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No evidence for an evolutionary trade-off between learning and immunity in a social insect

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**The immune response affects learning and
memory in insects. Given this and the known
fitness costs of both the immune system and
learning, does an evolutionary trade-off exist
between these two systems? We tested this by
measuring the learning ability of 12 bumble-bee
(*Bombus terrestris*) colonies in a free-flying
paradigm. We then tested their immune res-
ponse using the zone of inhibition assay. We
found a positive relationship between colony
learning performance and immune response,
that is, fast-learning colonies also show high
levels of antimicrobial activity. We conclude that
there is no *a priori* reason to demand an
evolutionary relationship between two traits that
are linked physiologically.**

1. INTRODUCTION

There is extensive communication between the ner-
vous system and immune system in mammals
(Dantzer 2004). Many responses to parasites, such as
fever, increased slow-wave sleep, reduced activity,
exploration and sexual behaviour in mammals are
orchestrated by immune products (proinflammatory
cytokines) released in response to the detection of
antigens (Maier & Watkins 1998). Links between
nervous and immune systems are not unique to
vertebrates. We have shown that both honeybees *Apis
mellifera* (Mallon *et al.* 2003*a*) and bumble-bees
Bombus terrestris (Riddell & Mallon 2006; Alghamdi
et al. 2008) perform poorly in learning assays when
their immune systems have been challenged by
lipopolysaccharide (LPS). LPS is a component of
gram-negative bacterial cell walls, which is a non-
pathogenic elicitor of the immune response (Moret &
Schmid-Hempel 2000). That is, we found that
learning and memory are impaired by the immune
response directly with no parasite present.

Given this physiological link between learning and
immunity, and that learning and immunity have
demonstrated fitness costs in insects (Kraaijeveld &
Godfray 1997; Mery & Kawecki 2003; Raine &

The first two authors contributed equally to this work.

Chittka 2008), it seems reasonable to hypothesize an
evolutionary trade-off between learning and immu-
nity. An evolutionary trade-off is where the evolution
of an increase in a given trait leads to a reduction in a
different trait. This could be due to the pleiotropic
effects of the genes involved, linkage disequilibrium
with deleterious mutations or resource allocation
during development (Schmid-Hempel 2005). While a
recent paper showed no evidence of a trade-off
between immunity and learning in different artificially
selected *Drosophila* lines in the laboratory (Kolss *et al.*
2006), it might be more ecological relevant to
examine natural levels of variation in these traits.

Bumble-bees are an obvious candidate for this
approach as colonies show natural variation in
learning performance (Raine *et al.* 2006*b*) and the
physiological relationship between learning and
immunity has been demonstrated in *B. terrestris*
(Riddell & Mallon 2006; Alghamdi *et al.* 2008). Also
there are ecological reasons to believe that learning
and immunity could be more costly in bumble-bees
than *Drosophila*, potentially leading to a higher
likelihood of a trade-off.

The demands of foraging from many different
flower species, which can vary dramatically in the
quantity and quality of rewards they offer, and the
need to find the nest after each foraging bout,
mean that bees have highly developed cognitive
abilities. Bumble-bees also learn from conspecifics,
so-called social learning (Leadbeater & Chittka
2007). Furthermore, we would also expect that
immunity would be a more important trait in social
species that have high-contact rates with genetically
close individuals leading to a greater chance of
infection (Cremer *et al.* 2007).

In this study, we used a free-flying floral choice
assay to test the learning abilities of bumble-bee
colonies. We took workers from these colonies
and tested their immune response using the antibac-
terial zone of inhibition (ZOI) assay. This allowed us
to identify any evolutionary relationship between
these two traits.

2. MATERIAL AND METHODS

We obtained 12 bumble-bee (*Bombus terrestris dalmatinus*) colonies
from Koppert Biological Systems (Berkel en Rodenrijs, The
Netherlands). All workers were uniquely marked with Opalith tags
(Christian Graze KG, Germany).

