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Immunochallenge reduces risk sensitivity during foraging in white-footed mice

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Foraging behaviour has the potential to impact interactions among species in a community and is influenced, in part, by individual condition and fear of predation. These relationships are exemplified by white-footed mice, *Peromyscus leucopus*, in mixed hardwood forest communities of the northeastern U.S.A., whose foraging behaviour can influence the population ecology of several prey items and the disease ecology of human Lyme disease and its tick vector. We examined whether dosage of an immunogen influenced foraging behaviour in wild white-footed mice at foraging arenas. Low-dose mice preferentially favoured safe (covered) food patches over risky food patches, whereas high-dose mice showed no preference based on patch safety. Immunochallenge did not alter foraging time in patches of equal risk. The results reveal fitness costs of an immune response, namely that immunochallenged mice favour increased energy consumption over safety from predation, presumably leading to greater mortality. Acceptance of risky patches for foraging by immunochallenged mice suggests that mice mounting this immune response will forage in a greater proportion of their home ranges and encounter a greater number of patchily distributed prey items and ticks carrying pathogens.

Injury or mortality through predation represents clear losses of future fitness in prey. Fear of predation (the perceived cost of injury or mortality; Brown & Kotler 2007) typically leads animals to forage with less risk by preferentially seeking relatively safe habitats for foraging or increasing vigilance when predators are present (Gilliam & Fraser 1987; Brown 1988, 1992; Lima 1988; Lima & Dill 1990; Morris & Davidson 2000; Laundré et al. 2001; Brown & Kotler 2004; Schwanz et al. 2011a). For example, many species of granivorous rodents preferentially forage in microhabitats covered by bush vegetation that offer more protection from aerial predators compared to open microhabitats (Brown 1988). These changes in foraging behaviour can have dramatic effects on interactions among species in a community (Abrams 1984; Beckerman et al. 1997; Ripple et al. 2001; Brown & Kotler 2007; Ripple & Beschta 2007; but see Kauffman et al. 2010).

Risk sensitivity is unlikely to be uniform among individuals, however, and should depend on individual condition and anticipated future fitness (McNamara & Houston 1986; Clark 1994; Brown & Kotler 2004, 2007). Animals in good condition are predicted to exhibit greater risk sensitivity (e.g. preference for safe habitats over risky habitats) than those in poorer condition if (1) they have greater anticipated future fitness to preserve (asset protection principle, Clark 1994), (2) they are in less need of additional resources (i.e. the marginal fitness value of energy is lower; Brown 1988, 1992), or (3) they are better able to assess variation in actual risk among habitats (Brown 1992; Brown & Kotler 2007). In support of this prediction, empirical studies have shown that risk sensitivity is higher for gerbils (*Gerbillus allenbyi* and *G. pyramidum*), juncos, *Junco hyemalis*, and guppies, *Poecilia reticulata*, with greater energetic reserves (Godin & Smith 1988; Lima 1988; Kotler 1997; but see Kotler et al. 2004).

Important components of individual condition include exposure and immune responses to parasitic infection, raising the possibility that immunochallenges play an indirect role in shaping communities by altering fear and foraging behaviour in hosts. If the costs of infection or immune response influence host risk sensitivity, then host behaviours and any correlated community interactions will be

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impacted. Little is known about the influence of parasites on anti-predator behaviour. Empirical evidence comes from three-spined sticklebacks, *Gasterosteus aculeatus*, and upland bullies, *Gobiomorphus breviceps*, where parasite load (cestodes and trematodes, respectively) causes foraging fish to be less sensitive to predation risk (Milinski 1985; Godin & Sproul 1988; Poulin 1993). As a contrasting example, infestation with fleas causes gerbils to forage with greater sensitivity to the risk of fox predation, possibly because flea bites distract gerbils from foraging and vigilance (Raveh et al. 2011). Mechanisms underlying these variable responses are poorly understood.

