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Maternal Transfer of Strain-Specific Immunity in an Invertebrate

Tom J. Little,* Benjamin O'Connor, Nick Colegrave, Kathryn Watt, and Andrew F. Read
Institute for Cell, Animal, and Population Biology
University of Edinburgh
Kings Buildings
West Mains Road
Edinburgh EH9 3JT
United Kingdom

Summary

The most celebrated component of the vertebrate immune system is the acquired response in which memory cells established during primary infection enhance the proliferation of antibodies during secondary infection. Additionally, the strength of vertebrate acquired immune responses varies dramatically depending on the infecting pathogen species or on the pathogen genotype within species [1, 2]. Because invertebrates lack the T-cell receptors and Major Histocompatibility Complex (MHC) molecules that mediate vertebrate adaptive immune responses, they are thought to lack adaptive immunity and be relatively unspecific in their interactions with pathogens [3–5]. With only innate immunity, invertebrate hosts are believed to be naïve at each new encounter with pathogens [1, 6]. Nevertheless, some forms of facultative immunity appear to be important in insects; some individuals have enhanced immunity due to population density [7], and some social insects benefit when their nest-mates have been exposed to a pathogen or pathogen mimic ([8, 9]; see [10] for a predation example.) Here we provide evidence for acquired strain-specific immunity in the crustacean *Daphnia magna* infected with the pathogenic bacteria *Pasteuria ramosa*. Specifically, the fitness of hosts was enhanced when challenged with a bacterial strain their mother had experienced relative to cases when mother and offspring were challenged with different strains.

Results and Discussion

Our experiment tested for the phenomenon of acquired immunity in an invertebrate. *Daphnia magna* females (the host) were exposed to strains of *P. ramosa* (these *Daphnia* received the "prior strain"), and their offspring were challenged with either the same or a different strain (offspring received the "challenge strain") (Figure 1). For homologous combinations (i.e., when the "prior" and "challenge" strain were the same), overall infectivity (the proportion of hosts that became infected) was lower as compared to that in heterologous challenges (logistic regression, challenge strain by prior strain interaction $\chi^2 = 4.81$, df = 1, p = 0.028). The principal effect of *P. ramosa* on hosts is fecundity reduction [11], and we therefore analyzed reproduction to assess whether the differences in infectivity represented fecundity differences. The number of newborns from homologous challenges was 21% (challenge strain G) or 6% (challenge strain A) higher than from heterologous challenges over the course of the experiment (Figure 2A). This effect was stronger when we considered only the first three clutches, attained on average about mid-way through the experiment, with 27% (challenge strain G) and 10% (challenge strain A) more newborns in homologous challenges. We further tested whether hosts from homologous challenges had higher fecundity due to earlier reproduction by examining the timing of each the six clutches recorded during the experiment. For the first two clutches, hosts from homologous infections had earlier reproduction than hosts from heterologous ones (Figures 2B and 2C). Later clutches showed no such interaction.

These strain-specific maternal effects on *Daphnia* immunity could generate a large fitness advantage. We combined the fecundity measures above (the number of offspring and the timing of clutches) into a single parameter, the number of offspring produced per day (Figure 3), which permitted the calculation of the intrinsic rate of population increase, *r*. During challenge with strain A, the intrinsic rate of population increase was 0.247 if the host's mother also received strain A (homologous challenge) and 0.227 if the mother had received strain G (heterologous challenge). During challenge with strain G, *r* for the homologous challenge was 0.206, but it was only 0.180 for the heterologous scenario. If one takes these performance differences to represent the benefit of transferring immunity to offspring, hosts possessing this maternal effect would see their population size double that of hosts lacking such flexibility in about 2–3 generations (6–8 weeks at 20°C).

Our observation of a strain-specific maternal effect on host resistance is unique for an invertebrate. We note that the effect we observed is similar in magnitude to, for instance, the strain-specific component of the secondary responses of rodents exposed to malaria [2], although the mechanisms underlying each are certainly different. Vertebrates may confer immunity to offspring by transferring antibodies, and it is plausible that invertebrate mothers exposed to pathogens might imbue their eggs with their immune system peptides. It is less clear how such maternal transfer could be accomplished in a manner directed toward specific pathogen genotypes. Indeed, the basis of pathogen specificity in nonacquired invertebrate immunity is poorly understood. In *Drosophila*, which has the best-studied arthropod immune system, the production of antimicrobial molecules is stimulated by peptides that distinguish between, for example, gram-negative and gram-positive bacteria by recognizing different pathogen cell surface signatures (e.g., peptidoglycans) [12, 13]. The level of specificity understood