(a) Learning assay

Results from this associative learning assay are reported in a
previous paper (Raine & Chittka 2008). Bees were pre-trained to
forage from 20 bicoloured, blue and yellow, artificial flowers in a
flight arena. During pre-training all flowers were rewarded with 50
per cent (w/w) sucrose solution providing previously colour-naive
bees with an equal chance to associate both colours with reward
(Raine *et al.* 2006*b*). Bees completing at least five consecutive
foraging bouts were selected for training. These foragers were
trained individually, in a flight arena containing 10 blue (Perspex
Blue 727) and 10 yellow (Perspex Yellow 260) artificial flowers
(each 24×24 mm). Yellow flowers were rewarding (each contained
15 µl of 50% (w/w) sucrose solution), while blue flowers were
empty (unrewarding). Bees were regarded as choosing a flower
when they either approached (inspected) or landed on it. Choosing
a yellow flower was regarded as 'correct', while choosing a blue
flower was deemed to be an 'error'. We recorded the choice
sequence made by each bee from the time it first entered the flight
arena. Recording flower choices ceased once a bee made 99 flower
choices after the first time it probed a rewarding (yellow) flower
(Raine *et al.* 2006*b*). Flowers were changed and their positions
re-randomized between foraging bouts to prevent bees using scent
marks or previous flower positions as predictors of reward. Flower

colours were selected so that bees had to overcome their strong, unlearned preference for blue, before associating one of their innately least favoured colours (yellow) with reward (Chittka *et al.* 2004; Raine *et al.* 2006*a*).

(b) Learning curves

The starting point for each bee's learning curve was the proportion of errors made (blue flowers chosen) before the bee first probed a rewarding (yellow) flower. Flower choices made by each bee after (and including) the first time it probed a rewarding (yellow) flower were evaluated as the number of errors (blue flowers chosen) in each group of 10 choices. Learning curves (exponential decay functions: $y = y_0 + Ae^{-x/t}$) were fitted to these 11 data points (i.e. the start point and subsequent 10 groups of 10 flower choices) for each individual bee, using MICROCAL ORIGIN (Chittka *et al.* 2004; Raine *et al.* 2006*b*). Here, x is the number of flower choices the bee made, starting with the first time it probed a yellow flower, and y is the number of errors. The saturation performance level (y_0) is the number of errors made by a bee after finishing the learning process, i.e. when reaching a performance plateau. The decay constant (t) is a measure of learning speed: with lower t-values corresponding to faster learning speeds. A is the curve amplitude: the maximum displacement (height) of the curve above y_0. Both amplitude (A) and saturation performance (y_0) were constrained between 0 and 10 for curve fitting.

(c) Zone of inhibition assay

This assay measures antibacterial activity: it is based on the ability of immune proteins to inhibit bacterial growth when placed onto an agar plate seeded with bacteria (*Arthrobacter globiformis* 10^5 bacteria per ml of agar). Workers from all 12 colonies were sacrificed after the learning assay and stored at $-20°C$ for later analysis. Each thorax was homogenized in 300 µl of sodium cacodylate solution. Two microlitres of the supernatant from the centrifuged solution ($1300g$ for 10 min at 4°C) were pippetted into a hole on the agar plate. This was incubated overnight (28°C). The resultant ZOI (mm) were measured as the mean of its longest and shortest axis (ZOI value).

3. RESULTS

As reported in Raine & Chittka (2008), there was significant variation among colonies in learning speed (t-value: one way ANOVA: $F_{11,160} = 1.900$, $p = 0.043$).

We tested the immune response of 55 bees from 12 colonies (mean number per colony ($\pm$s.d.) $= 4.58 \pm 0.67$) using the ZOI assay. There was a significant difference between colonies in their immune response (colony $F_{11,33} = 2.51$, $p = 0.020$), which could not be attributed to the effect of body size (head width $F_{10,33} = 2.2$, $p = 0.072$).

