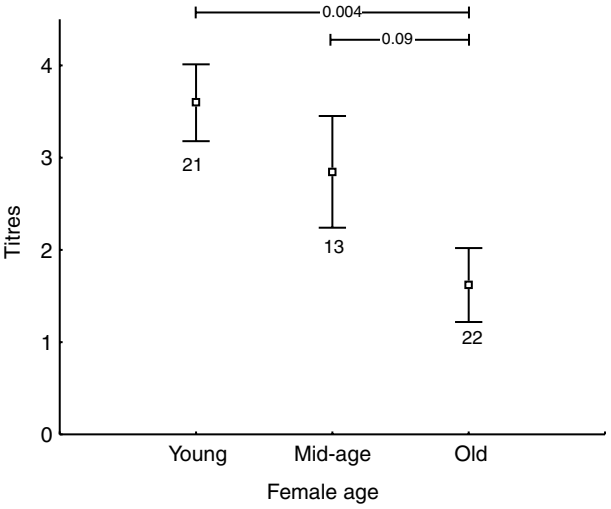

Fig. 1 Immune responses to sheep red blood cells of females from different age classes (mean ± SE). 'Young' are 1-year old, 'mid-age' 3-year old females and 'old' is 5–6-year olds. Numbers below whiskers denote sample size. Horizontal lines with numbers denote statistical significance after Tukey *post hoc* test.

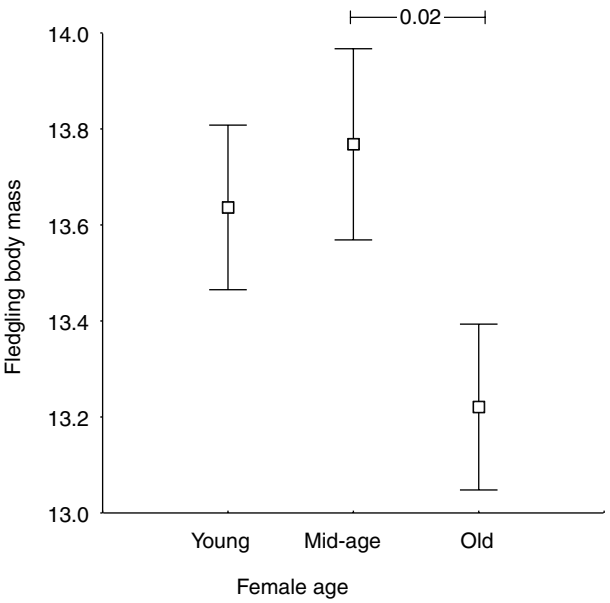

Fig. 2 Differences (mean ± SE) in body mass of fledglings raised by young, mid-age and old females. Horizontal lines with numbers denote statistical significance after Tukey *post hoc* test.

Discussion

In this paper we showed that in a wild population of Collared Flycatchers old females were inferior in terms of ability to mount a humoral immune response against SRBC. To our knowledge it is the first study reporting age-related differences in immune function in a free-living population. A substantial number of studies have previously demonstrated senescence of immune system in humans and in captive animals supplied food *ad libitum*, being free from predation and to large extend free from parasites (e.g. Goidl *et al.*, 1976; Hayashi *et al.*, 1989; Grossmann *et al.*, 1990; Powers & Belshe, 1993; Holliday, 1994; Zhao *et al.*, 1995; Miller, 2000). However, in contrast to humans and laboratory animals, individuals showing inferior ability to fight infections might not be surviving under natural condition. Thus, even old birds observed in natural populations might have had well functioning immune system. Our results do not confirm this prediction.

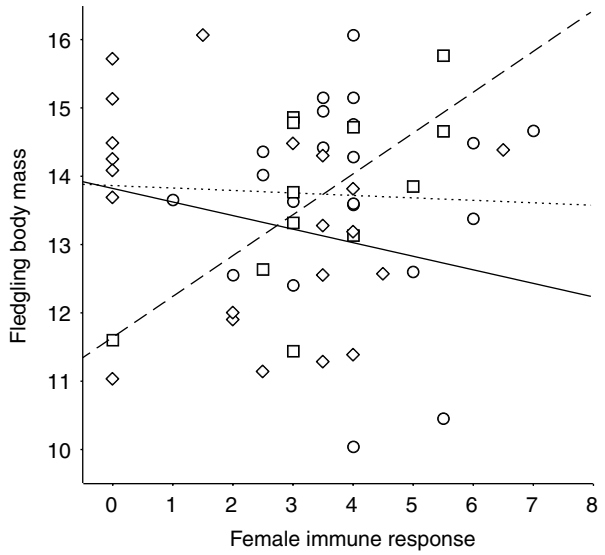

Fig. 3 Relationship between female immunocompetence and brood-average fledgling body mass separately for age classes (circles and dotted line are data for young females, squares and centered line for mid-age and diamonds and solid line for old females). The relationship is significantly positive for mid-age females ($r = 0.67$, $P = 0.02$) and negative but nonsignificant for young and old females. Lines are regressions showed only to visualize the relationships.

