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Invited reply

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1. INTRODUCTION

Flint & Franson (2009) comment on our recent paper about influenza A virus in wild mallards (*Anas platyrhynchos*; Latorre-Margalef *et al.* 2009). They acknowledge the quality of our data and analyses, but think that our interpretation of the results is incomplete. One can break down their comments into two parts: (i) the issue of assumed causality in our *Discussion* and (ii) the alternative hypothesis suggested by Flint and Franson, according to which body condition and immune status affect the likelihood of acquiring influenza A infection (rather than the latter affecting the former).

2. CAUSALITY

It is important to stress that the data in our study come from a large and long-term surveillance scheme carried out at Ottenby Bird Observatory in southeast Sweden since 2002. For our paper, we analysed approximately 11 000 mallard cloacal samples for the presence of influenza A virus in order to determine how long individual ducks excrete virus particles, and to relate infection status to body mass (corrected for individual structural size, i.e. a measure of body condition) and duration of staging on autumn migration. The sampling protocol was designed primarily to investigate natural variation in influenza A virus prevalence, and to collect virus isolates for phylogenetic and molecular studies (Munster *et al.* 2005, 2007; Olsen *et al.* 2006; Wallensten *et al.* 2007). As such, it is not an experimental set-up and we have not been able to vary the factors studied. For instance, we rely on natural infections and have neither experimentally infected any birds nor vaccinated or suppressed immunity in any of the birds. We agree with Flint and Franson that statistical analyses of such a dataset cannot establish causality, but may generate new ideas and hypotheses for further testing. Much of Flint and Franson's comment compares wordings between the *Result* and the *Discussion* sections of our paper in order to drive the point that we assume causality between influenza infection and body mass status and staging time. Their quotes are correct but, when taken out of context, do not convey the full message and moreover give a simplified interpretation. For example, in their criticism, Flint and Franson fail to appreciate our conclusion that 'this is a host-virus system where more is known about 'the two players', respectively, than about their interactions. Discerning such interactive patterns and pinning down their causality remain top priorities for avian ecology as well as for zoonotic research' (Latorre-Margalef *et al.* 2009). Among candidate hypotheses to be tested in future experiments, we believe that the one highlighted by us (i.e. that influenza infection incurs a cost in mallards) has solid epidemiological and theoretical foundations, which we discuss below.

The accompanying comment can be viewed at http://dx.doi.org/doi:10.1098/rspb.2008.1962.

3. ALTERNATIVE HYPOTHESES

Flint and Franson hypothesize that the pattern of lower relative body mass in influenza-infected mallards is not a direct effect of infection *per se*, but rather owing to birds in worse condition being more susceptible to acquiring infection. Flint and Franson argue that this 'condition-dependent' hypothesis can also explain increased staging time in infected juvenile ducks as well as the correlation between *Ct*-value (a relative index of virus shedding) and body mass. We agree that an experimental approach is needed to conclusively discriminate between these two hypotheses, among others. This is also the way forward proposed by us, as quoted above. Nevertheless, we argue that Flint and Franson overlook current information about influenza A virus infections in birds to assess the relative merits of these two hypotheses. According to the condition-dependent hypothesis, birds with lowered condition (for any reason) are likely to have a reduced immunocompetence. This, in turn, makes them more prone to acquire influenza infection, and also affects the severity of disease manifestations. It may seem intuitive that a bird in poor condition is more susceptible to disease than one in better condition and this is indeed the case for some pathogens mostly studied for latent infections. However, the shape of this relationship is likely to vary among pathogens and according to whether the disease is acute or chronic, as well as being dependent on the genetic background of the individual host (Westerdahl *et al.* 2005).