Stimulation of the immune system alone can trigger alterations in host physiology and behaviour (Adamo 1999; Zuk & Stoehr 2002; Derting & Compton 2003; Velando et al. 2006; Weil et al. 2006). Immunochallenge appears to reduce the risk sensitivity of foraging white-footed mice, *Peromyscus leucopus* (Schwanz et al. 2011a). In habitats in which an immunogen derived from the etiological agent of Lyme disease (LD; *Borrelia burgdorferi*) was deployed, microhabitat safety appeared to be less important for foragers than in habitats in which no immunogen was deployed. Schwanz et al. (2011a) observed these differences at the population level and did not investigate how the number of immunogen doses could alter individual behaviour. If this pattern was due to individual responses to immunochallenge, it would reveal hidden costs of the immune response by indicating that (1) immunochallenge reduced individual 'condition' and led to altered foraging behaviour and (2) altered foraging behaviour as a result of immunochallenge leads to a greater risk of predation. Moreover, such changes in the foraging behaviour of white-footed mice would have far-reaching implications for interspecific interactions and disease ecology in the surrounding forest community in northeastern U.S.A. (Schmidt & Schauber 2007). White-footed mice can regulate the populations of native ground-nesting birds and invasive gypsy moths, *Lymantria dispar* (Ostfeld et al. 1996a; Jones et al. 1998; Schmidt & Ostfeld 2003a). When mice are less selective of food patch quality, more of the space in their home range becomes profitable foraging space and mice become more likely to encounter these spatially clumped, incidental prey items (Schmidt et al. 2001; Schmidt & Ostfeld 2003b; Connors et al. 2005). White-footed mice additionally serve as one of the main reservoirs for *B. burgdorferi* (Levine et al. 1985; LoGiudice et al. 2003; Keesing et al. 2009), which is transmitted among hosts (including humans) via the bite of *Ixodes* ticks (in North America, most prominently the black-legged tick, *Ixodes scapularis*; Burgdorfer et al. 1982). Because ticks are spatially clumped (Ostfeld et al. 1996b, c), it is likely that mouse space use as reflected by foraging behaviour also influences tick–mouse encounter rates. Thus, the indirect impacts of mouse foraging behaviour on broader community and disease ecology may be large.

In this study, we directly tested the effect of immunochallenge on the risk sensitivity of individual white-footed mice that were foraging in the wild. Using an immunochallenge rather than live pathogens addresses the costs of an immune response and eliminates the possibility of changes in mouse behaviour being caused by manipulation by a live parasite to further its own propagation. We examined foraging behaviour, in risky and safe food patches, of mice that had experienced varying levels of immunochallenge. If highly immunochallenged mice (poor condition) have reduced future fitness or are in greater need of food, then we predict they will spend greater time than good-condition mice foraging in patches of a given level of predation risk. Moreover, for mice in poor condition, the change in future fitness and value of food should reduce the importance of variance in patch riskiness, leading to the prediction that immunochallenged mice will show less preference for safe patches over risky patches.

METHODS

Study System

The experiment was conducted in mixed hardwood forest on the property of the Cary Institute of Ecosystem Studies in Dutchess County, southeastern New York, U.S.A. As part of a larger study into the community and disease ecology of white-footed mice and *B. burgdorferi*, two 8×8 trapping grids were monitored (Canoe Gap, CG, and Field Lab, FL), with trapping stations 15 m apart and two Sherman traps placed at each trap station. Every night, Monday to Thursday, traps were opened around 1600 hours in the afternoon and were checked the following morning (0800 hours; maximum time in trap ca.12 h because mice are not active until dusk at ca. 2000–2100 hours). From 6 April to 14 August 2009 traps were baited with an oat and water mixture containing a *B. burgdorferi*-derived immunogen (see immunogen methods below) and the proportion of bait consumed by trapped *P. leucopus* was noted (as 0, 0.1, 0.25, 0.5, 0.75, 0.9 or 1). From 18 August until the end of the trapping season (11 September), traps were baited with oat without the immunogen. During the 2009 trapping season, 308 individual white-footed mice were trapped and tagged (see below) on the two focal trapping grids. Mice were freely permitted to enter the traps each night with a frequency of up to 4 nights per week, resulting in an average total number of trapping events per mouse of 6.4 ± 7.4 (range 1–49 trappings). Because *P. leucopus* have very low water requirements (Deavers & Hudson 1979) and water was mixed with the oat bait, additional water was not provided in the traps. All traps were covered with a wooden board to provide protection from rain, and cotton bedding was provided in the traps during cooler months. During the trapping season, which amounted to over 11 000 trap nights, 12 predation events occurred (0.1% predation rate, most likely by raccoons). Because white-footed mice can breed continuously during the summer, pregnant and lactating females were regularly trapped. Lactating *P. leucopus* in the field appear to spend nearly the entire night outside of their nest (Hill 1972), suggesting that trapping lactating females may not adversely affect litter survival. Neonates can maintain warm body temperatures for several hours when huddling with other littermates (reviewed in Hill 1983); however, the importance for litters of maternal visits to the nest during the course of the night is not known.

