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A direct physiological trade-off between personal and social immunity

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Summary

1. Recent work shows that organisms possess two strategies of immune response: personal immunity, which defends an individual, and social immunity, which protects other individuals, such as kin. However, it is unclear how individuals divide their limited resources between protecting themselves and protecting others.

2. Here, with experiments on female burying beetles, we challenged the personal immune system and measured subsequent investment in social immunity (antibacterial activity of the anal exudates).

3. Our results show that increased investment in one aspect of personal immunity (wound repair) causes a temporary decrease in one aspect of the social immune response.

4. Our experiments further show that by balancing investment in personal and social immunity in this way during one breeding attempt, females are able to defend their subsequent lifetime reproductive success.

5. We discuss the nature of the physiological trade-off between personal and social immunity in species that differ in the degree of eusociality and coloniality, and suggest that it may also vary within species in relation to age and partner contributions to social immunity.

Key-words: antibacterial, ecological immunology, insect, lysozyme, *Nicrophorus*, wounding

Introduction

Parasites are ubiquitous and can threaten the survival prospects of their hosts, dramatically impacting upon their fitness (Thomas, Guegan & Renaud 2009). In response to this threat, organisms have developed highly effective immune responses that recognize invaders and act to eliminate them. Organisms can defend their fitness from attack by parasites and pathogens with two different strategies of immune response: personal and social (Cremer, Armitage & Schmid-Hempel 2007; Cotter & Kilner 2010a). The well-characterized personal immune system comprises an innate cell-based and humoral response that can phagocytose parasites or inhibit their growth. In addition, vertebrates have an acquired response with immunological memory (Janeway *et al.* 2001). This personal immune response is typically deployed internally (but see Martin-Vivaldi *et al.* 2010) and mainly serves to defend an individual's survival, whereas the more recently identified social immune responses are typically deployed externally and have evolved mainly to protect the fitness of others. Social immune responses (*sensu* Cotter & Kilner 2010a) range from antibodies provided in mammalian milk that protect newborn offspring, to antimicrobial metapleural gland secretions in ants that can provide benefits to the whole colony (Cotter & Kilner 2010a).

Mounting an immune response can be costly in terms of energetic expenditure (e.g. Long 1977; Ots *et al.* 2001; Freitak *et al.* 2003) or in terms of the availability of specific nutrients such as protein (e.g. Beisel 1977; Povey *et al.* 2009; Cotter *et al.* 2011). How, then, do individuals allocate their limited resources between personal and social immunity? Is there a direct trade-off between the two strategies of immune response, as has been suggested for honeybees, who appear to possess fewer genes for personal immunity than non-social insects, but bear many genes for colony-level immune function (Evans *et al.* 2006; Wilson-Rich *et al.* 2009)? Or, in the face of an immune challenge, are both personal and social immune responses maintained at a cost to current or future reproduction?

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To answer these questions, we focused on the sub-social burying beetle *Nicrophorus vespilloides*, which rears its offspring on carrion (Pukowski 1933). Biparental care is typical; both parents care for the developing larvae, protecting them from predators and competitors and regurgitating pre-digested meat for them (Pukowski 1933), but in some cases more than two adults may breed on a single carcass (Muller *et al.* 2007). Burying beetles fight for access to carcasses and so are particularly prone to wounding (Trumbo & Wilson 1993; Steiger *et al.* 2012). An open wound is susceptible to infection and so rapid wound healing is an early line of defence for the immune system. In insects, this involves cell migration and adhesion (Fauvarque & Williams 2011), rapid clotting, which involves the immune enzyme phenoloxidase (Bidla *et al.* 2005; Haine, Rolff & Siva-Jothy 2007) and localized up-regulation of phenoloxidases and lysozymes which show antimicrobial activity (Haine, Rolff & Siva-Jothy 2007). Sterile wounding stimulates these responses, but they are enhanced by the presence of PAMPs (pathogen-associated molecular patterns) (Haine, Rolff & Siva-Jothy 2007). Any pathogens that manage to invade the body further stimulate antimicrobial peptide (AMP) production, phagocytosis and nodulation of microparasites and encapsulation of macroparasites (Hoffmann & Hetru 1992 and references therein).