If we hypothesize that mallard condition, as measured by body mass corrected for individual structural size, is related to the risk of acquiring an infection, one could assume that the epidemiology is characterized by disease outbreaks when and where mallards face adverse conditions. Strenuous migratory flight would be one such factor that affects condition negatively, but so could also physiological stress associated with breeding, periodic food shortages, low temperatures, starvation, concomitant disease or trauma. Most mallards at Ottenby are close to their wintering quarters, and some even winter locally (Fransson & Pettersson 2001; Latorre-Margalef *et al.* 2009); hence reserves needed to finish migration are

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expected to be lower than that in mallards preparing for longer remaining flights. Importantly, the prevalence of influenza A virus in our study population varies in a predictable manner, within as well as between years (Wallensten *et al.* 2007; Latorre-Margalef *et al.* 2009). Typically, prevalence is low during summer and increases to approximately 10–20% infected birds during September–November and then drops during December (Wallensten *et al.* 2007; Latorre-Margalef *et al.* 2009). A small, less consistent peak (4–10%) can also be seen during spring migration at Ottenby (Wallensten *et al.* 2007). Although juvenile birds make up most of our sample, adult mallards show similar, but less pronounced, prevalence patterns, with approximately 12 per cent prevalence in autumn and approximately 2 per cent in spring (Wallensten *et al.* 2007). Similar temporal patterns in autumn are found in other European and North American studies on ducks (Krauss *et al.* 2004; Olsen *et al.* 2006; Munster *et al.* 2007), with the consistently highest prevalence at northern latitudes, and a decrease in prevalence during late autumn and winter as birds move south to their winter quarters. The ultimate explanation needs to be verified by experimental studies, but such predictable patterns over large parts of the Holarctic, with no indication of eruptive outbreaks at other times of the year, are in our view less compatible with the condition-dependent hypothesis.

A major finding in our study is the short shedding time of infected ducks, where individuals normally cleared the infection within one week (Latorre-Margalef *et al.* 2009). Thus, for wild mallards, influenza is typically an acute, non-persistent infection. This fact, together with consistent high prevalence over the course of the autumn in the study population, indicates that over time, a large proportion of the birds will have had an influenza infection. This is also reflected in the serological evidence of infection, where the seroprevalence in mallards at Ottenby approaches 66 per cent in December, and where some re-sampled ducks seroconvert during their stay. This pattern is not restricted to Sweden, as similar values on seroprevalence are available also from wintering mallards in Italy (De Marco *et al.* 2003). We argue that high seroprevalence in late autumn and winter indicates that a major part of the population gets infected during the same autumn season, irrespective of initial body condition.

Interestingly, in duck infection models, low-pathogenic influenza A virus seems to produce only a transient, low-level humoral immune response (Olsen *et al.* 2006). The high seroprevalence in wild mallards might therefore be the result of repeated exposure to the virus. These facts, too, do not agree well with the hypothesis that the influenza virus primarily infects immune-suppressed individuals. Additional support come from experimental infections, which show that healthy ducks are permissive to influenza A virus regardless of the strain used (references in Latorre-Margalef *et al.* 2009).

4. CONCLUSIONS

Our recent paper (Latorre-Margalef *et al.* 2009) has provided a good starting point for discussions and further research on the ecology of influenza A virus in natural settings. Our study is one of the very few long-term surveillance schemes of a viral disease in its main natural host. We demonstrated that infected mallards were leaner than uninfected birds of the same structural size and that body mass in infected ducks was related to the amount of viruses shed by these ducks. The condition-dependent hypothesis of Flint and Franson may be correct, but, as discussed above, several established features of influenza A virus epidemiology indicate that the probability of acquiring infection is unlikely to be dependent mainly on the condition of the individual duck. The severity of infection, on the other hand, is more likely to be influenced by body condition. Which of these hypotheses, or others, is or are most valid will ultimately be determined by further research, descriptive and experimental. For the future, it is also worth considering the combined effects of influenza A virus and other pathogens. For instance, mallards at Ottenby in autumn are relatively often colonized with *Campylobacter* spp. (Waldenström *et al.* 2002), but seem not to be carriers of *Salmonella* (Hernandez *et al.* 2003). Ideally, an experimental approach should involve infecting free-living mallards with influenza A virus and comparing performance with that of uninfected birds. However, introducing disease in wild bird populations involves both ethical and epidemiological concerns, so it is more likely that a combination of laboratory infection models and experimental manipulation of factors related to immunocompetence in the field is the best way forward. Such studies might also provide general insights applicable in other host–pathogen interactions.

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