All *P. leucopus* were given ear tags (1005-1 self-piercing monel tags inserted with a model 1005s1 applicator, National Band & Tag Co., Newport, KY, U.S.A.), and sex, age and mass were recorded weekly, if recaptured. Beginning in June, PIT tags (12.5 mm, 134.2 Khz, sterile, TX1440ST from Biomark, Boise, ID, U.S.A.) were implanted, using sterile syringes and needles, between the shoulder blades in all trapped mice, with all animals except new captures having tags by late June. Neither ear tags nor PIT tags caused bleeding. Both have high retention rates and are not known to cause major detriment in small rodents (Gibbons & Andrews 2004; Fokidis et al. 2006). All live-trapping and tagging procedures were conducted in accordance with the guidelines approved by the American Society of Mammalogists (Sikes et al. 2011) and were approved by the Institutional Animal Care and Use Committee at Cary Institute (Protocol 09-01II).

Bait Immunogen

The oatmeal bait deployed in the field contained an immunogen consisting of *Escherichia coli* transformed with recombinant *B. burgdorferi* outer surface protein A (OspA) and induced with IPTG for protein expression (described in detail in Schwanz et al. 2011a). OspA is down-regulated in *B. burgdorferi* in the tick midgut before the

spirochete is transmitted to the host (de Silva et al. 1996). Thus, antibodies to this protein are negligible in wild infections (Hofmeister et al. 1999; Bunikis et al. 2004). Experimental consumption of OspA by mouse hosts induces the production of a high titre of specific antibodies which persist at least up to a month after termination of immunogen delivery (e.g. Fikrig et al. 1990; Luke et al. 1997; Gomes-Solecki et al. 2006). Dosage of this immunogen is positively related to antibody titres and levels of circulating white blood cells in wild white-footed mice (Schwanz et al. 2011a). No other adverse effects of the immunogen are known.

Foraging Measures

When a foraging animal encounters a food patch, the rate of food acquisition is highest at the initiation of feeding, and declines as the density of food items is depleted and search time for the increasingly scarcer food items in the patch increases (Charnov 1976). The quitting harvest rate (QHR) is the rate of food acquisition at which a forager leaves the patch and is predicted to have an optimal value depending on the costs of predation, foraging and missed opportunities (e.g. mating; Brown 1988, 1992). Provided that the substrate (e.g. soil) of the patch is held constant, the QHR can be estimated and compared among patches by measuring the time a forager spends in a patch or the giving-up density (GUD) of a food patch, which is the amount of food left behind when a forager leaves a food patch (Brown 1988, 1992). Empirical research on foraging animals supports the prediction that food patches with higher costs of predation (i.e. riskier habitats) show higher GUDs, indicating that foragers have a higher QHR in these patches and thus require greater food intake rates to remain foraging in a risky habitat (Brown 1988; Kotler et al. 2004). In our study, we measured foraging behaviour of mice on the trapping grids using GUDs of seed and time spent foraging at experimental seed trays that were either risky (uncovered) or safe (covered) patches (Schwanz et al. 2011a).

