Eco-Immunology:
Establishing a new research field

Let's Go Wild

A wild relative of C57BL/6

Photo: Fotolia/ Wotan

In some aspects, humans are nothing more than a wild animal. So why is most biomedical research only done on laboratory animals? In recent times, more and more immunologists have packed up their gear and ventured into the far more thrilling wilderness.

"When two become one" – it's a 'phenomenon' that doesn't only apply to human relations but apparently occurs in science as well. Throughout the last two decades, two very different and independent fields of research, ecology and immunology, have gotten in touch, become acquainted and are now finally making the arrangements to move in with each other for good. Their marital name on the front door: eco-immunology (sometimes known as 'wild immunology', too).

The foundations for this relationship were laid in the mid-nineties, when one of the field's seminal papers "Ecological Immunology: costly parasite defences and trade-offs in evolutionary ecology" by Ben Sheldon and Simon Verhulst, back then at the University of Uppsala, Sweden, was published (*TREE,* 11:317-21). In wild populations, it had been observed that the immune system doesn't react in a rigid but in a very flexible way. In their paper, Sheldon and Verhulst came up with explanations of what could be the cause of those varying responses, a question that is still at the heart of eco-immunology research.

The two authors argue that "immune function is costly" (in terms of calories and proteins). In order to mount an immune response, to fight off a nasty parasite, for example, resources (e.g. energy) need to be sucked from elsewhere. Sheldon and Verhulst speculate that "the operation (of immune function) requires resources that the host might otherwise have used for some other function". A 'trade-off' seems to be the only option and depending on every species' personal evolutionary history (life history) in concert with current biotic and abiotic environmental factors (reproductive state, co-infection, season etc.), this could be responsible for the observed immunological flexibility.

It's all about fitness

You simply can't have it all at the same time or else you'll end up in league with the devil. A so-called Darwinian Demon is a hypothetical model organism that doesn't have to put up with any "trade-offs". It has maximised all its fitness parameters (and by fitness we're not talking about strength and endurance training). Fitness in the evolutionary sense is defined by the ability of an organism to both reproduce and survive. If all resources were available without any limits, our hypothetical demon would live indefinitely, anywhere, and produce as many offspring as possible right after its own "birth" and continue this pattern throughout its existence. No such being exists (or hasn't yet been found) because all living organisms are held at bay by life itself. Life, as we all know, isn't always 'peaches and cream'. Amy Pedersen, 'wild immunologist' at the University of Edinburgh explains, "Life in the wild is tough. Individuals face ecological pressures that will affect their health and fitness. Many of these pressures will also affect immune function and the costs associated with mounting an immune response."

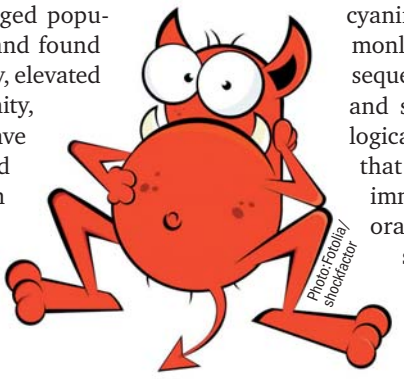
So, if the immune system is so easily influenced by our very surroundings, why not take immunological research to the outside world? Pedersen reasons, "The vast majority of our knowledge of the immune system is from the lab where animals have a constant supply of food and water, limited genetic variability and a more or less completely controlled environment. If we are to tackle the pressing issues of human, domestic animal and wildlife health that we currently face, one important step may be to test our vast knowledge of laboratory immunology in the wild, where conditions are far from controlled but may represent the true challenges of humans and wild animals." In her latest paper, simply entitled 'Wild Immunology' and co-authored by her colleague Simon Babayan (also from the University of Edinburgh), Pedersen has clearly set the agenda for future immunological research: "It is time to take immunology to the wild."

Even before arriving at this conclusion, eco-immunological research has put forward quite a number of scientific papers. In an editorial for *Functional Ecology,* Martin *et al.,* attest that "the field has grown rapidly"

over the last few years. They counted that 658 eco-immunologically inclined papers were published between 1991 and 2010, according to the ISI Web of Science database, and the authors furthermore found that the Sheldon & Verhulst paper alone has "up until now been cited over 700 times" (*Funct Ecol,* 25:1-4). Quite impressive! And, not to forget: two scientific journals, *Philosophical Transactions of the Royal Society B,* in 2008, and just recently, *Functional Ecology* ran a "special theme issue" featuring several articles about eco-immunology.