The finding that old females have a reduced immune response raises the question of how old females in a natural environment survive and cope with an inferior humoral immunity. Old females may already have large immunological experience, as they would probably have encountered an array of antigens during their life and they can still keep the memory cells that enable them quick recognition and development of immune response to already known antigens. We can suspect that only recognition of new antigens, but not immune response to already known antigens could be affected in old individuals. In fact, bursa of Fabricius, which is primarily responsible for proliferation of B-cells and presentation of antigens, tend to shrink gradually in birds just after maturity (e.g. Eerola *et al.*, 1987). Antigens are unable to induce antibody response in animals in which bursa fails to generate specific B-cells (Roitt *et al.*, 1998). The immune response to such antigens as SRBC relays also on the functions of the spleen (Jeurissen, 1993) and this organ also shrinks with age. Moreover, the presentation of antigens may be retarded in old animals (Miller, 1996, 2000). It is also possible that the innate immunity compensates for age related deterioration in acquired immunity. We lack information as to whether the function of the whole immune system was inferior in old females or only their humoral response.

The inferiority of immune response at old age might result from age-related physical deterioration of immune system, but it may also result from an optimal reduction in immune function later in life. If one considers immune system as a self-maintenance mechanism one should expect a decline in immune function with age as a share of resources devoted to self-maintenance should shrink. More and more resources should instead be channelled to reproduction with advanced age and an increase in reproductive effort should be expected later in life (Cichoń, 2001). At the end of life an organism should shut down self-maintenance and should make a so-called terminal investment in reproduction (Williams, 1966; Schaffer, 1974; Pianka & Parker, 1975; Pärt *et al.*, 1992). In light of these arguments we should expect a negative relationship between immunocompetence and reproductive effort, which should be more pronounced among old individuals. However, as a gradual decline in physiology affects also reproduction, comparisons of reproductive effort between age classes are very difficult: high reproductive effort does not necessarily need to be translated to high reproductive success. Here, we employed fledgling body mass as a measure of reproductive effort and looked whether it was related to female humoral immune response. We did not find any relationship across age classes, but there was an interaction between age classes that was close to significance, which implied that the relationship differed between age classes. However, the relationship for old females, although negative, appeared nonsignificant, while it was significantly positive among mid-age females. Thus, the extent to which the observed reduction in immune function in old ages results from optimal resource allocation strategy, aiming at terminal investment in reproduction, cannot be determined with the current data.

The inferiority of humoral immune response at old age may alternatively result from adaptive down-regulation of innate immune function. Difficulties in antigen recognition because of accumulation of somatic mutations in immunocompetent cells may cause a risk of autoimmune reactions (Walford, 1969). As the efficiency of the immune system critically depends on the ability of distinguishing of self from nonself antigens, failure of this system may lead to self-destruction. Thus, shutting down the humoral immune functions at old age when proper antigen recognition becomes problematic should be adaptive. However, testing such alternative is very difficult and impossible with the current data set.

The present results have some important implications for studies in the field of ecological immunology. The commonly observed difficulties in showing any effect of various environmental factors on immune function, such as for example, any effects of elevated reproductive effort may, to some extent, arise because of the effect of age (Nordling, 1998; Cichoń *et al.*, 2001). If individuals from different age classes are involved in the study, it could be difficult to reveal any significant effects of environmental factors because of high variance in immunocompetence associated with differences between age classes.

This study shows that humoral immune function may deteriorate with age in wild populations. Thus, dysfunction of the immune function with age can no longer be attributed to artificial laboratory condition. Future studies should determine whether innate immunity could compensate for the observed deterioration of acquired immunity. Moreover, cellular immunity may be as more important as acquired immunity as it deals with intracellular microorganisms (Roitt *et al.*, 1998). It is also very important to perform similar studies outside the breeding period. This could help to understand to what extent reproductive activities mediate malfunction of the immune system at old age classes. There is a possibility that young and old females show similar level of response outside the breeding period and that the observed immunosuppression in old females attending their broods may result from stress related to high reproductive effort, if old females are making their terminal investment. Thus, more studies are needed to understand whether the observed pattern in age-related decline in humoral immune response results from deterioration of immune system with age or it results from optimal resource allocation strategy promoting reproduction in favour of immunity at older ages.

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