We deployed foraging arenas on nontrapping nights between 28 June and 31 August. Arenas were established roughly 2 m from a trap site and consisted of two seed trays (20 × 28 cm; Perma-nest Plant Tray, Growers Supply Co., Inc., Dexter, MI, U.S.A.) placed 1 m apart. Each pair of seed trays contained two (randomly assigned) tray treatments: covered and uncovered. Covered trays had an opaque shade cloth suspended 5–10 cm above the top edge of the tray, whereas uncovered trays had no shade. Each tray contained 1.5 litres of play sand and 4 g of millet seed mixed into the sand. Each week we established four trap stations to target novel individuals. Each foraging arena was 'set' (sand and seed accessible to foragers) for 1–2 nights prior to the experimental run as a 'prebait' to ensure that mice had located the seed trays. At dusk (ca. 2000 hours) of the night of the experimental run, we selected an arena that showed signs of activity during the prebait (presence of footprints) and activated it by putting 4 g of fresh seeds in each tray and setting automated PIT tag readers (FS2001F-ISO, Biomark, Boise, ID, U.S.A.) for each tray. Each reader consisted of a circular antenna located underneath the tray and connected to a data logger and a 12 V battery (Model ES17-12 MKbattery.com). The reader was set to read PIT tags every 5 s; however, if an additional mouse entered the tray its tag would be read instantaneously. The data logger stored the PIT tag number of each individual that visited the tray together with a time stamp of the visit. At dawn the next morning (ca. 0630 hours), the readers were stopped and the remaining seed in each tray was sifted out of the sand and weighed to the nearest 0.01 g. Because white-footed mice are the only nocturnal granivores at our field site, having the trays open only at night guaranteed that only white-footed mice foraged at the trays. At the time of establishing each foraging arena, we estimated vegetation cover around each seed tray (see Schwanz et al. 2011a).

Data Analysis

Twenty of 43 experimental foraging arenas provided no data because of rain on the trays or because no mice foraged in them. From the 23 stations that had foraging visits, we first examined the relationship between total time in the tray and seeds remaining in the tray by fitting a negative exponential regression model to the data (Seeds remaining $= a \times \exp(-b \times \text{Time})$). We determined whether the relationship was the same in the covered and uncovered trays by asking whether the parameter estimates differed by more than two standard errors. Apart from the interest in the shape of this relationship, we wanted to confirm that a clear relationship existed and was similar among treatments, in which case we could interpret the time spent at a tray as a good proxy for foraging time.

To examine the influence of immunogen dose on individual foraging behaviour, we limited the data to those records where the first (or only) individual mouse to visit the foraging arena was alone and a second mouse did not overlap in time with the first mouse in the arena (21 of 23 arenas). Thus, our goal was to include only records where we assumed that a single mouse encountered 4 g of seed and made foraging decisions independent of interference competition from other mice. Owing to limited data on females, we included only males and we excluded the first of any repeated samples of the same mouse (nine of 21 arenas excluded). We calculated the cumulative time an individual mouse spent in each tray at the foraging arena. We estimated risk sensitivity by calculating the preference for covered trays as the ratio of time spent foraging in the covered tray to all time spent foraging in a tray (C/[C + U] or C/T). Values greater than 0.5 indicate a mouse preferred the covered tray to the uncovered tray and thus showed risk aversion. Values near or less than 0.5 indicate a mouse did not prefer the covered tray and was therefore largely risk insensitive.

We used ANCOVA models to determine whether variation in the response variables (time spent foraging in uncovered trays, time in covered trays or risk sensitivity (C/T)) is explained by immunogen dose. We also included in the model as predictors the site identity (CG or FL) and vegetation cover above the foraging arena, which may influence risk perception. After fitting the models we examined the residuals to determine whether the data complied with the assumption of normality and homoscedasticity. In addition, we further tested the effect of immunogen dose on risk sensitivity using bootstrapping to calculate confidence intervals for the coefficient estimates of the parameters in the model. This involved randomizing the residuals of the linear regression model 10 000 times using the boot package in R (Canty & Ripley 2009). The function boot.ci was used to generate the adjusted bootstrap percentile interval with the bias-corrected and accelerated approach (BCa). All the analyses were conducted using the R statistical program (R Development Core Team 2009).