Nicrophorus vespilloides show the typical insect haemolymph personal immune responses of constitutive phenoloxidase activity (S. Cotter unpublished data), an enzyme involved in wound healing and the melanization of encapsulated invaders, and AMPs produced after bacterial challenge (Cotter, Ward & Kilner 2011; Vogel, Badapanda & Vilcinskas 2011). However, during reproduction, adults also invest heavily in lysozyme-like antibacterial activity in their anal exudates, which are smeared on the carcass to protect it from microbes (Cotter & Kilner 2010b). This is a social immune response because it benefits others, namely the burying beetle larvae, whose growth rate and survival prospects are severely reduced if reared on a carcass that has been heavily compromised by microbial infestation (Rozen, Engelmoer & Smiseth 2008). This social immune response is phenotypically plastic, it is produced only during reproduction (Cotter & Kilner 2010b), it is tailored to the perceived state of the carcass and is costly to up-regulate (Cotter *et al.* 2010). It is likely, therefore, to compete for limiting resources with the internally deployed personal immune responses.

We challenged the personal immune system of female burying beetles during reproduction and measured their subsequent investment in the social immune response. We also quantified the lifetime reproductive success (LRS) of our challenged individuals to assess whether females choose to maintain investment in both forms of immunity at a cost to future reproduction, or whether the costs of a personal immune response are paid for by a reduction in the social immune response.

Materials and methods

NICROPHORUS VESPILLOIDES COLONY

The burying beetle colony was established in 2005 and a pedigreed, outbred population maintained as described previously (Cotter & Kilner 2010b). Briefly, adult beetles were maintained in individual containers and fed twice weekly on minced beef. For breeding, a female was placed with a non-sibling male in breeding chambers comprising clear plastic boxes measuring $17 \times 12 \times 6$ cm containing a 2 cm depth of moistened compost. A freshly defrosted mouse carcass was weighed and placed in each breeding chamber. The breeding chambers were stored in a dark cupboard to simulate underground conditions. Carcass preparation, mating and egg laying occur during the first 3 days, eggs hatch in ~3 days and larvae disperse from the carcass after ~5 days of feeding, making the time from egg laying to dispersal ~8 days at 21 °C. After breeding, adults were transferred back to individual containers and dispersed larvae were placed in 25-cell petri dishes, covered with moist soil and left to pupate. Field-caught beetles were bred into the population every summer to maintain the genetic variability in the population.

EXPERIMENT 1: CHARACTERIZING THE ANTIBACTERIAL ACTIVITY OF EXUDATES THROUGHOUT A BREEDING BOUT

We have previously shown that the antibacterial activity of beetle exudates is phenotypically plastic and is only switched on when beetles are presented with a carcass (Cotter & Kilner 2010b). Here, we wanted to assess in more detail how activity levels change across the 8 days of the breeding bout in unmanipulated females. To do this, 80 young virgin females (mean age in days (SE) = 25·26 (1·45)) were paired as described above. After 2 days, the male was removed to ensure that the female was mated, but that his presence did not interfere with her investment in exudate antibacterial activity later in the breeding cycle (as has been shown previously (Cotter & Kilner 2010b)).

Of the 80 pairs set up, 54 bred successfully. Anal exudate was collected from these females on days 0, 2 (male removed), 4, 6 and 8 of the breeding bout using capillary tubes, blown into eppendorf 1·5 mL reaction tubes and stored at −20 °C until they could be subjected to further testing (Cotter & Kilner 2010b). Burying beetles readily produce an anal exudate when handled, and gentle tapping of the abdomen is generally sufficient to encourage beetles to produce enough exudate for collection and analysis. However, in some cases a beetle cannot be coerced to produce an exudate sample. Therefore, we did not successfully collect exudate from every female at every sampling point resulting in 195 samples in total of a possible 270. All females produced at least one exudate sample, with the median number of samples produced per beetle being three.