*Correspondence: tom.little@ed.ac.uk

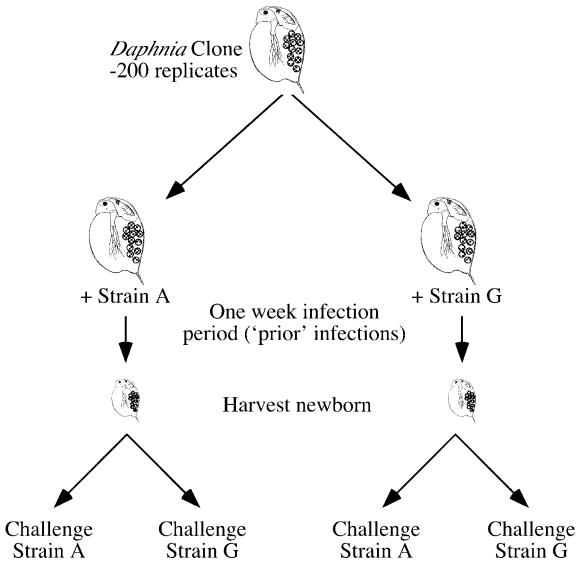

Figure 1. Experimental Design to Test for Strain-Specific Immunity as a Maternal Effect

Daphnia reproduce clonally, and thus all replicates are genetically identical.

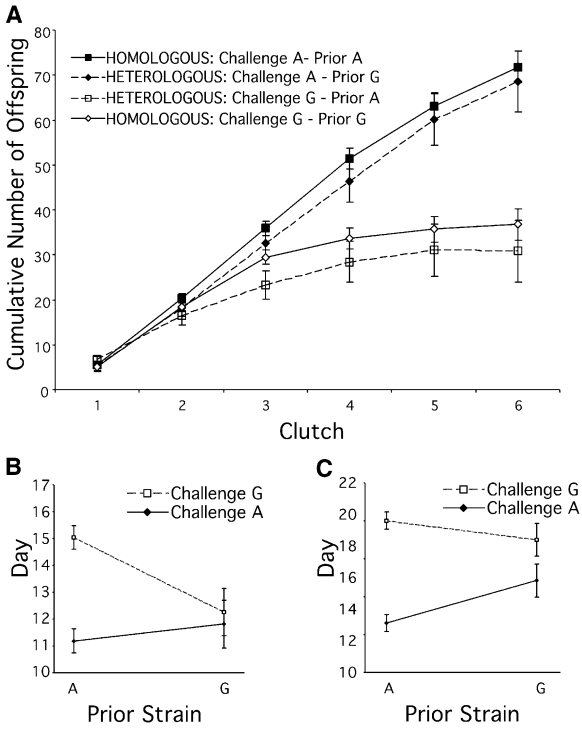

Figure 2. Fecundity Measures for *D. magna* Indicated that Hosts from Homologous Challenges Had Higher Reproductive Output than Hosts from Heterologous Challenges

(A) The number of offspring over six clutches revealed a significant interaction among the challenge strain, prior strain, and clutch size (repeated-measures ANOVA for three-way interaction: $F_{5, 368} = 2.6$ $p = 0.026$), and inspection of the data shows that, for five of six clutches, hosts from homologous challenges had more offspring than hosts from heterologous ones. Graph (A) shows the cumulative number of offspring, although statistical analysis was performed on the number of offspring produced at each clutch.

(B) The age (in days) at which females had their first clutch was earlier for homologous challenges: Poisson regression, challenge strain*prior strain interaction ($\chi^2 = 6.43$, df = 1, $p = 0.011$).

(C) The age at which females had their second clutch was earlier for homologous challenges: Poisson regression, challenge strain*prior strain interaction ($\chi^2 = 7.12$, df = 1, $p = 0.008$). A test on clutches four to six revealed no significant effects.

from these studies of immunological mechanisms is, however, extremely broad as compared to that detected in the present study or other whole-organism studies of arthropods [14–16]. Still, the potential diversity of pathogen cell surface signatures is immense, and it will be interesting to determine the extent to which receptors of arthropod immune systems match this diversity.

It will also be important to consider the impact of facultative immunity on the maintenance of genetic polymorphism for virulence or the extent to which pathogens can drive arms races. In this regard, it may be that clonal organisms such as *Daphnia* are more likely to show maternally effected plasticity of immune responses than purely sexual taxa. One argument as to why invertebrates ought to lack acquired immunity is their short lifespan; most invertebrates will have lived and died before a secondary exposure occurs [17]. In clonal organisms, however, when mothers experience the primary exposure but offspring experience the secondary exposure, the pathogen will have faced the identical genetic environment at both times. By contrast, sexual taxa, through recombination, constantly produce alternative genetic environments to which pathogens must newly adapt. Recombination is therefore thought to provide a sort of flexibility that hosts require to thwart virulence [18]. A maternally transferred, phenotypically plastic effect would allow clonal hosts to gain pliancy lost by forgoing recombination.