RESULTS

We found a strong relationship between GUDs (g of seeds found at the tray the next morning) and the total amount of time that all mice combined foraged in a seed tray (Fig. 1; 13 of 23 foraging arenas were visited by more than one mouse). In covered and uncovered trays, the amount of seed remaining was a negative exponential function of foraging time. The relationship between seed remaining and foraging time was not different for covered and uncovered trays as indicated by the similarity of the parameter coefficient estimates (Table 1). These results clearly indicate that the amount of time that foragers spent in the tray reflects foraging behaviours, and that foraging rate was similar in both tray types. That is, individuals occupied the trays to search for food, and not for shelter or other activities.

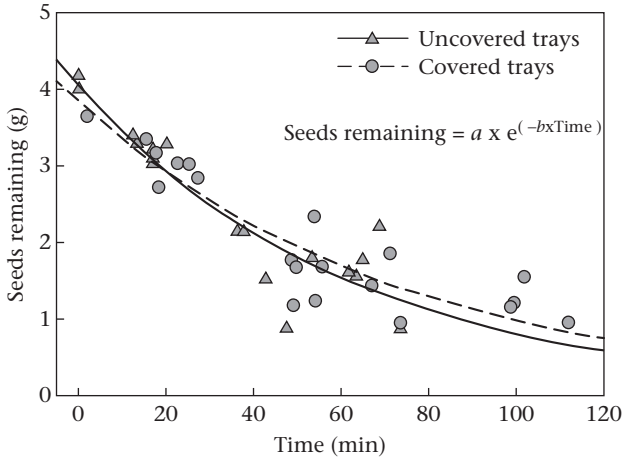

Figure 1. Relationship between the amount of seeds remaining at a tray (GUDs) and the total amount of time that all mice combined were recorded on a tray during the night.

Contrary to our prediction, immunogen dose did not influence foraging time in risky or safe patches (ANCOVA: uncovered trays: dose: $F_{1,11} = 0.30$, $P = 0.60$; vegetation cover: $F_{1,11} = 0.99$, $P = 0.35$; site: $F_{1,11} = 0.20$, $P = 0.67$; $N = 12$; covered trays: $F_{1,11} = 0.55$, $P = 0.48$; vegetation cover: $F_{1,11} = 0.46$, $P = 0.52$; site: $F_{1,11} = 1.65$, $P = 0.23$; $N = 12$). Given that vegetation cover did not provide any explanatory power for foraging behaviour, it was excluded from the statistical models used to explain risk sensitivity.

In accordance with our prediction, the ratio of time spent foraging in covered trays to total time spent foraging tended to decline as immunogen dose increased (Model 1, Table 2, Fig. 2a). That is, mice with a greater immunogen dose showed less preference for covered trays than low-dose mice, suggesting they were less risk sensitive. The 95% bias-corrected interval obtained by bootstrapping indicates that we can be 95% confident that the coefficient of the effect of immunogen dose on risk sensitivity would be between −0.036 and −0.0014, not overlapping zero, which suggests that this behavioural response was statistically significant.

Because immunogen dose was necessarily associated with trapping events, we considered two alternative hypotheses for the above pattern that could complicate our interpretation. First, the pattern could be explained if being trapped repeatedly causes mice to become less risk sensitive. The number of times a mouse was trapped between first capture and recording of foraging behaviour was often but not always the same as the immunogen dose ($r^2 = 0.91$, $F_{1,11} = 104$, $N = 12$, $P < 0.001$). This was mainly because four mice were recorded in foraging arenas after deployment of the immunogen in the bait had ceased. To test this hypothesis, we examined the same model of risk sensitivity above, with immunogen dose replaced with number of trappings. The proportion of foraging time spent in the covered tray was not related to the number of trappings (Model 2, Table 2), suggesting that immunogen dose per se and not the number of trappings accounts for the measured effect on mouse foraging behaviour.

