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Induced maternal response to the Lyme disease spirochaete *Borrelia burgdorferi sensu lato* in a colonial seabird, the kittiwake *Rissa tridactyla*

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Mothers are predicted to invest in their offspring depending on the quality of their mate, their opportunity to invest in future reproduction and the characteristics of the habitat in which their offspring will be born. Recent studies have suggested a transfer of maternal immunity to offspring as an induced response to the local presence of parasites in the environment, but evidence has been indirect. Here, we show the presence of antibodies against the Lyme disease agent *Borrelia burgdorferi sensu lato*, a spirochaete transmitted by the seabird tick *Ixodes uriae*, in the eggs of kittiwakes *Rissa tridactyla*. We report higher prevalence of antibodies against *Borrelia* in eggs from breeding areas with higher prevalence and abundance of ticks. Further, high repeatabilities of antibody-positive eggs within clutches and between first and replacement clutches show that, within a breeding season, females differ consistently with respect to the expression of this induced maternal response. Our results suggest that mothers can alter investment in their young depending on local conditions. Such maternal effects clearly have implications for the ecology and evolution of host–parasite interactions.

Keywords: eggs; host–parasite; immunity; maternal effect; epidemiology; spatial heterogeneity

1. INTRODUCTION

Individuals live in environments varying in space and time at different scales, and theory predicts that several mechanisms have evolved to cope with changing local conditions (May & Southwood 1990). In the presence of parasites, these mechanisms can take the form of induced immune defences (Sheldon & Verhulst 1996), behavioural responses (Barnard & Behnke 1990) or the modification of life history traits (Møller 1997). One response with important ecological and evolutionary implications is the capacity for a mother to transmit immunity to her offspring against specific parasites to which they may be exposed (Price 1998). This maternal investment is likely to be costly for females, due to the price of mounting an immune response (Sheldon & Verhulst 1996; Saino *et al.* 1997; Ilmonen *et al.* 2000; Lochmiller & Deerenberg 2000), but is expected to increase fitness of their offspring if they are exposed to the parasite. Recently, the transfer of antibodies to offspring through the egg has been suggested in different bird–ectoparasite systems (Boulinier *et al.* 1997; Heeb *et al.* 1998). In particular, it has been shown that parents exposed to parasitic fleas before egg laying produced young better able to cope with local parasite presence (Heeb *et al.* 1998). However, no evidence of the presence in the eggs of antibodies against the parasite was provided in this study. Other studies on domestic or captive-reared birds have shown that specific antibodies can be passed directly through eggs to offspring (Grazyk *et al.* 1994; Smith *et al.* 1994), but to our knowledge this transfer has never been shown to occur in a host–parasite system under natural conditions.

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As part of a study examining the role of host response in the dynamics of host–parasite interactions (Boulinier *et al.* 1997; Danchin *et al.* 1998), we investigated the presence of maternally transferred antibodies against a micro-parasite, *Borrelia burgdorferi sensu lato*, in a spatially structured host–ectoparasite system consisting of the seabird tick *Ixodes uriae* and the kittiwake *Rissa tridactyla*. The spirochaete *B. burgdorferi* is the causative agent of Lyme disease, the most common and medically important vector-borne disease in Europe and the USA (Barbour & Fish 1993). Different strains of this bacterium have been shown to circulate in distinct transmission cycles involving ixodid ticks and mammalian and avian hosts (Kurtenbach *et al.* 1998). At least three genospecies within the *B. burgdorferi s.l.* group are pathogenic to humans, *B. burgdorferi sensu stricto*, *B. afzelii* and *B. garinii*. Of these, *B. garinii* has been found in seabird colonies in both hemispheres, and seabirds are considered to be important reservoir hosts for the maintenance and spread of this disease agent (Olsen *et al.* 1993, 1995; Gylfe *et al.* 1999).

