the success of this programme is that all the microorganisms are maternally inherited. Preliminary evidence suggests that *Wolbachia* mediate CI in tsetse flies, and Aksoy believes that this can be used to drive genetically engineered s-symbionts into the tsetse fly population. The research is progressing well – the s-symbionts are in culture and express foreign genes and, after microinjection into pregnant flies, are transmitted to the progeny. The search is now on for a suitable gene product that will enable the s-symbionts to disrupt the transmission of the trypanosome parasite.

One of the main problems confronting the use of *Wolbachia* to drive foreign genes into insect populations is the possibility that a target pest will already harbour one, or even two, *Wolbachia* strains. Work in the laboratory of Scott O'Neill (Dept of Epidemiology and Public Health, Yale University) has addressed this problem by establishing a triple infection in an already double-infected *Drosophila* population. The triple infection is stable and will replace both single and double infections, indicating that these 'superinfections' could be used to repeatedly sweep genes into insect populations. O'Neill is also concerned that many multiple infections are missed in the field because the current PCR-primers used to diagnose infection do not target a sufficiently variable gene. His laboratory has recently cloned a gene encoding a major surface protein from *Wolbachia* (the *wsp* gene), which is highly variable and should prove useful in selecting different *Wolbachia* strains to combine in multiple infections. Development of the *wsp* gene has already broken new ground; using antibodies raised to the recombinant protein, O'Neill has determined the tissue distribution of *Wolbachia* in several insect species. Contrary to previous assumptions, *Wolbachia* are not restricted to the gonads but are distributed throughout the somatic tissues, including the gut, salivary glands and haemolymph. In O'Neill's words, '*Wolbachia* occupy the perfect cellular compartments for expressing and secreting gene products which would interfere with the ability of insects to transmit disease agents'.

Of course, there is a long way to go before any benefits are realized from the use of *Wolbachia* in insect control. In particular, foreign genes have yet to be expressed in *Wolbachia*, and inconsistencies between field and laboratory trials need to be resolved. Nevertheless, these are exciting times for *Wolbachia* research, not least because the resurgence of several vector-transmitted diseases in recent years has highlighted the need for novel approaches to combat insect pests. If the current momentum can be maintained, and all the signs from this recent symposium are that it can, then *Wolbachia* could prove the ideal candidate to fulfil this need.

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Local adaptation and host–parasite interactions

The environment and, in particular, the biotic component of the environment experienced by every organism is usually variable in space and time. This is particularly true for parasites: their environment (the host) is predominantly biotic; this can be highly heterogeneous both qualitatively (different genotypes, host races and host species) and quantitatively (abundance of host individuals). The question of how organisms evolve in heterogeneous environments was explored at a recent workshop on local adaptation in host–parasite interactions. The workshop was held in January at the Université P. & M. Curie, Paris, France, was co-organized by Yannis Michalakis (Université P. & M. Curie) and Susan Mopper (University of Southwestern Louisiana, Lafayette, USA) and was jointly funded by the French Centre National de la Recherche Scientifique and the United States National Science Foundation. The presentations featured very different host–parasite systems, such as copepods on flatfish (Thierry de Meeus, University of Montpellier, France), phytophagous insects (Isabelle Olivieri, University of Montpellier), viruses (Gregory Dwyer, University of Chicago, IL, USA), and parasitic cuckoos (Anders Pape Møller, Université P. & M. Curie).

Adaptations to spatially heterogeneous environments

As pointed out by Robert Holt (University of Kansas, Lawrence, USA), four general adaptive mechanisms can be observed among parasites that evolve on spatially heterogeneous hosts. The most efficient way to adapt to a host is to settle actively on the most suitable host. This host preference was demonstrated for insects on their plant hosts by Kim Waddell (University of Maryland, College Park, MD, USA) who presented studies of a woodboring beetle (*Brachys tessallatis*) on oak tree (*Quercus*) hosts. Michael Singer (University of Texas, Austin, USA) presented long-term data on checkerspot (*Euphydryas*) butterflies that exhibit host choice for either *Veronica* or *Plantago* (plantain)[1].

In many cases, however, the infection is random and parasites are not able to choose their host actively. In this situation, different strategies can evolve. First, individuals can adopt a plastic behaviour that allows them to exploit each resource efficiently by changing their own phenotype in different habitats – Møller showed that parasitic cuckoos can adopt a plastic virulence strategy that depends on the level of host resistance[2]. By being more virulent on more-resistant hosts, this facultative strategy might counteract the evolution towards higher resistance in the host[3]. Second, individual organisms can evolve towards a very general exploitation strategy. For example, Richard Lenski (Michigan State University, East Lansing, USA) demonstrated that several bacteriophage species can evolve host-range extension (i.e. generalism) to counter bacterial resistance.

Another example of a parasite generalist strategy was presented by Michael Polak (Syracuse University, NY, USA), in which mites (*Macrocheles subbadius*) parasitize several *Drosophila* species. However, if generalist strategies do not evolve, but

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individuals exhibit different performances across habitats, then selection can promote the emergence of an adaptive genetic structure. As emphasized by John Thompson[4] (Washington State University, Pullman, USA), adaptive structure resulting from coevolutionary processes at the landscape scale can result in a geographic mosaic of locally adapted or maladapted species complexes, depending on the specific mechanisms involved (Box 1).