Table 1
Parameter estimates for the nonlinear relationships (for each tray treatment) between GUDs and the total amount of time that all mice combined were recorded on a tray during the night based on the negative exponential model: Seed remaining $= a \times e^{(-b \times \text{time})}$, $df = 38$

Treatment	Parameter	Estimate	SE	t	Pr(>|t|)
Covered	a	3.838	0.243	15.8	<0.001
	b	0.014	0.002	8.5	<0.001
Uncovered	a	4.055	0.178	22.7	<0.001
	b	0.016	0.002	9.6	<0.001

Table 2
Multiple regression models for explaining the variation in risk sensitivity as a function of the number of immunogen doses the mouse received (Models 1 and 3) or number of times the mouse was trapped (Model 2), and sampling site

	Parameter	Coefficient estimate	SE	t	Pr(>|t|)
Model 1	Intercept	0.71	0.12	5.91	<0.001
	Immunogen dose	−0.02	0.01	−2.04	0.072
	Site (FL)	0.16	0.14	1.14	0.285
Model 2	Intercept	0.73	0.13	5.42	<0.001
	Number of trappings	−0.02	0.01	−1.83	0.101
	Site (FL)	0.14	0.14	0.98	0.352
Model 3	Intercept	0.78	0.06	12.46	<0.001
	Immunogen dose	−0.03	0.01	−4.89	0.001
	Site (FL)	0.10	0.07	1.38	0.204
	Trap happiness index	0.09	0.02	5.18	0.001

Risk sensitivity was measured as the proportion of time spent foraging at the covered tray over total amount of time foraging. The trap happiness predictor included in Model 3 is the residuals of a linear regression between number of trappings and number of sampling nights. $N = 12$.

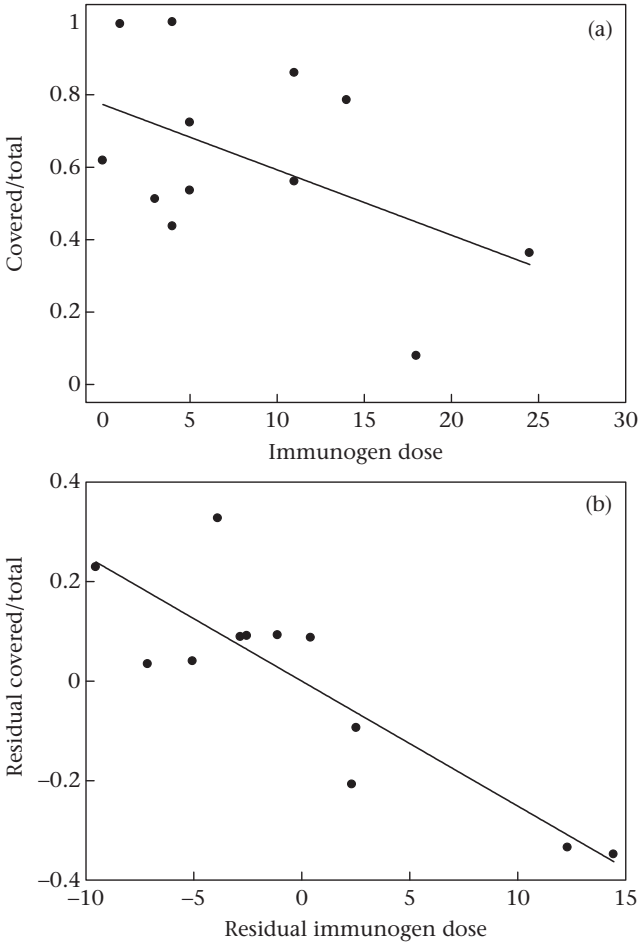

Figure 2. Preference for covered trays while foraging (time in covered/total time) as a function of immunogen dose. (a) Simple scatterplot; (b) partial regression plot from Model 3, showing residuals of covered/total controlling for site and trap happiness plotted against residuals of immunogen dose controlling for site and trap happiness. Each data point represents a single mouse visiting a station. $N = 12$.

