OIKOS 101: 291–298, 2003

Dynamics of an immune response in house sparrows *Passer domesticus* in relation to time of day, body condition and blood parasite infection

C. Navarro, A. Marzal, F. de Lope and A. P. Møller

Navarro, C., Marzal, A., de Lope, F. and Møller, A. P. 2003. Dynamics of an immune response in house sparrows *Passer domesticus* in relation to time of day, body condition and blood parasite infection. – Oikos 101: 291–298.

The T-cell mediated response to a challenge of the immune system with phytohemagglutinin (PHA) is a common measure used in ecological studies of host-parasite interactions or parasite-mediated selection. We investigated the temporal dynamics of this response in house sparrows *Passer domesticus* in order to determine factors that contribute to temporal and individual variation in PHA response. After an initial significant increase in response from injection to six hours post-injection, there was no further significant change in mean response after 12, 24, 36, 48 and 72 hours. Responses at night were consistently stronger than during daytime. Individuals may benefit from producing both a strong and a quick immune response to parasite attacks, although this may be impossible because of the observed positive relationship between maximum response and latency to maximum response. House sparrows with *Haemoproteus* infections had lower PHA responses and smaller maximum responses than individuals without infections. Individuals in prime body condition had stronger PHA responses than individuals in poor condition. House sparrows with large diurnal fluctuations in body mass had a weaker maximum response than individuals with small fluctuations. Since individuals with *Haemoproteus* infections have large fluctuations in body mass, this result suggests that infected individuals with large fluctuations in mass are unable to mount a strong immune response.

C. Navarro, A. Marzal, F. de Lope, Depto de Biología Animal, Univ. de Extremadura, E-06071 Badajoz, Spain. – A. P. Møller, Lab. d'Ecologie Evolutive Parasitaire, CNRS UMR 703, Univ. Pierre et Marie Curie, 7 quai St. Bernard, Case 237, F-75252 Paris Cedex 05, France (amoller@hall.snv.jussieu.fr).

Parasites have been implicated as important selective agents in the evolution of host life history, sexual selection and the evolution of sex (Hamilton 1980, Hamilton and Zuk 1982, Folstad and Karter 1992, Hochberg et al. 1992, Møller 1997). Hence, the ability of hosts to defend themselves efficiently against parasites is of utmost importance. Recently, immunity and the ability to produce efficient immune responses have been suggested to have important consequences for a diverse array of scientific questions in ecology. That is the case for an understanding of host-parasite interactions (Frank 1991, Gandon et al. 1996), but also for understanding host life history (Martin et al. 2001), host sexual selection (Hamilton and Zuk 1982, Folstad and Karter 1992, Møller et al. 1999), host migration (Møller and Erritzøe 1998), and parent-offspring conflict (Saino et al. 2000).

Immune responses are the most sophisticated and diverse host defences that have evolved (Klein 1990, Roitt et al. 1996, Wakelin 1996). However, when it comes to testing ecological and evolutionary questions in the field, there is relatively little information on the efficiency of immune responses. Intuitively, it seems likely that individuals, which are able to produce both strong and quick responses, will be at a selective advantage. Stronger responses provide hosts with a higher

Accepted 11 February 2002

ISSN 0030-1299

probability of survival, as suggested by higher survival rates of individuals that produce stronger responses (Christe et al. 1998, 2001, Birkhead et al. 1999, González et al. 1999, Horak et al. 1999, Soler et al. 1999, Merino et al. 2000). However, it remains unknown whether it is also advantageous to produce a fast response, although this seems a likely possibility. Yet, we know very little about the relationship between strength of immune responses and latency to maximum response. Since it is likely that it is beneficial to produce both a strong and a fast response, we can predict a trade-off. If it is beneficial to produce both a strong and a fast response, which are the phenotypic correlates of these? Since immune responses show condition-dependence (Chandra and Newberne 1977, Gershwin et al. 1985, Møller et al. 1998), we should expect that individuals in prime condition would be better able to produce fast and strong responses than individuals in poor condition.

A particular popular measure of immune response in the field is the T-cell mediated immune response to a challenge with phytohemagglutinin (PHA) arising from undifferentiated T-cells to undergo blastogenic transformation. This popularity arises from the fact that the response can be estimated without sophisticated equipment and after a very short time interval. Furthermore, it represents an estimate of T-cell dependent immunity, which is an important component of immunocompetence (National Research Council 1992). There is a significant additive genetic component to the PHA response (Saino et al. 1997, Brinkhof et al. 1999, Christe et al. 2000). The PHA response reflects the ability of individual birds to survive under field conditions (Christe et al. 1998, 2001, Birkhead et al. 1999, González et al. 1999, Horak et al. 1999, Soler et al. 1999, Merino et al. 2000). Furthermore, it also reflects the impact of parasites on nestling mortality across species of birds (Martin et al. 2001). In addition, the stress induced by the challenge of the immune system with PHA is weak as determined from very low levels of heat shock protein induction caused by the injection (Merino et al. 1999). Finally, it is independent of experimentally created levels of ectoparasitism (Saino et al. 1998, Brinkhof et al. 1999), which suggests that it is an inherent measure of the ability of an individual to raise a response. Thus it is not surprising that since the initial use in the beginning of the 1990's, more than 30 papers have been published in ecology, evolution and behaviour using the PHA test in free-living animals.