EXPERIMENT 2: TESTING FOR A TRADE-OFF BETWEEN PERSONAL AND SOCIAL IMMUNITY

Young virgin females (mean age (SE) = 25·89 (±0·35) days) were randomly assigned to one of the three experimental groups: bacteria-challenged ($n = 30$), sterile-challenged ($n = 31$)

and controls (unchallenged; $n = 34$). The treatments were designed to challenge two components of the personal defence system independently: wound healing in response to piercing of the cuticle (sterile-challenged and bacteria-challenged treatments) and increased antibacterial activity in the haemolymph caused by injection of bacteria (bacteria-challenged treatment only). Beetles in the challenged treatment groups were pierced with a needle either dipped in ethanol that had been allowed to air dry (sterile-challenged) or a solution of *Micrococcus lysodeikticus* (bacteria-challenged), which was made up of 50 mg of lyophilized cells (Sigma) in 100 ul of sterile water. Piercing was carried out in such a way as to minimize haemolymph loss, just the tip of the needle pierced the cuticle, and in the majority of cases there was no bleeding at all. *M. lysodeikticus* is a common soil bacterium that beetles should encounter in their natural environment. It is not pathogenic to the burying beetles, but causes up-regulation of the antibacterial response in the haemolymph (mean diameter of zone of bacterial inhibition on test plates in mm: sterile-challenged = 6·84 ± 0·86, bacteria-challenged = 10·00 ± 0·68; $F_{1,28} = 8{\cdot}15$, $P = 0{\cdot}008$; (Cotter, Ward & Kilner 2011)). Haemolymph collected from unchallenged beetles has no measurable antibacterial activity (mean diameter of clear zone in mm = 0.)

Directly after the immune challenge treatment, each female was paired with a non-sibling male (day 0) as described above. The males were removed before exudate collection on day 2 so that we could focus on the female response without any confounding effects of partner compensation (Cotter & Kilner 2010b). At this point, all beetles had prepared the carcass ready for the arrival of larvae.

Anal exudate was collected from the females on days 2, 4 and 6 of the breeding bout (we chose these sampling points after analysing the data from experiment 1 and finding that this was when social immune activity peaked). Samples were collected with capillary tubes, blown into eppendorf containers and stored at −20 °C until they could be subjected to further testing. A random subset of the beetles was then repeatedly bred without further immune challenge, using the same protocol as above, until death, with a new virgin male for each breeding attempt (bacteria-challenged ($n = 16$), sterile-challenged ($n = 16$) and controls ($n = 17$)). Females were returned to their own containers and allowed to rest for 3 days between each breeding bout. The exudates were collected on days 2, 4 and 6 of each breeding bout. For each brood, the number and weight of larvae dispersing from the carcass were recorded to ascertain the LRS of each female. All larvae from all treatments dispersed by day 8 of each breeding bout, indicating that females were not slowing reproduction in response to the immune challenge. As for experiment 1, not all females gave exudate samples at every time point. In total, 388 samples were collected of a possible 646, but these were evenly distributed across the treatment groups (control = 140, sterile = 127, bacteria = 121). The success rate varied with brood with 45% of the possible samples collected in broods 1 and 4 and 78% and 86% of samples collected in broods 2 and 3 respectively.

ANALYSING THE LYTIC ACTIVITY OF ANAL EXUDATES

The antibacterial activity of the exudates was analysed using a lytic zone assay against *Micrococcus lysodeikticus* (Cotter & Kilner 2010b). In brief, agar plates were prepared with 0·75 g of freeze-dried *M. lysodeikticus*, 1·5 g agar, 100 mL distilled water and 50 mL 0·2 M potassium phosphate buffer (pH 6·4). Holes were punched in the set agar and 1 μl of defrosted exudate was pipetted into each hole, two replicates per sample, with each replicate on a different plate. The plates were incubated overnight at 33 °C and photographed the following day. The diameter of the clear zones around each hole, indicating lysis of bacterial cells, was measured using Image J software (http://rsbweb.nih.gov/ij/). The diameter of the clear zone is indicative of the concentration of lysozymes in the sample.

STATISTICAL ANALYSES

As females were sampled repeatedly, data were analysed using linear mixed effects Restricted Estimate Maximum Likelihood (REML) models, including female ID as a random effect. All interactions were considered and final models were determined using stepwise deletion; the *P*-values of the retained terms were determined by dropping individual terms from the minimum adequate model. Exudate antibacterial activity was measured as the diameter of the clear zone around the sample. These data were log-transformed prior to analysis to approximate normality. The term Day had unequal variances and so these were allowed to vary in the model.

For experiment 2, exudate antibacterial activity was analysed separately for the first brood, directly after the immune challenge, using data from all 95 females. For the assessment of antibacterial activity over future breeding bouts and the reproductive output by brood data, we restricted this analysis to the random subset of females that had been bred repeatedly ($n = 49$). We excluded data after brood 4 to avoid biasing the data set, due to a large reduction in successful broods after this point (brood 5 onwards, $n < 15$). However, data for LRS included all broods produced by repeatedly bred females.