Second, if mice vary in risk sensitivity because of something other than immunogen dose (e.g. age, natural infection, personality) and risk sensitivity influences trap happiness (Biro & Dingemanse 2008), then mice that are less risk sensitive may be trapped more frequently and consume more immunogen. This hypothesis proposes the reverse causality in the pattern that risk sensitivity influences immunogen dose. To examine this hypothesis, we assumed that the frequency of trappings relative to the number of nights the traps are set is a good measure of trap happiness. This assumption holds if there is no trap saturation on the nights the mice are not trapped. We confirmed in our trapping data that in all instances there was at least one trap free in the area used by the focal mice. We performed a linear regression between the number of trappings prior to recording of foraging behaviour and the number of sampling nights between first capture and recording of foraging behaviour ($r^2 = 0.91$, $F_{1,11} = 104.7$, $N = 12$, $P < 0.001$; the proportion of nights trapped ranged from 0.43 to 1). The residuals of this regression would be indicative of trap happiness (positive residuals) or trap shyness (negative residuals). These residuals were not related to immunogen dose ($r^2 = 0.05$, $F_{1,11} = 0.48$, $N = 12$, $P = 0.50$), indicating that dose was not related to trap happiness and suggesting that risk sensitivity is a product rather than a cause of immunogen exposure. Moreover, including trap happiness in the model that relates risk sensitivity to immunogen dose to account for uncontrolled individual variation reveals an even stronger effect of immunogen dose that is highly significant and less dependent on the high-dose observations (Model 3, Table 2, Fig. 2b).

DISCUSSION

Parasites and immunochallenge are known to alter reproduction and behaviour of animals (Holmes & Zohar 1990; Poulin 1994; Zuk & Stoehr 2002), and some behaviours, particularly foraging behaviours, appear to have unanticipated effects on community structure (Kotler & Brown 2007). Together, these suggest that parasites have the potential to influence community ecology indirectly via alterations in host behaviour (Thomas et al. 1999; Wood et al. 2007; Tompkins et al. 2011). We investigated this question within an important ecological system that includes white-footed mice, the etiological agent of Lyme disease, *B. burgdorferi*, and the mixed hardwood forest community within which they live in the northeastern United States.

We found that individual, wild mice that had received a higher dosage of a *B. burgdorferi*-derived immunogen were less risk sensitive than mice with a low dose, supporting our predictions and the results from the few other empirical studies conducted on the question (e.g. Milinski 1985; Godin & Sproul 1988; Poulin 1993; Schwanz et al. 2011a; but see Raveh et al. 2011). Reduced risk sensitivity in animals facing greater immunochallenge indicates that these animals place less value on avoiding predation compared to animals with lower immunochallenge. In the formulation of Brown (1988, 1992), the optimal QHR is predicted to depend on the sum of the energetic costs of foraging, the missed opportunity costs (e.g. mating), and the costs of predation. Considering the costs of predation, the QHR (and GUD) is predicted to decrease when the instantaneous risk of predation or the future fitness of a forager decreases, or when the marginal value of acquiring more food increases (Brown & Kotler 2004). Thus, a change in the response to experimentally induced instantaneous risk (tray treatment) suggests that immunochallenged mice (1) detect a reduction in future fitness and are less compelled to 'preserve' their life, or (2) are in greater need of immediate energetic resources (Brown 1988, 1992; Clark 1994).

These changes should also lead to an overall reduction in QHR (increase in foraging time) in patches of equal risk (i.e. within tray type). However, similar to the results of our previous study (Schwanz et al. 2011a), we found no effect of immunochallenge on foraging time when predation risk was equal. This suggests that an additional predictor of QHR has also been altered by the immune response, such as an increase in the energetic costs of foraging (e.g. if immunochallenged mice are lethargic), an increase in the missed opportunity costs (e.g. if immunochallenged mice would optimally spend more time searching for mates), or an increase in the instantaneous risk of predation (e.g. if immunochallenged mice have reduced predator detection or escape ability; Raveh et al. 2011). Some evidence for a negative effect of immune response on predator escape ability and energy levels is provided by data showing that greater immunogen doses lead to lower voluntary running speeds of wild white-footed mice held in the laboratory (Schwanz et al. 2011a). Alternatively, the collective results may be explained more simply by immunochallenge reducing a forager's real or perceived variance in the instantaneous risk of predation between covered and uncovered trays. This may occur, for example, if cover no longer aids an immunochallenged mouse in predator escape because of increased lethargy (see Raveh et al. 2011 for more examples). Teasing these effects apart would require more fine-scaled data on individual foraging behaviour.