Sheep and wild mice

When ***Lab Times*** asked Amy Pedersen and Mark Viney, parasitologist at the University of Bristol, about what they think has so far been the most important finding, the opinions were divided. Amy Pedersen thinks, "The most significant piece of work to be published thus far was by Andrea Graham and colleagues in *Science* in 2010, titled 'Fitness correlates of heritable variation in antibody responsiveness in a wild mammal'. The authors investigated a wild/unmanaged population of Soay sheep and found that, counter-intuitively, elevated markers of auto-immunity, which other studies have found to be associated with lower infection burdens, were correlated with higher fitness. This is one of the first studies to link robust laboratory immunological tools to a measure of host fitness in a natural population" (*Science,* 330:662-5).



Could this be the elusive Darwinian Demon?

For Mark Viney, however, it's rather the finding that when it comes to immune responses, wild mice differ considerably from their lab-held brothers and sisters. In his own recent paper, Viney and co. caught some wild mice dwelling in a "multipurpose barn on a dairy farm near Bristol" and subjected them to some immunological experiments. The team immunised the mice with keyhole limpet haemocyanin (KLH), an antigen commonly used in the lab, and subsequently analysed their blood and spleen cells for immunological parameters. They found that wild mice made stronger immune responses than a laboratory strain, the highly responsive C57BL/6 strain. In more detail, the group revealed a higher baseline level of the immunoglobulins IgG and IgE and a more potent short term response; all pointing to a greater immune function in general. The authors also found a greater inter-individual variation in the immune response, which is, however, not very surprising when comparing outbred with highly inbred animals (*Mol Ecol,* 20(5):881-92).

So, ever more pointers make it increasingly clear that immunologists really should be boldly going where not many of them have gone before, i.e. into the wild. "Ab-

solutely!", Viney immediately responds. "Our study and the studies of some other colleagues have raised some interesting questions. The crucial question is why do these wild mice have the greater immune responses compared to laboratory mice? That's the next big question." He adds, "But I think one can never just work on mice in the wild, one needs to work on mice in the lab and in the wild – the comparison is interesting."

Out of the laboratory

Obviously, there are other advantages, too, when taking your studies to the field. When you're not too busy with office or other lab work, you can spend some time outside of the cramped confines of a usually dull and boring laboratory – become a trapper or simply enjoy a deep breath of fresh air. Study objects can be found near and far. In the past, a vast amount of eco-immunological research has focused on birds and insects. Richner *et al*. found, for example, that when they experimentally enlarged the clutch size of *Parus major* (Great Tit) so that the birds had to put more parental effort into their unhatched offspring, males had higher malaria infections (*Proc Natl Acard Sci,* 92:1192-4). And bumblebee (*Bombus terrestris*) workers that were allowed to forage had a reduced immune response (encapsulation response) to a novel antigen than workers, whose wings were clipped to prevent them from foraging and flying (*Proc R Soc Lond,* B260, 225-7).

photo: Fotolia/tiero

Many tools are still needed to continue work in the wild

But it's not only the birds and the bees that spark the interest of eco-immunologists. Studies are also conducted on other invertebrates like *Daphnia (Proc Biol Sci,* 277(1698):3291-7); on voles (*Microtus agrestis, Mol Ecol,* 2010 Nov 9) or reptiles like the ornate tree lizard *(Urosaurus ornatus)*. Outside the safe and cosy laboratory environment, female lizards showed a reduced wound healing response during vitellogenesis, an energetically costly stage during reproduction (*Gen Comp Endocrinol,* 155(1):148-56). Even plants have to pay for their defense efforts, in *Arabidopsis thaliana* this is reflected in trichome density and the total concentration of glucosinolates, a natural product that protects the plant against overly greedy animals (*Am Nat,* 151(1):20-8). Simply put, anything goes as long as it has some kind of immune system. On a side note, at least in the UK, wild animals are under the same legal protection as lab animals. One just needs to have the right permission.

Where are my tools?

However, not everything looks so rosy for our newly paired-up scientific fields just yet. And it's not the money that's causing sleepless nights, as both Mark Viney and Amy Pedersen receive their funding from traditional funding agencies like charities and/or UK research councils. "I think even though one could call this a new field, the questions that need to be addressed are all questions of biology. Some of the methods and approaches are new but the scientific endeavour is what everyone else is doing as well," Mark Viney points out. The biggest problem of eco-immunology lies elsewhere: "Tools need to be developed. It takes time and money but that is what needs to be done," he says.

For example, the field desperately needs reagents for non-model species. Antibodies are there but many of them only work on mouse or rat samples and usually have little cross-reactivity. However, to do proper immunological studies on wild animals you really need to have working tools. But then there's the question of what to measure exactly and how to interpret your data in a complex setting like the wild, where so many different factors contribute to the outcome of an immune response? Graham *et al.* gave a fitting example of the dangers of drawing conclusions from just one measure – "the immune measure for the dead". When *Daphnia* are infected by the bacterium *Pasteuria ramosa* they mount an immune response, which can be measured by counting the number of certain immune cells called plasmatocytes. However, *Pasteuria* not only infects but also sterilises its host, so it is no longer able to reproduce – one aspect that constitutes host fitness and "thus, a strong immune response is not associated with high fitness, but rather is tightly linked to genetically dead" (*Funct Ecol*, 25:5-17). Graham *et al.*, therefore conclude that eco-immunologists should routinely "measure three intertwined parameters: host fitness, parasite density **and** relevant immune responses".