The number of broods females produced over their lifetime was analysed with GLM using Poisson errors. Data were not overdispersed; estimating the dispersion parameter gave values < 1 and did not change the significance of the results. Where included, the terms Day and Brood were coded as factors. All data were analysed in Genstat 13 (VSN International, Hemel Hempstead, UK) and the assumptions of the models were tested by visual inspection of the diagnostic plots produced by Genstat.

Results

EXPERIMENT 1: CHARACTERIZING THE ANTIBACTERIAL ACTIVITY OF EXUDATES THROUGHOUT A BREEDING BOUT

The antibacterial activity of the exudates increased to day 4 then decreased again to day 8 (REML, day: $F_{4,56} = 41{\cdot}57$, $P < 0{\cdot}001$; Fig. 1). Exudate activity was not significantly affected by carcass weight (REML, carcass weight: $F_{1,123} = 0{\cdot}05$, $P = 0{\cdot}818$) nor by the number, nor weight of larvae that the females produced (REML, number of larvae: $F_{1,68} = 1{\cdot}37$, $P = 0{\cdot}245$; weight of larvae: $F_{1,73} = 1{\cdot}72$, $P = 0{\cdot}194$).

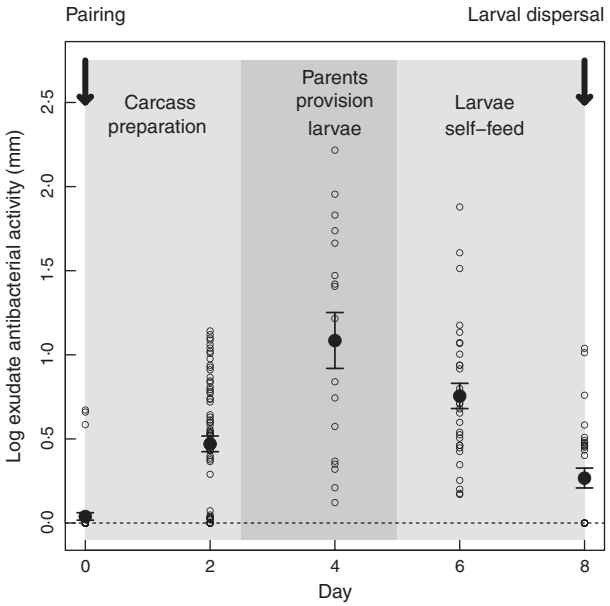

Fig. 1. Antibacterial activity of female anal exudates over the course of the first reproductive bout in unmanipulated females. The open circles are the raw data; the filled circles are the predicted means and SEs from a REML model controlling for female identity.

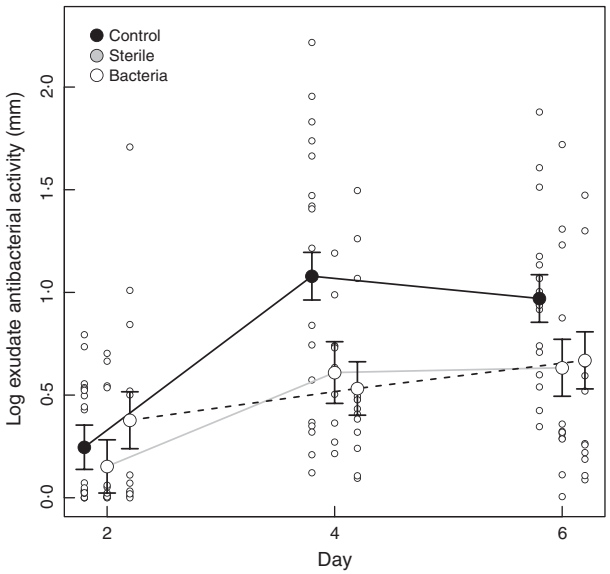

Fig. 2. Antibacterial activity of female anal exudates over days 2, 4 and 6 of the first reproductive bout in unmanipulated females and those whose immune systems have been challenged by wounding with a sterile or bacteria-dipped needle. The open circles are the raw data; the filled circles are the predicted means and SEs from a REML model controlling for female identity. The data points for each treatment have been offset to improve the clarity of the figure.