Scientists habitually measure the PHA response after 24 hours, since the initial paper by Goto et al. (1978). However, we know very little about the temporal dynamics of immune responses (see Goto et al. 1978 for exception). This lack of information about temporal dynamics of immune responses is complicated by the fact that many parasites are nocturnal in their activity because hosts are relatively immobile during night (Noble and Noble 1976, Marshall 1981, Cox 1982). Furthermore, the body temperature of homeothermic vertebrates generally decreases during night (e.g. Whittow 1986), which may be an opportunity for bacteria and other micro-parasites that have multiplication rates that are inhibited by fever or high temperatures (Noble and Noble 1976, Cox 1982, Banet 1986, Blateis 1986, Kluger 1991). Thus, we should expect that immune responses have evolved to become stronger during night than during daytime.

The aims of the present study were to (1) describe the temporal dynamics of the phytohemagglutinin-induced T-cell response; (2) determine whether there are diurnal fluctuations in immune response; (3) determine the relationship between maximum strength of response and latency to maximum response; and (4) determine the phenotypic correlates of maximum strength of response and latency to maximum response. We did this by studying house sparrows that were kept in aviaries during the study.

Previous studies of PHA responses in house sparrows have shown that individual in better body condition have stronger responses (González et al. 1999). Furthermore, this immune response is condition-dependent as shown by increased responses in individuals provided with high quality nutrition (González et al. 1999). The PHA response appears to be effective in preventing parasitism since infection with *Haemoproteus* blood parasites is significantly negatively related to the PHA response (González et al. 1999). Finally, individuals that survive in captivity with ad libitum access to food and water had stronger PHA responses than non-survivors (González et al. 1999).

Material and methods

We captured 44 house sparrows (24 males and 20 females) in mist nets during December 2000–January 2001 and randomly put them in eight outdoor aviaries sized $3.5 \times 1.1 \times 2.5$ m, with 3–7 individuals in each aviary. The aviaries contained food (a mixture of commercial seeds for seed eating birds) and water ad libitum. Perches were provided, and each aviary contained two nest boxes. The aviaries were exposed to the normal day light schedule.

As a measure of immune response we used the T-cell mediated immune response to a challenge with phytohemagglutinin (PHA). This is a standard estimate from the poultry literature of the ability to produce a T-cell mediated immune response (Goto et al. 1978, McCorkle et al. 1980, Parmentier et al. 1993, Dietert et al. 1996). Injection with PHA results in local activation and proliferation of T-cells, followed by local recruitment of inflammatory cells and increased expression of major histocompatibility complex molecules (Goto et al. 1978, Abbas et al. 1994, Parmentier et al. 1998).

The night before the experiment the birds were sleeping in a small cage ($1 \times 0.5 \times 0.5$ m) to reduce the amount of stress caused by capture. House sparrows were injected 0.05 ml of 0.2 mg phytohemagglutinin (PHA-P) in one wing web and 0.05 ml of physiological water in the other wing web on March 8th, 9th, 11th and 13th 2001. All individuals were injected in the morning between 0800 and 1200. The dose of PHA used in this study is similar to that used in numerous other studies of free-living or captive birds (Lochmiller et al. 1993, Saino et al. 1997, Christe et al. 1998, 2000, 2001, Birkhead et al. 1999, Brinkhof et al. 1999, González et al. 1999, Horak et al. 1999, Soler et al. 1999, Merino et al. 2000).

We measured the thickness of the patagium injected with PHA and with physiological water before injection and after 3, 6, 12, 24, 36, 48 and 72 hours, using a pressure-sensitive calliper (Digimatic Indicator ID-C Mitutoyo Absolute cod. 547–301 Japan), with an accuracy of 0.01 mm. This measure has a very high repeatability as shown previously for the house sparrow (González et al. 1999, F. de Lope unpubl.). In the subsequent analyses we used the increase in the thickness of the wing injected with PHA minus the increase in the thickness of the wing injected with physiological water as a measure of the intensity of the phytohemagglutinin-induced immune response (hereafter PHA response). Body mass was recorded with a Pesola spring balance to the nearest 0.1 g on the same occasions. We measured tarsus length on the first capture with a digital calliper to the nearest 0.01 mm. Badge size of males was estimated from the length and the width of the visible badge (the part that is not covered with white feather tips) with a ruler to the nearest mm after pressing the feathers against the body. The length and the width of the total badge (including the part that was hidden by white feather tips) was recorded in a similar way. These measures of badge size have previously been shown to be highly repeatable between measurement events (González et al. 1999). All measurements were made by a single, experienced person, which reduces the variance in the data due to inter-observer variability. Between 0 and 12 hours the sparrows were kept in the previously described small cages to avoid stress caused by re-capture. After 12 hours the sparrows were freed in the corresponding aviaries.