There was a significant negative correlation between the median t-value of a colony and its mean ZOI value (Spearman's rank: $r = -0.608$, $n = 12$, $p = 0.036$; figure 1). As high t-values correspond to slower learning speeds, this is a positive relationship between the ability of a colony to learn and the strength of its immune response.

4. DISCUSSION

We found a positive correlation between the ability of a colony's workers to learn and the strength of their immune response. Our initial hypothesis that learning ability and immune response would be in an evolutionary trade-off was not supported. Our result is in broad agreement with that of Kolss *et al.*'s (2006) artificial selection experiment, and expands our understanding of this potential learning and immunity trade-off by examining natural variation in both traits in unmanipulated organisms.

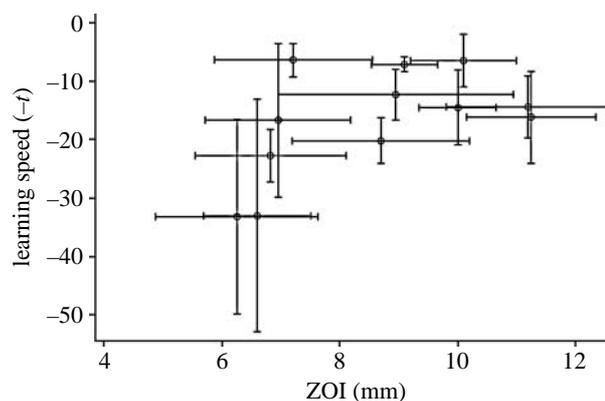Foraging activity has been shown to decrease the immune response of bumble-bee workers (König &

Figure 1. The relationship between median learning speed (negative *t*-values) and mean ZOI response of the 12 colonies. As high *t*-values correspond to slower learning speeds, we have plotted negative *t*-values to make clear the positive relationship between the colony learning ability and the strength of its immune response. Each point represents a colony. Vertical error bars represent median absolute deviation. Horizontal error bars represent standard error.

Schmid-Hempel 1995). All workers tested in the learning assay and subsequently used for ZOI assays had similar levels of foraging experience in the laboratory flight arena. Hence, as all our bees were foragers this could not explain variation in immune response. Potential exposure to pathogens which could induce stimulation of the immune system was identical for all 12 colonies that came directly from the bee breeder and were not exposed to field foraging conditions before this experiment.

Phenotypic correlations are generally seen as weak evidence for evolutionary trade-offs (Reznick *et al.* 2000). However, along with Kolss *et al.*'s selection experiment, we can ask why is there no evidence for an evolutionary trade-off when a physiological connection has been found repeatedly? Below we discuss three mutually non-exclusive possibilities.

First, it could be argued that we have incorrectly generalized antimicrobial response to some measure of overall immunocompetence. The various parts of the insect immune system (antimicrobial peptides, encapsulation, nitric oxide production, etc.) are known not to necessarily correlate (Mallon *et al.* 2003*b*). Hence, other parts of the immune response may show an evolutionary trade-off with memory if tested. However, as the physiological trade-off has been found repeatedly with ZOI measures, we felt this was the most likely place to find an evolutionary trade-off.

Second, there is the possibility that genetic variation exists not only in resource allocation but also in resource acquisition (Reznick *et al.* 2000). If there was more variation in allocation and less in acquisition, we would expect to see a negative correlation between any two life-history traits. Vice versa, we would expect to see a positive correlation (Van Noordwijk & Dejong 1986).

Third, Schmid-Hempel (2005) outlined the differences between the evolutionary and the activation cost of the immune system. Evolutionary costs are the fitness effects of possessing an immune system of a given strength. Evolutionary costs can occur due

to the pleiotropic effects of resistance genes, linkage disequilibrium with deleterious alleles or changes in resource allocation during development. Activation costs are simply the effect on other physiological systems of generating the immune response from an organism's immune system. Although a physiological connection may lead us to look for an evolutionary trade-off there is no *a priori* reason to demand one.

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