EXPERIMENT 2: TESTING FOR A TRADE-OFF BETWEEN PERSONAL AND SOCIAL IMMUNITY

Wounding negatively affected the up-regulation of exudate antibacterial activity over the course of the first brood, with the control group producing exudates with much higher antibacterial activity than either the sterile-challenged or bacteria-challenged females (REML, treatment*day: $F_{4,78} = 2{\cdot}80$, $P = 0{\cdot}032$; Fig. 2). The identity of the female from which the exudates were collected explained a small amount of the variance (Variance Component = $0{\cdot}093 \pm 0{\cdot}034$); however, neither the weight of the carcass, nor the weight of the brood reared on that carcass, had any effect on the antibacterial activity of the exudates (REML, carcass weight: $F_{1,60} = 2{\cdot}96$, $P = 0{\cdot}091$, brood weight: $F_{1,53} = 0{\cdot}16$, $P = 0{\cdot}691$).

We then examined whether this trade-off between personal and social immunity continued over the subsequent broods (reared with no further challenge to the mother's personal immune system). We found that antibacterial activity of the anal exudates increased in all females from brood 1 to brood 2, but that the increase was far greater when females were immune-challenged due to their much lower levels of investment in social immunity in brood 1 (brood*treatment: $F_{6,290} = 6{\cdot}20$, $P < 0{\cdot}001$; Fig. 3). By broods 3 and 4, antibacterial levels in all three treatment groups tended to fall off slightly and the differences between the groups disappeared (Fig. 3). There was also an interaction between day and brood number, suggesting that the pattern of up-regulation over days 2–6 changed with different broods (brood*day: $F_{6,268} = 2{\cdot}75$, $P = 0{\cdot}013$; Fig. 4); however the three-way interaction was not significant (brood*day*treatment, $F_{12,252} = 1{\cdot}23$, $P = 0{\cdot}264$). The identity of the female from whom the exudates were collected explained only a small amount of the variance as can be seen by the marginal estimated variance component (VC = $0{\cdot}011 \pm 0{\cdot}005$).

To test whether the differences between the treatments in broods 2–4 were significant, we reanalysed the results from broods 2–4 only. In this case, the interaction between brood number and treatment was no longer significant (brood*treatment: $F_{4,230} = 0{\cdot}64$, $P = 0{\cdot}635$; Fig. 3), suggesting that the level of antibacterial activity in the exudates differed between the treatment groups only in the first brood directly after the immune challenge. However, the interaction between day and brood number was still significant (brood*day: $F_{4,207} = 4{\cdot}09$, $P = 0{\cdot}003$; Fig. 4). Again the variance component estimated for individual females was small (VC = $0{\cdot}013 \pm 0{\cdot}006$).

To test for a correlation between exudate lytic activity and fecundity, we had to look at each day separately as there were up to three exudate measures per female per breeding bout, but only one measure of brood weight. Interestingly, if we just consider those broods that were successful, there was a significant negative effect of brood weight on lytic activity, independent of treatment, on day 4 only (REML: Day 4, brood weight, $F_{2,68} = 5{\cdot}23$, $P = 0{\cdot}025$; treatment, $F_{2,68} = 4{\cdot}53$, $P = 0{\cdot}014$; treatment* brood weight, $F_{2,69} = 0{\cdot}11$, $P = 0{\cdot}90$; Days 2 and 6,

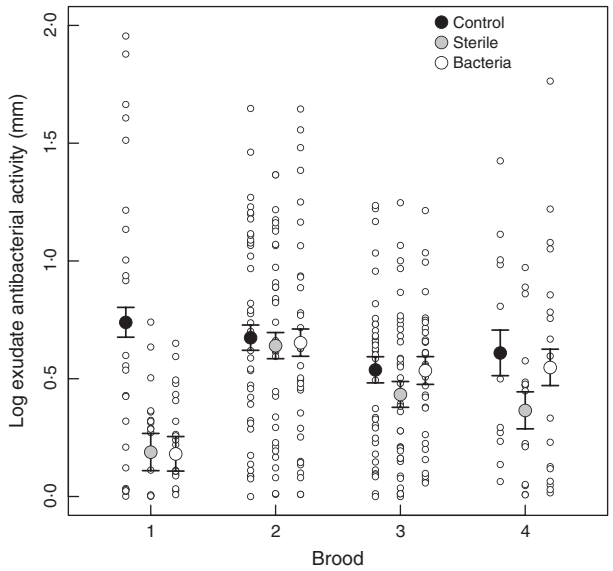

Fig. 3. Exudate antibacterial activity for each brood in unmanipulated females and those whose immune systems have been challenged by wounding with a sterile or bacteria-dipped needle. Females experienced immune challenge prior to the first brood only and were bred repeatedly until death. The *n* for each brood are as follows: 1 (49), 2 (45), 3 (41) and 4 (33). The open circles are the raw data; the filled circles are the predicted means and SEs from a REML model controlling for female identity. The data points for each treatment have been offset to improve the clarity of the figure.