The transmission of *B. burgdorferi s.l.* among seabirds depends on the presence of its vector, the tick *Ixodes uriae*, in the host environment (Olsen *et al.* 1993). This generalist ectoparasite of seabirds has a three-stage life cycle (larvae, nymph, adult) usually corresponding to one stage per year. In each stage, *I. uriae* attaches to its host for a single, long, blood meal and then returns to the host nesting substrate to overwinter (Eveleigh & Threlfall 1974). It has previously been shown that tick prevalence and abundance are autocorrelated in both space and time at the scale of the host breeding cliff (Danchin *et al.* 1998; McCoy *et al.* 1999). As kittiwakes, like many seabirds, are highly philopatric to their breeding sites between years (Coulson & Thomas 1985), ticks become a predictable part of their breeding environment. Given this, we

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expected a positive correlation between the prevalence and abundance of ticks present in a breeding cliff and the proportion of clutches with antibodies to *Borrelia*. As kittiwakes lay a single clutch of one to three eggs per season and can lay a replacement clutch if the first one disappears, we also predicted a high repeatability of antibody-positive or negative eggs for a given female.

2. MATERIAL AND METHODS

Fieldwork was conducted on the island of Hornøya, northern Norway (70°22′ N, 31°10′ E) where approximately 21 000 pairs of kittiwake *Rissa tridactyla* breed. Eggs were collected in nests from 12 separate breeding cliffs divided among three different sectors of the island, as part of an experiment on habitat selection in this species. Egg yolks were isolated and directly frozen until the end of the season. Once thawed, yolks were homogenized and extraction procedures followed the protocol of Mohammed *et al.* (1986). The yolk was diluted 1:1 in phosphate-buffered saline (PBS) and mixed, then 2 ml of reagent-grade chloroform was added to 1 ml of the mixture. The yolk–chloroform mixture was then centrifuged at 18 000 rpm for 6 min, and the clear supernatant was used for the immunological assay tests.

The first test performed was a passive haemagglutination-inhibition technique produced for the serodiagnosis of Lyme disease in humans (LYMAG, Diagast, Loos, France). Tests were carried out directly according to manufacturer's instructions. This test was used by Gauthier-Clerc *et al.* (1999) for studying the seroprevalence of *Borrelia burgdorferi* in king penguin *Aptenodytes patagonicus*. The second test performed was a *Borrelia*-specific enzyme-linked immunosorbent assay (ELISA) test using an ELILYME-G/M kit (Diagast). Because this kit was manufactured for human use and was designed to recognize mammalian antibodies, we replaced the IgG antibody of the kit by a chicken IgG antibody. Tests were performed according to kit instructions. The optical density (OD) of the resulting solution was read at a wavelength of 492 nm. Egg extracts were considered positive for *Borrelia* when they were positive for the haemagglutination test and when their OD was greater than the OD value of the upper limit of the 95% confidence interval drawn from 17 eggs negative to the haemagglutination test.

Repeatabilities between eggs from within a single clutch and between first and replacement clutch eggs were assessed with the phi coefficient of association (r_ϕ; Siegel & Castellan 1988) and using Fisher's exact test. For analyses within single clutches, only two eggs were considered per clutch as most clutches contained two eggs (for the few clutches with three eggs, two eggs were randomly selected).

The level of tick infestation was determined for each breeding cliff the year prior to egg collection, as levels of infestation are determined using parasite loads on chicks (Boulinier *et al.* 1996; Danchin *et al.* 1998; McCoy *et al.* 1999). For some cliffs, chicks were also searched for ticks the year of egg collection, which permitted us to increase sample sizes by pooling data for the two years. Local levels of infestation of kittiwake cliffs by *I. uriae* are autocorrelated between successive years and counts of ticks on individual chicks are highly repeatable (Boulinier *et al.* 1997). Tick abundance of a given cliff was expressed as the average number of ticks per chick per nest. The prevalence of ticks was the proportion of nests with at least one chick with ticks (chicks within a nest are not independent regarding their infestation by ticks; Boulinier *et al.* 1996).