A blood smear was made from a drop of blood collected from the brachial vein upon first capture. The slide was air dried and then fixed in absolute ethanol and stained with Giemsa for 45 min. Smears were scanned at $1000\times$ magnification. The intensity of infection was quantified as the number of *Haemoproteus* blood parasites observed per 10 000 red blood cells. Intensity was $\log_{10}(x+1)$-transformed.

We estimated mean body mass and mean PHA response as the mean value from all eight (seven for PHA response) measurements. Maximum fluctuations in body mass was defined as the maximum body mass minus the minimum body mass. Maximum PHA response was defined as the largest of the seven values, while latency to maximum response was the number of hours post-injection until the maximum was recorded.

We used repeated-measures ANOVA to test for significant differences in immune response and body mass for individuals on different measurement occasions, while taking the effect of aviaries into account. Thus, the PHA responses and body masses of each individual were the repeated measures, the individual birds were subjects and aviary was a factor in these analyses. None of analyses of effects of sex (factor) or effects of badge size (covariate) were statistically significant in a repeated-measures ANCOVA, and these variables were therefore not included in the final models. None of the tests showed significant aviary effects, with the single exception of body mass (Table 1). Hence, aviary is unlikely to have been a factor confounding the conclusions reported in the Results.

Results

The temporal dynamics of the response to PHA injection differed among individuals, as exemplified for 8 individuals in Fig. 1a. Some individuals produced a quick response while others showed a later peak response. Mean response increased significantly from injection to 6 hours post-injection and remained stable afterwards (Fig. 1b). These temporal differences were highly significant, but independent of aviary, as demonstrated by a repeated-measures ANOVA (Table 1). The interaction between aviary and individual was non-significant (Table 1). Correlations between PHA responses of individuals measured at different times were positive with Pearson correlation coefficients r ranging from 0.16 to 0.82, with values above 0.30 being statistically significant. If initial responses after three hours were deleted, r ranged from 0.28 to 0.82, with only one value not being significant, while elimination of responses

Table 1. Repeated-measures ANOVA for PHA response and body mass of 44 house sparrows in relation to aviary and subject.

Factor	df	MS	F	P
PHA response				
Aviary	7	0.092	0.463	0.855
Subject	36	0.199		
PHA response	6	0.392	15.460	<0.001
PHA response × Aviary	42	0.025	1.001	0.478
PHA response × Subject	216	0.025		
Body mass				
Aviary	7	17.53	0.80	0.592
Subject	36	21.89		
Body mass	6	23.93	47.31	<0.001
Body mass × Aviary	42	1.84	3.63	<0.001
Body mass × Subject	216	0.51		