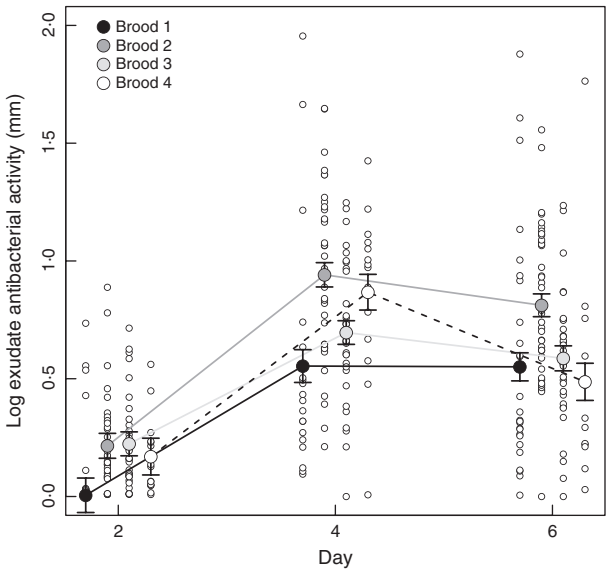

Fig. 4. Mean exudate antibacterial activity for each brood by the day that the exudate was sampled. The *n* for each brood are as follows: 1 (49), 2 (45), 3 (41) and 4 (33). The open circles are the raw data, the filled circles are the predicted means and SEs from a REML model controlling for female identity. The data points for each treatment have been offset to improve the clarity of the figure.

$F < 0{\cdot}19$, $P > 0{\cdot}66$) indicating a possible trade-off between the size of the brood and the maximal amount of lytic activity a female can produce.

THE EFFECT OF TREATMENT ON FECUNDITY

The immune challenge treatments did not affect the total number of broods attempted, nor the number of those broods that successfully produced offspring (GLM, total broods: $\chi^2_2 = 0{\cdot}58$, $P = 0{\cdot}74$; successful broods: $\chi^2_2 = 0{\cdot}49$, $P = 0{\cdot}78$; Table 1). Moreover, immune challenge did not affect the beetles' LRS (REML, total weight of offspring, treatment effect: $F_{2,41} = 0{\cdot}29$, $P = 0{\cdot}75$). However, females confronted with a bacterial immune challenge differed from the other two treatments in the way they invested in each brood (REML, treatment*brood: $F_{6,448} = 9{\cdot}60$, $P < 0{\cdot}001$; Fig. 5). The bacteria-challenge treatment caused females to increase their investment in the first brood relative to the other two groups, with this brood attaining a greater mass, but investment fell sharply thereafter, with very low brood weights by the 4th breeding attempt. By contrast, the other two treatment groups slightly increased their investment in their brood the second time they bred, but gradually reduced their investment levels when raising broods 3 and 4 (Fig. 5). The variance component estimated for females was high (VC = $0{\cdot}657 \pm 0{\cdot}168$), suggesting that females consistently produced either larger or smaller broods.

Table 1. The total number of broods and the number of broods that were successful for each treatment group. Values are means ± SE

Treatment	Total number of broods	Number of successful broods
Control	$3{\cdot}8 \pm 0{\cdot}27$	$2{\cdot}0 + 0{\cdot}24$
Sterile-challenged	$4{\cdot}2 \pm 0{\cdot}28$	$2{\cdot}1 + 0{\cdot}30$
Bacteria-challenged	$4{\cdot}1 \pm 0{\cdot}41$	$2{\cdot}4 + 0{\cdot}36$

Discussion

Our study reveals a trade-off between one aspect of the burying beetle's personal immune defence and one component of its social immune response. By inflicting wounds on female burying beetles, and thus challenging the personal immune system prior to reproduction, we caused a down-regulation in the antibacterial activity of their anal exudates (Fig. 2). Down-regulation was only temporary, however, because by the next breeding bout the social immune response was restored to control levels (Fig. 3).