Or as Amy Pedersen and Simon Babayan simply suggest in their latest paper: "to measure as many factors as possible". This does sound reasonable, considering the circumstances but, at the same time, it also sounds very time-consuming and pricey. "This is absolutely true. To do wild immunology, it will take a significant investment in creating appropriate tools and techniques. But once these immunological, genomic and/or post-genomic tools are developed, they should be applicable to many eco-immunological questions and possibly several host-parasite systems. These costs may be great, at least initially, but are probably unlikely to be more expensive than current laboratory immunological studies that include extensive animal husbandry."

Measuring everything

Mark Viney also believes that companies will join in and help to develop new tools, "They might be surprised at how widely those tools can be used, actually. I think there's a potential there." He adds, "Some of us are planning to develop our own tools just to get on with the science."

And in order to get on even more quickly, in the US, ecologists, evolutionary biologists and immunologists have banded together, and recently founded the Research Coordination Network in Eco-immunology. This network, which also has some European researchers in it, is funded by the National Science Foundation and aims at "developing new tools and techniques, enhancing breadth and depth of research questions, establishing new interdisciplinary collaborations, and stimulating outreach and training opportunities for scientists, high school teachers and the public broadly". This is planned to be achieved by annual workshops, training exchanges between labs, website development to facili-

tate communication of methods and techniques (the website *www.ecoimmunology.org* already features a collection of buffer recipes and immunological protocols) and the organisation of symposia. The network's first-ever meeting "Standardizing methods and theory in eco-immunology" took place at the University of South Florida, Tampa only last year.

Networks and communities

Incidentally, the second meeting of the Research Coordination Network titled "The Costs of Immunity" will take place in the UK on June 30th. Even though, according to Mark Viney, who is also a participant in the US network, a similar network isn't in the plans for Europe just yet, "Those of us in the UK who started to work on immune function and infection in wild animals have been or are in the process of putting together an application to work together more. I think there is a community developing."

A small community has already developed in the north of Great Britain, in Edinburgh. Here the Centre for Infection, Immunity & Evolution (CIIE), funded by the Wellcome Trust, has been established by the city's university. The Centre's mission is to "bring together research leaders in infectious disease, bridging across from immunology and immunity to infection, to mathematical and evolutionary biology". Part of the CIIE is also the recently formed "Wild Immunology group", in which eight groups, amongst them the groups of Amy Pedersen and Andrea Graham, will unite their eco-immunological research interests. "The University of Edinburgh has a strong tradition of research in both evolutionary biology and infection and immunology, as well as interdisciplinary projects between these two groups," explains Pedersen. But also, elsewhere (at the universities of Bristol, Nottingham, Liverpool, Kiel, Bourgogne [Dijon], Modena and Reggio Emilia), researchers are 'going wild' to solve the mysteries of immune function in natural systems.

photo: Fotolia/Leonid Smirnov

One possible method to catch wild mice.

As one could imagine, establishing a new field is not exactly a piece of cake. "Yes, it is hard to start a new project, a new field. Even though our experiments went very well, there were things we attempted that didn't work so well because we were trying it for the first time. But science also relies on people to do new things for the first time," Mark Viney confirms. With everything that is new and fresh, there's also "a lot of excitement and enthusiasm" to find out more, as Amy Pederson has observed. This momentum could give the new discipline the push it needs from backstage to the main stage of the scientific research scene.

The future of eco-immunology?

What do Amy Pedersen and Mark Viney expect and prophesy for the future of eco-immunology? Amy Pedersen hopes that "bringing the tools and techniques of immunology into wild systems will firstly, contribute to our understanding of the dynamics of natural populations and secondly, inform the development of medical intervention strategies for humans, whom are about as genetically and environmentally diverse as the wild systems we study." Whereas Mark Viney thinks, "The field will grow and come to be seen to be central to infectious disease biology and immunology research. Such that perhaps in 20 years' time, nobody will think about doing the immunological studies solely in the laboratory and always consider working in wild populations as well."

So it seems like the two research fields were, after all, meant for each other. If everything goes as planned, this happy and, hopefully, fruitful liaison will bring forth many new insights into our own wild nature.

Kathleen Gransalke

The full-length interview with Amy Pedersen and Simon Babayan is available on our website *www.lab-times.org*.

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