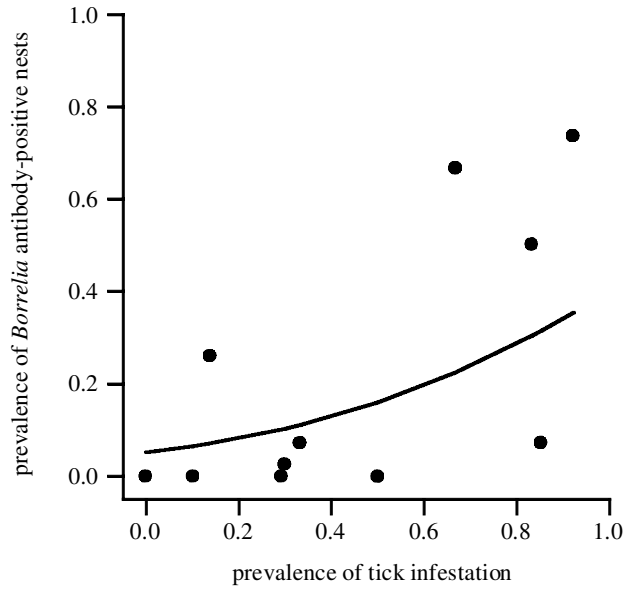

Figure 1. Prevalence of nests with *Borrelia* antibody-positive clutches and prevalence of tick infestation of kittiwake breeding cliffs. Symbols represent the proportion of antibody-positive or tick-infested nests (prevalence) within a given cliff. The performed logistic regression indicated that the prevalence of nests with antibody-positive eggs was significantly related to the prevalence of tick infestation ($\chi^2_1 = 15.87$, $p < 0.001$). The regression analysis was weighted for the number of examined clutches in each cliff. Note that two cliffs are represented at the tick prevalence of 0.1.

Logistic regressions (Proc GENMOD, binary response variable) were performed to test for a relationship between the proportion of *Borrelia* antibody-positive clutches and local tick prevalence or abundance. Statistical tests were carried out using SAS software (SAS Institute 1990).

3. RESULTS

Out of the 349 eggs from 177 nests examined for *B. burgdorferi* s.l.-specific antibodies, 14.9% were found to be positive by the two independent immunological assay tests. There was a high repeatability of antibody-positive or negative eggs both within a single clutch ($r_\phi = 0.77$, $p < 0.001$ for the Fisher's exact test) and between successive clutches ($r_\phi = 0.65$, $p < 0.002$ for the Fisher's exact test). Thus, mothers were consistent with respect to the expression of this induced response. Overall, the proportion of clutches with at least a single positive egg was not different between first (16.06%, $n = 22$) and replacement clutches (19.44%, $n = 14$; $\chi^2_1 = 0.315$, $p > 0.5$). Nevertheless, over the 22 nests with negative first clutches, 22.73% changed status and contained at least a single positive egg in the second clutch (five over 22). Conversely, all six nests with positive first clutches produced positive second clutches. Examined eggs came from 12 breeding cliffs spread across three sectors of the study area and the spatial distribution of nests with antibody-positive eggs was heterogeneous at these two scales (sector: $\chi^2_2 = 44.47$, $p < 0.001$; cliff: $\chi^2_{11} = 68.22$, $p < 0.001$), suggesting spatial heterogeneity of maternal effects.

The level of tick infestation was determined for each breeding cliff by searching chicks for ticks. Tick

infestation levels varied significantly among cliffs ($\chi^2_{11} = 72.83$, $p < 0.001$) and infestation was temporally autocorrelated between years ($r = 0.812$, $p = 0.0014$, $n = 12$). As predicted, there was a significant positive relationship between the prevalence of nests with *Borrelia* antibody-positive eggs and tick prevalence (figure 1). This relationship was also significant ($\chi^2_1 = 23.86$, $p < 0.001$) after accounting for an effect of sector ($\chi^2_2 = 74.30$, $p < 0.001$). A similar relationship was found between the prevalence of nests with anti-*Borrelia*-positive eggs and the local abundance of ticks ($\chi^2_1 = 20.57$, $p < 0.001$; individual sector effect: $\chi^2_2 = 39.87$, $p < 0.001$).