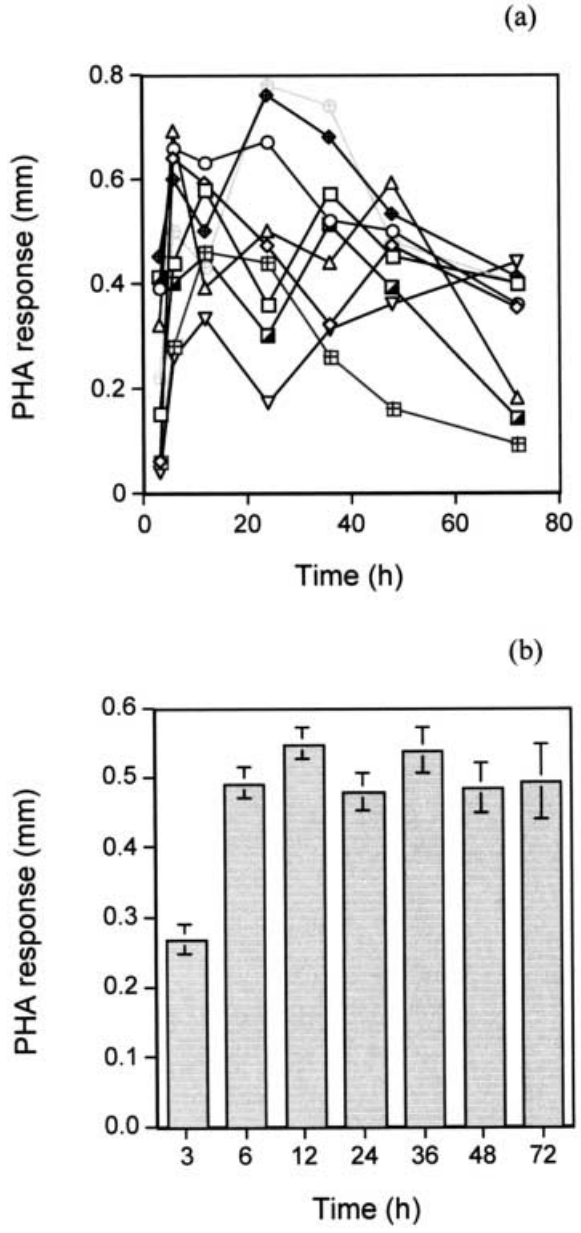

Fig. 1. (a) Temporal change in phytohemagglutinin (PHA) response (mm) in 8 different house sparrows, as examples of the temporal dynamics of the response of individuals. (b) Temporal change in mean phytohemagglutinin (PHA) response (mm) in 44 house sparrows. Values are means (SE).

shorter than 12 hours resulted in r ranging from 0.45 to 0.82. This implies that responses remained relatively stable after the initial increase.

There were clear diurnal changes in immune response (Fig. 1b; 12 and 36 hours). Values at night were consistently higher than values during day-light hours (Fig. 2). The mean difference between values at night and the mean diurnal value before and after was 12.6%. While pairwise differences in immune response were non-significant when the two samples were both from the day or the night ($t < 0.52$, $df = 43$, $P > 0.608$), pairwise comparisons between values during the day (just before or after the value at night was obtained) and the night were all significant ($t > 2.48$, $df = 43$, $P < 0.017$). Since mean values at night were significantly larger than mean values during the day, both when the day-time values were obtained before and after the nocturnal value, this implies that in the present study the difference between day and night does not depend on the number of hours elapsed since injection.

Maximum PHA response was 0.710 mm (SE = 0.037, $N = 44$), which was significantly larger than the mean response (0.473 mm (0.037)) and the response after 24 hours (0.478 mm (0.027)). Latency to maximum response was 30.4 hours (SE = 3.79, $N = 44$). There was a strongly positive relationship between maximum response and latency to maximum response (Fig. 3; $F = 25.83$, $df = 1,43$, $r^2 = 0.375$, $P < 0.0001$, slope (SE) = 0.006 (0.001)). This implies that strong responses took longer time to develop than weak responses.

Condition-dependent immune responses should show a positive relationship with body condition indices. The PHA response at 36 hours was the only one that was positively correlated with body mass pre-injection (Fig. 4; $F = 4.10$, $df = 1,43$, $r^2 = 0.089$, $P = 0.049$, slope (SE) = 0.045 (0.022)), and this relationship only accounted for 9% of the variance. Mean body mass was 25.2 g (SE = 0.25, $N = 44$). Maximum fluctuations in body mass among measurements were on average 2.97 g (SE = 0.27). Residual maximum PHA response (residuals from the regression in Fig. 3) was negatively

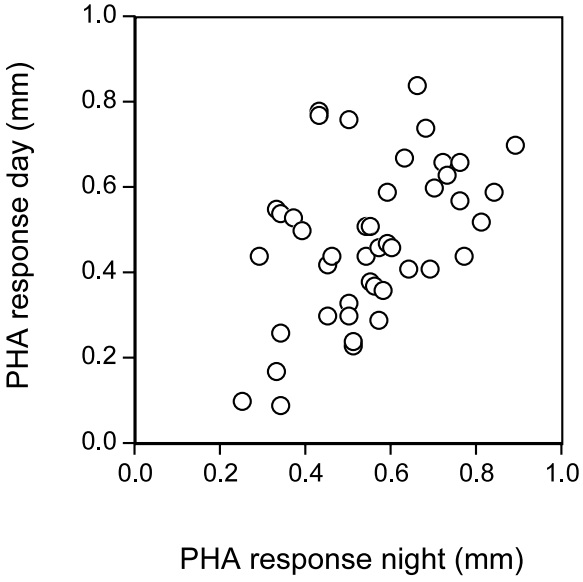

Fig. 2. Phytohemagglutinin (PHA) response (mm) during day (24 hours after injection) in relation to response during night (12 hours after injection) in 44 house sparrows.