Experimental Procedures

Replicate hosts of a single clone were exposed to strains of *P. ramosa* (these hosts received the "prior strain"), and their offspring were challenged with either the same or a different strain (offspring received the "challenge strain") (Figure 1). The host clone was grown for three generations in a 10 liter tank under constant food conditions (we feed *Daphnia* on chemostat-grown cultures of the green algae *Scenedesmus sp.*) and with the water changed every other day. On the first day of the experiment, all *Daphnia* that had been born within the previous 18 hr (i.e., we began the experiment 18 hr after a water change in which only adults were retained) were collected. Female newborns were then randomly distributed to the treatment jars, each of which contained 100 ml water and a tablespoon of fine sand (sand mimics sediment in pond bottoms). There was one newborn per glass treatment jar and initially 100 replicates per treatment. The treatments were *P. ramosa* strain A (3×10^3 spores added per *Daphnia*) and strain G (3×10^6 spores added per *Daphnia*). These were the "prior" treatments. The different spore doses reflect the different sensitivities the host clone has to these two strains. After one week (a period known to be adequate for infections to establish [19, 20]) of exposure to *P. ramosa*, *Daphnia* were removed from the spore-containing jars, placed in fresh media, and monitored for newborns. We took two newborns from each female and exposed

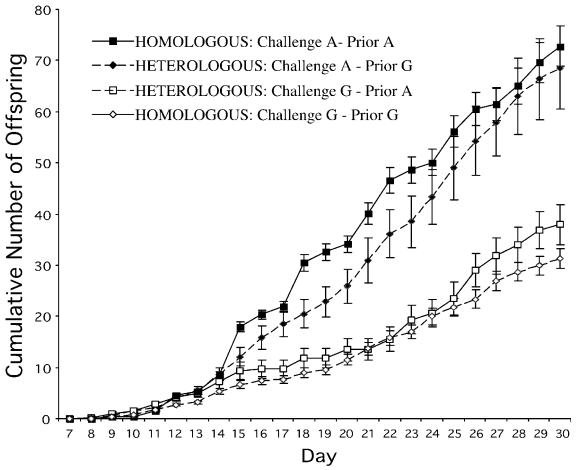

Figure 3. Fecundity Measures for *D. magna* Indicated that Hosts from Homologous Challenges Had Higher Reproductive Output than Hosts from Heterologous Challenges

Shown here is offspring born per day. This relationship incorporates both the number of offspring and the timing of clutches (see Figure 2) and permitted the calculation of the intrinsic rate of increase for a hypothetical population of each treatment (see results in text).

one to strain A and the other to strain G (these were the "challenge" treatments) under the same conditions as above. Note that spores are not released naturally from hosts until host death, and therefore the only exposure to parasites was from our administration of spores. Food levels were 2×10^6 algal cells per *Daphnia* per day during the infection period, and double this amount at all other times. The lower food during the infection period encourages browsing on the sand and thus increases uptake of pathogen spores.

We assayed the "challenge" hosts for 30 days and recorded the proportion that had became infected by day 30 (called infectivity in this study), the timing of clutches, and the number of newborns produced. Statistical analyses were performed with SAS [21]. We tested the main effects of the "prior strain," the "challenge strain," and the interaction between them. Note that for most tests it is the interaction that is of primary interest, i.e., it was important to demonstrate that the effect of a particular strain administered presently (challenge strain) was dependent on the strain administered previously (prior strain). For infectivity, because the response variable is binary (an individual host either becomes infected or not), binary logistic regression (SAS procedure GENMOD, dist = BIN) was performed. For the timing of clutches, each clutch was analyzed separately, and the response variable was multinomial. For example, most hosts had their first clutch on day 7, 8, 9, 10 11, or 12, with a small number of hosts having their first clutch on later days. Thus, the timing of each clutch was tested with Poisson regression (SAS procedure GENMOD, dist = POISS). The number of newborns produced was compared among treatments with a repeated measures analysis of variance to incorporate the fact that offspring were produced through six consecutive clutches (SAS procedure GLM).

To estimate the magnitude of fitness advantages due to acquired immunity, we calculated the number of offspring produced per day and then estimated the intrinsic rate of population increase (r) [22, 23] for each treatment. We took generation time to be the average age of a mother and used this to obtain an initial approximation of r. This approximation was used as a starting point for determining an exact solution with the Euler equation [23].

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