To our knowledge, this is the first study to find a direct physiological trade-off between these two strategies of immune response, experimentally induced by increased investment in personal immunity. Recently reported experiments on the congeneric burying beetle *N. orbicollis* similarly sought evidence of a trade-off between the social immune response and personal immunity, but found none (Steiger *et al.* 2011). Perhaps this is because these experi-

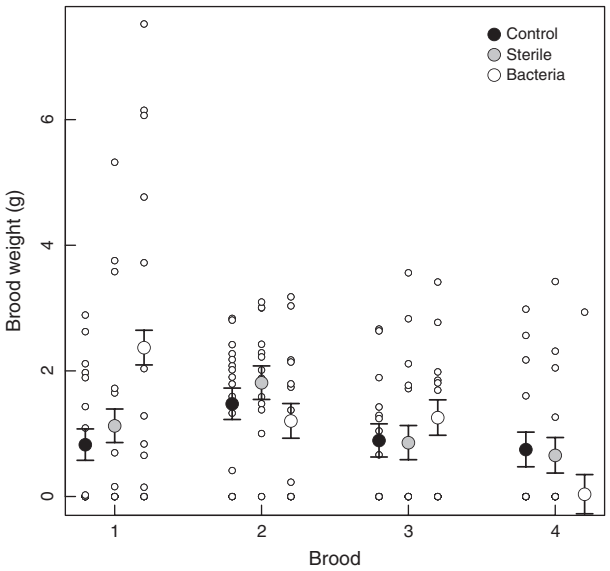

Fig. 5. Brood weight for each brood in unmanipulated females and those whose immune systems have been challenged by wounding with a sterile or bacteria-dipped needle. Females experienced immune challenge prior to the first brood only and were bred repeatedly until death. The *n* for each brood are as follows: 1 (49), 2 (45), 3 (41) and 4 (33). The open circles are the raw data; the filled circles are the predicted means and SEs from a Restricted Estimate Maximum Likelihood model controlling for female identity. The data points for each treatment have been offset to improve the clarity of the figure.

ments focused on different components of the personal immune system (in a different species). Or perhaps the data conceal a trade-off which might have been detected had they included an unchallenged treatment group for comparison (Steiger *et al.* 2011). Whilst a negative correlation between traits can indicate a trade-off, a positive correlation between traits does not necessarily mean that the two traits do not trade-off. If there is more variation between individuals in the levels of resources than in how those resources are allocated between traits, this can generate a positive correlation between traits despite an underlying trade-off (van Noordwijk & de Jong 1986). It is possible that challenged individuals up-regulated their personal immune response and down-regulated their social immune response whilst still maintaining a positive correlation between the two. Studies with other insect species have yielded results that are more consistent with our own. For example, honeybees appear to possess fewer genes for personal immunity than non-social insects, but bear many genes for colony-level immune function (Evans *et al.* 2006; Wilson-Rich *et al.* 2009). Similarly, certain resins have antimicrobial properties and so are collected by ants and bees for use in the nest. In wood ants, the presence of the resin decreases both the bacterial and fungal load in nest material, and this resulted in lower lytic and AMP activity in worker haemolymph (Castella, Chapuisat & Christe 2008). In honeybees, the presence of this resin also decreases bacterial load and so decreases expression of genes connected with personal immunity (Castella, Chapuisat & Christe 2008; Simone, Evans & Spivak 2009). In both these cases, it may have been the reduction in pathogens that led to the decrease in the immune response.

Interestingly, the direct bacterial challenge to the haemolymph did not cause further down-regulation of social immunity, even though wounding alone is known to elicit a weaker AMP-based personal immune response than the bacterial challenge (Cotter, Ward & Kilner 2011). One possible explanation is that any form of antibacterial immune up-regulation (whether through wounding alone or through bacterial injection) is sufficient to trigger a down-regulation in the antibacterial activity of the anal exudates, and that down-regulation of the social immunity in this way is an all or nothing response. Alternatively, it is possible that the separate components of the personal immune system exhibit different trade-offs with the separate components of the social immune system. Or perhaps wounding alone is a good general indicator of the risk of infection for species that live in microbe-rich environments, such as burying beetles (Plaistow *et al.* 2003), and so is the sole trigger for down-regulation of the social immune response. In addition, it is worth noting that we used dead bacteria for our immune challenge to avoid confounding the effects of immune up-regulation and the illness induced by an actively replicating parasite. However, a next step would be to consider the effects of live pathogenic challenge on social immunity. Due to the additional costs associated with fighting a live infection, we might expect a more dramatic reduction of social immunity during live infection, and possibly stronger effects on female fecundity. It would also be interesting to test whether or not this trade-off is apparent in both directions; in other words, does stimulating the social immune response reduce an individual's capacity to defend themselves against parasites?