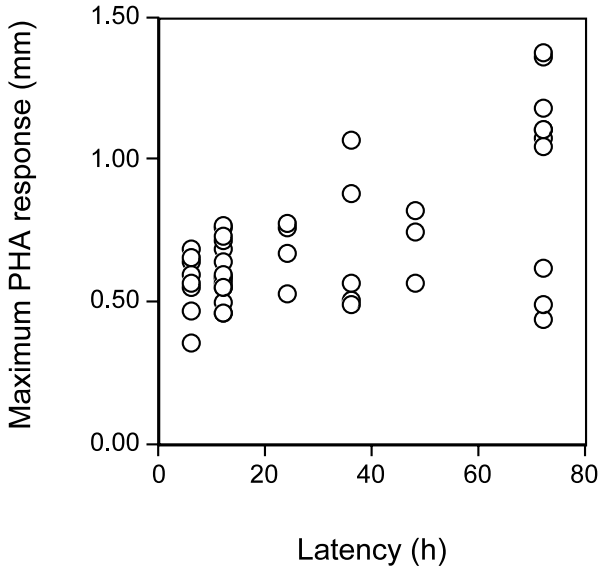

Fig. 3. Maximum phytohemagglutinin (PHA) response (mm) in 44 house sparrows in relation to latency to maximum response (h).

related to maximum fluctuations in body mass (Fig. 5; $F = 4.46$, df = 1,43, $r^2 = 0.096$, $P = 0.041$, slope (SE) = -0.020 (0.009)). This relationship was independent of extreme values as shown by a similar relationship for a rank correlation analysis (Spearman $r_S = -0.340$, $z = 2.23$, $P = 0.026$). Thus, house sparrows that had the largest fluctuations in body mass produced the weakest PHA responses.

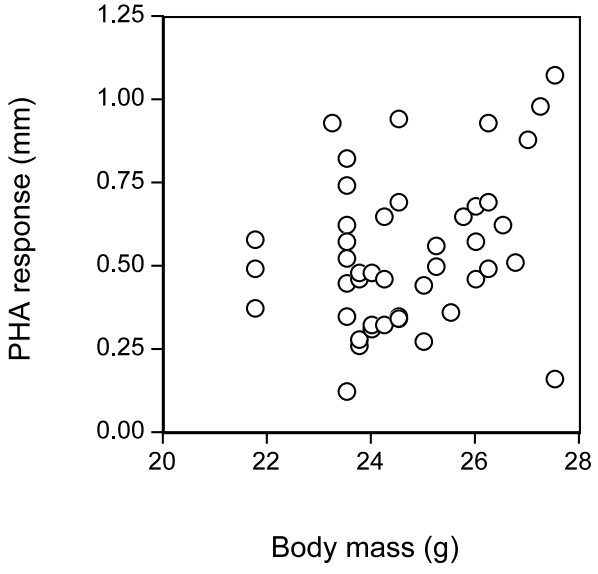

Fig. 4. Phytohemagglutinin (PHA) response (mm) in 44 house sparrows 36 hours after injection in relation to initial body mass (g).

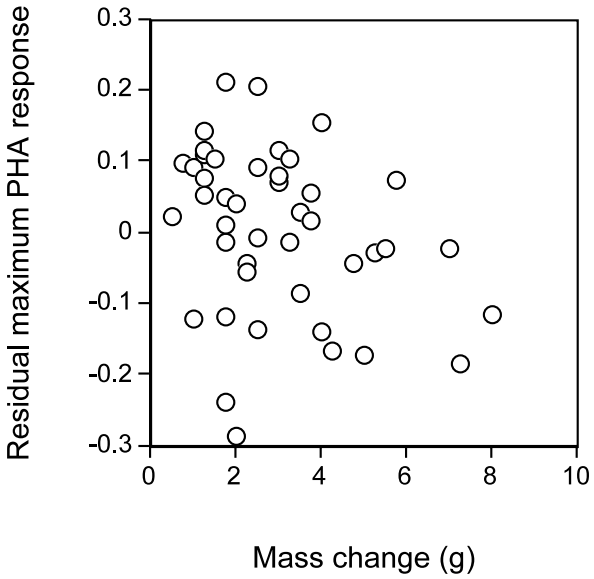

Fig. 5. Residual maximum phytohemagglutinin (PHA) response (mm) in 44 house sparrows in relation to maximum fluctuation in body mass (g). Residual response was calculated as residuals from a regression of maximum response on latency to maximum response.

Prevalence of *Haemoproteus* infection was 43.2% of 44 individuals, and mean intensity was 17.9 (SE = 5.1). There was no significant sex effect on infection ($F = 0.24$, df = 1,43, $P = 0.63$). House sparrows with strong PHA responses had lower intensity of infection ($F = 4.27$, df = 1,43, $r^2 = 0.092$, $P = 0.045$, slope (SE) = -0.064 (0.031)). House sparrows with high intensities also tended to have large diurnal fluctuations in body mass ($F = 4.04$, df = 1,43, $r^2 = 0.088$, $P = 0.049$, slope (SE) = -0.831 (0.413)).