4. DISCUSSION

It has been shown experimentally that ectoparasites can have deleterious effects on local host reproductive success (Møller 1997). However, the cost of living in an environment with ectoparasites is greater than just the possible direct costs of coping with the parasites themselves (e.g. Wang & Nuttall 1994). The presence of ticks means the potential presence of vectored micro-organisms such as *Borrelia*. These pathogens can impose additional costs on mothers: the cost of dealing with potential disease and the increased reproductive cost of giving protection to offspring. In the present study, we show that such transfer of antibodies against a specific microparasite in the eggs exists in a natural host–parasite system and that its occurrence is related to the local exposure of individuals to the ectoparasitic vector. There have been several studies showing evidence of a trade-off between reproductive effort and immunocompetence (Lochmiller & Deerenberg 2000; Norris & Evans 2000). Here, the reproductive effort was increased for individuals that laid a replacement clutch (Nager *et al.* 1999), yet there was no evidence suggesting that the induced host response changed. However, further study is required to investigate the relationship between the quantity of specific antibodies invested in offspring and parental reproductive effort.

To transmit *Borrelia* antibodies to their eggs, mothers must have had an active infection prior to the egg-laying period. It is possible that birds may have been exposed to infected ectoparasites in the weeks before egg laying after returning to land for the breeding season. However, presumably low tick activity due to seasonal conditions makes it difficult to understand how this might occur. Recently it was shown that dormant *Borrelia* infections could be reactivated in birds after periods of high stress, such as after migration (Gylfe *et al.* 2000). Thus, to transfer protective antibodies into their eggs, it is possible that birds retain infections and/or maintain high antibody levels over long periods of time, or have infections reactivated after periods of energy stress. In the seabird–*Borrelia* system, the dynamics of infection and antibody production need to be followed over time in order to understand potential transmission patterns between the three species involved and the potential cost of these infections. Likewise, it remains to be tested whether chicks from antibody-positive eggs have higher fitness (i.e. whether this maternal-induced defence is adaptive; Rossiter 1996) or whether the transfer of specific antibodies is a side effect of the avian immune system. Protection against *Borrelia* infection by antibodies of *Borrelia duttonii*, the causative agent of human relapsing fever, and against *Borrelia burgdorferi* has been experimentally shown in rodents (Morshed *et al.* 1993; Fikrig *et al.* 1990).

In kittiwakes, high tick infestation levels in breeding cliffs have been suggested to be responsible for increased dispersal of breeding adults (Boulinier *et al.* 2001). This suggests that there are two possible strategies: individuals may stay and cope with parasites or disperse to parasite-free locations. The choice birds make may depend on differential costs of dispersal, individual experience or specific life-history traits (e.g. prior exposure, ability to tolerate). As the presence of ticks is spatially heterogeneous and tick dispersal at inter-colony scales is host-mediated, host movements will alter tick dispersal patterns in the environment (McCoy *et al.* 1999). Thus, the spatial heterogeneity of the different species involved in this system and the frequency of seabird dispersal will have important consequences for the spread of *Borrelia* at different scales and, in turn, will affect the predictability of potential human exposure to Lyme disease (Van Buskirk & Ostfeld 1998).

At present, little consideration has been given to the effect of induced maternal protection in the epidemiology of tick-borne diseases in natural populations, but also more generally in the ecology and evolution of host–parasite interactions (Grenfell & Dobson 1995). Clearly, the ability of parents to afford immunological protection to their offspring can have profound potential implications in these fields. More experimental work, incorporating longitudinal surveys of individual immuno-histories in a spatially structured context, is required if we are to understand the importance of pathogens such as *Borrelia* to host populations, and their significance and consequences for the evolution of life-history traits. Similarly, if we are to better predict the potential spread of emerging infectious diseases such as Lyme disease, it may be important to incorporate the spatial dynamics of immunity of individuals of the different species involved into epidemiological models (Grenfell & Harwood 1997).

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