Although we have evidence that the concentration of lysozyme activity decreased in immune-challenged individuals, we do not know whether females change the quantity of exudates they produce. Although we found no consistent patterns in the amounts of exudate we were able to collect from females in the different treatment groups (S. Cotter personal observation), it is possible that females compensate for decreased lytic concentration of the exudates by producing a greater quantity of exudates, but this has yet to be tested.

Whatever the precise details of the trade-off between personal and social immunity, we found no evidence that mounting a personal immune response of any sort impaired LRS. Instead, by down-regulating expression of their social immune response, and thus moderating its considerable effect on their future fecundity (Cotter *et al.* 2010), challenged females were able to defend their LRS. However, we did find that females with large broods produced a lower peak lytic activity (day 4). Lysozyme activity peaks at the time the young larvae are at their most

demanding; perhaps the costs of caring for a large number of larvae limits the lytic activity that females can produce. To test this hypothesis, brood size would have to be experimentally manipulated and the effects on lytic activity measured.

Our findings contrast with a recent study on carpenter ants, which found that workers challenged with lipopolysaccharides or heat-killed bacteria showed an *increased* social immune response, with raised antimicrobial activity in the regurgitates they passed to nestmates (Hamilton, Lejeune & Rosengaus 2011). Perhaps the difference in results can be attributed to the sterility of the carpenter ant workers and the greater threat of disease that results from colonial living with genetically similar individuals (Cremer, Armitage & Schmid-Hempel 2007). For sterile workers of eusocial species, a personal immune challenge potentially represents a threat to the colony as a whole and defending inclusive fitness far outweighs the benefits of defending personal life span. Under these conditions, it is not surprising that a personal immune challenge up-regulated social immune defences. In contrast, burying beetles, which can reproduce and are not colonial, gain more by down-regulating their social immune response when their personal immunity is challenged because heavy investment in social immunity compromises their LRS (Cotter *et al*. 2010).

Although the beetle larvae did not appear to suffer from down-regulation of social immunity in our lab setting, it is likely that under more natural microbial conditions this would result in an inferior carcass, something that has been shown to reduce offspring quality (Rozen, Engelmoer & Smiseth 2008). So why might females effectively choose to sacrifice the survival of their current brood in favour of mounting a personal immune response? One possibility is that under conditions of biparental care, males may compensate for the reduced social immunity of their partner, thus mitigating its impact. We have shown previously that females bear the greater burden of the social immune response, but that males will increase their antibacterial output if experimentally widowed (Cotter & Kilner 2010b). The mechanism underpinning this flexible response to widowing may involve the perceived level of microbial activity on the carcass. Reduced activity by the female would then cause bacteria on the carcass to multiply which could in turn cause the male to produce more antimicrobial exudates.

A second possibility is linked to the residual reproductive value (RRV) of our experimental females, which were all young virgins. We have shown previously that young females have a high RRV and so should prioritize their future fecundity over their current brood, all else being equal (Ward, Cotter & Kilner 2009). However, we have also shown that a bacterial challenge to the immune system causes young females to behave as if their RRV is very low, perhaps because they perceive a greater risk of death (Cotter, Ward & Kilner 2011). This might explain why the bacteria-challenged females increased their immediate reproductive output compared with either controls or sterile-challenged females (Fig. 5, and see Cotter, Ward & Kilner 2011); unlike females in the other two treatments, the bacteria-challenged females prioritized their current brood over their future fecundity.

In conclusion, we provide the first evidence for a direct physiological trade-off between components of personal and social immunity, which allows females to defend their LRS when immune-challenged in their first breeding attempt. In future work, it would be interesting to compare the nature of this trade-off across species with varying degrees of coloniality and eusociality. Further studies are also required to assess how the trade-off is affected by the presence of a partner and whether it changes with age at first reproduction.

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