Discussion

The temporal dynamics of the T-cell dependent response to injection of house sparrows with phytohemagglutinin (PHA) showed an initial significant increase during the first hours post-injection, but no significant increase after six hours (Fig. 1). This finding is similar to that reported for domestic chickens *Gallus gallus* by Goto et al. (1978). Values of PHA responses of individual house sparrows recorded at different intervals were significantly positively correlated with each other, in particular if responses after short intervals of three and six hours were eliminated. Thus, PHA responses of individuals remained relatively stable once individual differences in the initial increase had been accounted for. The temporal consistency of PHA response of individual house sparrows, as demonstrated by the highly significant effect of the repeated measure in the repeated-measures ANOVA (Table 1), implies that individuals tend to show a similar response at

different time intervals since injection. These temporal patterns of development of the PHA response have the practical implication that it is possible to capture birds in the field in the evening and measure the response in the morning at first light before release.

We found evidence of a diurnal pattern of PHA response, with responses at night being on average 12.6% stronger than responses during daytime (Fig. 2). Sleep is generally considered an activity that restores physiological function (McFarland 1981, Amlaner and Ball 1983). We suggest that this novel pattern of diurnal variation in cell mediated immune response may have a non-adaptive and at least two different adaptive explanations. The non-adaptive explanation is that birds at rest simply have more resources available for immune responses. However, this suggestion does not explain why resources are put into immune function rather than kept as storage, unless there was a need for a stronger immune response during night. Next we consider the two adaptive explanations. First, many ectoparasites are mainly nocturnal and negatively photo-tactic (Marshall 1981). Thus, extraction of blood by ectoparasites mainly takes place during the night when the host is resting or sleeping. Martin et al. (2001) have shown in a comparative study of avian hosts that the magnitude of the PHA response is strongly positively correlated with the impact of ectoparasites on mortality of host nestlings. This finding implies that stronger PHA responses have evolved in host species with greater parasite-induced mortality. A maximum level of immune response during night will provide the strongest protection against the diurnal peak of parasite activity. Second, homeothermic vertebrate hosts, but also heterothermic vertebrates, defend themselves against micro-parasite attacks by raising their body temperature when infected. Such fever tends to reduce the multiplication rate of the parasite and hence favour recovery by the host (Banet 1986, Blateis 1986, Kluger 1991). Homeothermic vertebrate hosts, but also heterothermic vertebrates, have low body temperatures during the night (e.g. Whittow 1986), which should benefit parasite attacks. A stronger immune response during the night would counteract such an temperature-dependent advantage for parasites.

Both a strong and a fast immune response would be beneficial for an efficient host defence against parasite attack. A strong response should be more efficient at combatting a given invading pathogen inoculum than a weak response. Empirical data show that host individuals with the strongest immune responses indeed have an advantage in terms of survival (Christe et al. 1998, 2001, Birkhead et al. 1999, González et al. 1999, Horak et al. 1999, Soler et al. 1999, Merino et al. 2000). Similarly, a fast response should be able to more efficiently control a pathogen in the initial stages of infection compared to a slow response. We hypothesise that there is a selective advantage from production of a fast immune response, although that remains to be tested. We found a positive relationship between strength of PHA response and latency to maximum response (Fig. 3). This novel relationship suggests a trade-off between maximum response and latency to maximum response. If such a trade-off exists, hosts cannot both develop a strong response and rapid response to parasite attack, and strength and latency will have to be optimised to limit damage caused by parasitism.

Immune responses are condition-dependent, as shown by numerous studies of humans and domesticated animals (Chandra and Newberne 1977, Glick et al. 1981, 1983, Willis and Baker 1981, Gershwin et al. 1985, Tsiagbe et al. 1987, Lochmiller et al. 1993), but also by studies of free-living animals (Saino et al. 1997, Møller et al. 1998, González et al. 1999). In the present study we only found a weak relationship between body condition, estimated as residual body mass, and PHA response. Fluctuation in body mass during the day was a much better predictor of maximum immune response than body condition estimated as residuals from a regression of body mass on the cube of tarsus length. This implies that individuals that vary dramatically in body mass during the diurnal cycle are unable to produce a strong response. Several studies of passerines in captivity have shown that sub-ordinate birds have larger fluctuations in body mass than dominant individuals (Ekman and Hake 1990).

Previous studies of house sparrows have demonstrated a negative relationship between PHA response and infection with *Haemoproteus* blood parasites (González et al. 1999). To the best of our knowledge, this is the only study of avian blood parasites showing a correlation between host immune response and infection status. Here we replicated this finding for the same population of house sparrows in a different year, suggesting that this finding is robust. Furthermore, we showed that individual house sparrows with large diurnal fluctuations in immune response also had intense infections with blood parasites. Two interpretations are possible: Large fluctuations in body mass predispose hosts to blood parasite infections. Alternatively, infection results in large diurnal fluctuations in body mass. We have no possibility of discriminating among these possibilities at the moment, but planned experimental vaccination against blood parasites may provide a means to resolve this problem.