The results of our study reveal a hidden fitness cost of immunochallenge. Activation of components of the immune system can be energetically expensive (Lochmiller & Deerenberg 2000; Demas 2004; Klasing 2004), can lead to reductions in other immune components and reproduction (Zuk & Stoehr 2002; Martin et al. 2006), and leads to the production of potentially hazardous free radicals (Bertrand et al. 2006). However, immunochallenge or pathogenic infection often appear to have little or conditional effect on measured phenotypes of an animal (Munger & Karasov 1991, 1994; Zuk & Stoehr 2002; Derting & Compton 2003; Nilsson et al. 2007; Schwanz et al. 2011b). The fitness effects of these stressors become even more difficult to measure in wild animals under field conditions (e.g. Munger & Karasov 1994). Our results demonstrate that foraging behaviour can be used as an indicator of parasite and immunochallenge effects in the field (Schmidt & Schauber 2007), suggesting in our study that mounting an immune response causes individual white-footed mice to accept greater mortality risks to compensate for the associated costs. Moreover, the change in foraging behaviour would probably produce a fitness cost of immune response in the form of higher mortality through predation. Importantly, these compensatory changes in foraging behaviour may prevent detectable changes in traits easily measured in the field, such as body mass and reproductive condition.

Because this study was conducted on wild mice in the field, we could not control for additional traits that may influence foraging behaviour. However, we were able to discount statistically the two alternative hypotheses for our observed pattern. Repeated trapping itself did not appear to produce risk sensitivity in our study. We also considered the possibility that individual attributes such as body condition, reproductive opportunities or personality might influence risk sensitivity in such a way that increased trap happiness (and therefore immunogen dose) and reduced preference for safe foraging sites (Biro & Dingemanse 2008), thus creating a secondary correlation between immunochallenge and foraging behaviour. We found no support for this explanation: trap happiness actually led to a greater preference for covered trays (greater risk sensitivity), and accounting for this attribute statistically reinforced the importance of increasing immunogen dose in decreasing risk sensitivity.

The connections of white-footed mouse foraging behaviour to community ecology in northeastern U.S. mixed hardwood forests are clear. A reduction in risk sensitivity and acceptance of risky patches for foraging mean that mice will use a greater proportion of their home range for foraging. As a result, they are likely to have

a higher encounter rate with patchily distributed incidental prey items, such as native, ground-nesting birds and invasive gypsy moths (Schmidt et al. 2001; Schmidt & Ostfeld 2003b; Connors et al. 2005). Thus, immunochallenge should indirectly enhance the control of some moth and bird populations by white-footed mice through behavioural alterations (Ostfeld et al. 1996a; Jones et al. 1998; Schmidt & Ostfeld 2003a). Similarly, because *I. scapularis* ticks are patchily distributed (Ostfeld et al. 1996b, c), mice with reduced risk sensitivity are more likely to encounter these ticks and tick-borne pathogens. The immune system therefore may play an important and underappreciated indirect role in community and disease ecology.

Although we have examined the behavioural response of white-footed mice to one specific immunogen, our results indicate that parasites and the immune response in general may have unanticipated indirect effects in communities. White-footed mice at our study site are infected with a variety of parasites (e.g. *B. burgdorferi*, other tick-borne pathogens, bot flies; Burns et al. 2005; Brunner et al. 2008); thus the mice in our study were experimentally exposed to live *E. coli* expressing OspA in addition to any concurrent natural infections. The behavioural responses to infection with live parasites may be specific to the type of parasite or to host traits (e.g. age; Adamo 1999; Velando et al. 2006). In particular, because *B. burgdorferi* does not express OspA inside the mammalian host, we do not anticipate the immune response to a live infection to be the same as to our OspA immunochallenge. Regardless, if infection with any parasite alters foraging behaviour in white-footed mice, it is likely to have indirect effects on songbird and gypsy moth populations and may indirectly facilitate or impede encounter rates with ticks and alter the epidemiology of the pathogens they carry.

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