In conclusion, we found consistent temporal changes in PHA response of individual house sparrows during the course of the response, but also between day and night. Such temporal differences in immune responses are likely to be evolved responses of an adaptive nature. The positive relationship between maximum PHA response and latency to response suggests that it is impossible to produce both a strong and a fast response. Since individual house sparrows that fluctuated the most in body mass during the diurnal cycle, associated

with blood parasite infections, also produced the weakest responses, we suggest that only individuals in prime condition are able to develop the strongest responses. We recommend that similar studies are made on other species to allow generalisations about the nature of temporal dynamics of immune responses.

Acknowledgements – Ignacio García and Fernando Mateos helped with the field work. The study was supported by grants from the Spanish Ministry of Science and Technology BOS2000-0293 and Junta de Extremadura IPR00A021 for FdL.

References

Abbas, A. K., Lichtman, A. H. and Pober, J. S. 1994. Cellular and molecular immunology. – Saunders.

Amlaner Jr, C. J. and Ball, N. J. 1983. A synthesis of sleep in wild birds. – Behaviour 87: 85–119.

Banet, M. 1986. Fever in mammals: is it beneficial? – Yale J. Biol. Med. 59: 117–124.

Birkhead, T. R., Fletcher, F. and Pellatt, E. J. 1999. Nestling diet, secondary sexual traits and fitness in the zebra finch. – Proc. R. Soc. Lond. B 266: 385–390.

Blateis, C. M. 1986. Fever. Is it beneficial? – Yale J. Biol. Med. 59: 107–116.

Brinkhof, M. W. G., Heeb, P., Kölliker, M. and Richner, H. 1999. Immunocompetence of nestling great tits in relation to rearing environment and parentage. – Proc. R. Soc. Lond. B 266: 2315–2322.

Chandra, R. K. and Newberne, P. M. 1977. Nutrition, immunity, and infection. – Plenum Press.

Christe, P., Møller, A. P. and de Lope, F. 1998. Immunocompetence and nestling survival in the house martin: "The tasty chick hypothesis". – Oikos 83: 175–179.

Christe, P., Møller, A. P., Saino, N. and de Lope, F. 2000. Genetic and environmental components of phenotypic variation in immune response and body size of a colonial bird, the house martin *Delichon urbica*. – Heredity 85: 75–83.

Christe, P., de Lope, F., González, G. et al. 2001. The influence of weather conditions and ectoparasites on immune responses, morphology and recapture probability of nestling house martins (*Delichon urbica*). – Oecologia 126: 333–338.

Cox, F. E. G. 1982. Modern Parasitology. – Blackwell.

Dietert, R. R., Golemboski, K. A., Kwak, H. et al. 1996. Environment-immunity interactions. – In: Davison, T. F., Morris, T. R. and Payne, L. N. (eds), Poultry immunology. Carfax, Abingdon, pp. 343–356.

Ekman, J. B. and Hake, M. K. 1990. Monitoring starvation risk: adjustments of body reserves in greenfinches (*Carduelis chloris* L.) during periods of unpredictable foraging success. – Behav. Ecol. 1: 62–67.

Folstad, I. and Karter, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. – Am. Nat. 139: 603–622.

Frank, S. A. 1991. Ecological and genetic models of host-pathogen coevolution. – Heredity 67: 73–83.

Gandon, S., Capowiez, Y., Dubois, Y. et al. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. – Proc. R. Soc. Lond. B 263: 1003–1009.

Gershwin, M. E., Beach, R. S. and Hurley, L. S. 1985. Nutrition and immunity. – Academic Press.

Glick, B., Day, E. J. and Thompson, D. 1981. Calorie-protein deficiencies and the immune response of the chicken. I. Humoral immunity. – Poultry Sci. 60: 2494–2500.

Glick, B., Taylor, R. L., Martin, D. E. et al. 1983. Calorie-protein deficiencies and the immune response of the chicken. I. Cell-mediated immunity. – Poultry Sci. 62: 1889–1893.

González, G., Sorci, G., Møller, A. P. et al. 1999. Immunocompetence and condition-dependent sexual advertisement in male house sparrows (*Passer domesticus*). – J. Anim. Ecol. 68: 1225–1234.

Goto, N., Kodama, H., Okada, K. and Fujimoto, Y. 1978. Suppression of phytohaemagglutinin skin response in thymectomized chickens. – Poultry Sci. 52: 246–250.

Hamilton, W. D. 1980. Sex versus non-sex versus parasite. – Oikos 35: 282–290.

Hamilton, W. D. and Zuk, M. 1982. Heritable true fitness and bright birds: A role for parasites? – Science 218: 384–387.

Hochberg, M. E., Michalakis, Y. and de Meeus, T. 1992. Parasitism as a constraint on the rate of life history evolution. – J. Evol. Biol. 5: 491–504.

Horak, P., Tegelmann, L., Ots, I. and Møller, A. P. 1999. Immune function and survival of great tit nestlings in relation to growth conditions. – Oecologia 121: 316–322.

Klein, J. 1990. Immunology. – Oxford University Press.

Kluger, M. 1991. The adaptive value of fever. – In: Mackowiak, P. (ed.), Fever: basic mechanisms and management. Raven Press, pp. 105–124.

Lochmiller, R. L., Vestey, M. R. and Boren, J. C. 1993. Relationship between protein nutritional status and immunocompetence in Northern Bobwhite chicks. – Auk 110: 503–510.

Marshall, A. G. 1981. The ecology of ectoparasitic insects. – Academic Press.

Martin, T. E., Møller, A. P., Merino, S. and Clobert, J. 2001. Does clutch size evolve in response to parasites and immunocompetence? – Proc. Natl. Acad. Sci USA 98: 2071–2076.

McCorkle Jr, F., Olah, I. and Glick, B. 1980. The morphology of the phytohemagglutinin-induced cell response in the chicken wattle. – Poultry Sci. 59: 616–623.

McFarland, D. (ed.). 1981. The Oxford companion to animal behaviour. – Oxford University Press.

Merino, S., Martínez, J., Møller, A. P. et al. 1999. Phytohaemagglutinin injection assay and physiological stress in nestling house martins. – Anim. Behav. 58: 219–222.

Merino, S., Møller, A. P. and de Lope, F. 2000. Seasonal changes in immunocompetence and mass gain in nestling barn swallows: A parasite-mediated effect? – Oikos 90: 327–332.

Møller, A. P. 1997. Parasites and the evolution of host life history. – In: Clayton, D. H. and Moore, J. (eds), Host-parasite evolution: general principles and avian models. Oxford University Press, pp. 105–127.

Møller, A. P. and Erritzøe, J. 1998. Host immune defence and migration in birds. – Evol. Ecol. 12: 945–953.

Møller, A. P., Christe, P., Erritzøe, J. and Mavarez, J. 1998. Condition, disease and immune defence. – Oikos 83: 301–306.

Møller, A. P., Christe, P. and Lux, E. 1999. Parasite-mediated sexual selection: effects of parasites and host immune function. – Q. Rev. Biol. 74: 3–20.

National Research Council. 1992. Biologic markers in immunotoxicology. – National Academy Press, Washington, D.C.

Noble, E. R. and Noble, G. A. 1976. Parasitology, Fourth edition. – Lea and Febige, Philadelphia.

Parmentier, H. K., Schrama, J. W., Meijer, F. and Nieuwland, M. G. B. 1993. Cutaneous hypersensitivity responses in chickens divergently selected for antibody responses to sheep red blood cells. – Poultry Sci. 72: 1679–1692.

Parmentier, H. K., de Vries Reilingh, G. and Nieuwland, M. G. B. 1998. Kinetic immunohistochemical characteristic of mitogen-induced cutaneous hypersensitivity responses in chickens divergently selected for antibody responsiveness. – Vet. Immunol. Immunopathol. 66: 367–376.

Roitt, I., Brostoff, J. and Male, D. 1996. Immunology. – Mosby, London.

Saino, N., Calza, S. and Møller, A. P. 1997. Immunocompetence of nestling barn swallows in relation to brood size and parental effort. – J. Anim. Ecol. 66: 827–836.
Saino, N., Calza, S. and Møller, A. P. 1998. Effects of a dipteran ectoparasite on immune response and growth trade-offs in barn swallow (*Hirundo rustica*) nestlings. – Oikos 81: 217–228.
Saino, N., Ninni, P., Calza, S. et al. 2000. Better red than dead: Carotenoid-based mouth coloration reveals infection in barn swallow nestlings. – Proc. R. Soc. Lond. B 267: 57–61.
Soler, M., Martín-Vivaldi, M., Marín, J. M. and Møller, A. P. 1999. Weight lifting and health status in the black wheatear. – Behav. Ecol. 10: 281–286.
Tsiagbe, V. K., Cook, M. E., Harper, A. E. and Sunde, M. L. 1987. Enhanced immune responses in broiler chicks fed methionine-supplemented diets. – Poultry Sci. 66: 1147–1154.
Wakelin, D. 1996. Immunology to parasites, Second edition. – Cambridge University Press.
Whittow, G. C. 1986. Regulation of body temperature. – In: Sturkie, P. D. (ed.), Avian physiology. Springer-Verlag, pp. 221–252.
Willis, G. M. and Baker, D. H. 1981. Interaction between dietary protein/amino acid level and parasitic infection: Morbidity in amino acid deficient or adequate chicks inoculated with *Eimeria acervulina*. – J. Nutr. 111: 1157–1163.