Box 1. Reciprocal transplant experiments in host–parasite systems

The reciprocal transplant experiment is a test to detect the existence of adaptive genetic structure. It compares the performance of parasites (e.g. infectivity, within-host growth rate and survival) on native and novel environments (which can be different host individuals, populations, or species). Controls are required for the conditioning effects of parasites during development on individual hosts, or of hosts in different abiotic environments. Because models predict that host–parasite systems are intrinsically highly variable in space and time, general predictions of local adaptation hold only on average[5,6]. Therefore, studies should ideally consider a large number of host and parasite populations, and individuals should be sampled from sites located at different distances from each other. This will show the spatial scale at which local adaptation occurs and whether local adaptation is correlated with distance (a condition predicted by many host–parasite models).

There are two distinct ways to test for local adaptation in a reciprocal transplant experiment:

(1) Compare the performance of a parasite population in different environments (hosts) – local adaptation occurs when the performance of the parasite population in its native environment is greater, on average, than its performance in novel environments.

(2) Compare the performance of a parasite population in its native environment with other parasite populations transplanted there from different environments – local adaptation occurs when the performance of the 'native' parasite population is greater than the average performance of the transplanted populations.

In practice, it is difficult to choose between these two tests. Both give different information on the occurrence of adaptive genetic structure, and both should indicate local adaptation, despite their fundamental differences. An alternative method pools the information by averaging over the whole transplant experiment. In this situation, local adaptation is no longer a property of a given population, but a general pattern that describes the adaptive genetic structure of both the host and the parasite. Several examples for each classification of local adaptation have been determined, yet they each characterize different situations – it is thus important for studies to clarify the criteria being used.

When do we expect local adaptation?

A prerequisite for local adaptation is spatial heterogeneity of the environment. If the host does not evolve in response to the parasite (i.e. there is spatial variability but no temporal variability in the host population), the classic prediction is that local adaptation only occurs when the migration rate of the parasite is very low. Mark Kirkpatrick (University of Texas, Austin) illustrated this idea using a model in which the host population is continuous in space but has a defensive trait that varies along a gradient[7]. In this situation, the increased gene flow of the parasite counteracts adaptation to local conditions.

When the host coevolves with the parasite, the environment is changing both in space and in time, and the role of migration is more complicated. Yannis Michalakis presented a model in which migration can actually promote the emergence of local adaptation, because gene flow introduces genetic variability upon which selection can act (an idea pursued in source–sink models presented by Holt[8]). In this model[5,6], the migration rate of the parasite, relative to that of the host, determines local adaptation. If the parasite migrates more than the host, it should become locally adapted. Conversely, if it migrates less than the host, the parasite will be locally maladapted.

A more complex and realistic way to introduce spatial heterogeneity is to consider that both biotic and abiotic environments (e.g. the quality or amount of resource available for the host) vary in space. Michael Hochberg (Université P. & M. Curie) formalized this question and showed that, in productive environments, hosts allocate more energy to parasite resistance and, consequently, parasites adopt a more virulent strategy[9]. Reciprocally, in less productive environments, both the host and the parasite invest very little in coevolutionary interaction (i.e. hosts are poorly resistant and parasites are avirulent).

When does local adaptation occur?

The most thorough way of testing local adaptation employs reciprocal transplant experiments (Box 1). Serge Morand (Université de Perpignan, France) reported results from such experiments that provided evidence for local adaptation in several *Schistosoma*–snail interactions. Mike Antolin (Colorado State University, Boulder, USA) demonstrated that populations of the parasitoid wasp *Diaeretiella rapae*, which attack a number of hosts, are genetically subdivided at a small spatial scale and that they perform better on their natal host environment relative to a novel host environment. Hans-Joachim Carius (University of Basel, Switzerland) transferred parasites (the microsporidium *Glugoides intestinalis*) to novel hosts (*Daphnia magna*), showing that parasite fitness was strongly reduced on allopatric hosts. Peter Van Zandt described a meta-analysis showing that, in general, phytophagous insects tend to be locally adapted to their host plants (although, in several cases, no local adaptation was detected). Oliver Kaltz (Université Paris Sud, France) presented the results of a transplant experiment using a system where the host (white campion, *Silene latifolia*) has much higher gene flow than the parasite (*Microbotryum violacea* – anther-smut disease). Contrary to most of the other transplant experiments, he found that the parasite was locally maladapted, corroborating the prediction that if there is coevolution, low parasite gene flow prevents local adaptation of the parasite[5,6].

Another way to study local adaptation is to follow the process of adaptation over several generations. Although these tests require long-term studies, they demonstrate evolutionary patterns more clearly. Using trees of known ages, and experimentally manipulating the extinctions of leafminer populations, Susan Mopper showed how the level of local adaptation of leafminer populations to their individual host tree changed drastically over time[10]. Organisms with shorter generation times may further clarify the mechanisms that promote local adaptation over evolutionary time scales. Dieter Ebert (University of Basel) reviewed evidence from serial passage experiments of pathogens in multiple hosts, which indicated that parasites generally evolve towards higher growth rates within hosts but lose the ability to infect novel hosts, thereby becoming locally adapted. The system presented by Richard Lenski for studying evolutionary relationships between bacteria and bacteriophages is also useful for studying local adaptation, because coevolutionary processes can be followed over several hundred generations in a short time[11].

Perspectives

The study of local adaptation in host–parasite systems is a growing field of research, and discussions at the workshop resulted in many new directions. Timothy Mousseau (University of South Carolina, Columbia, USA) emphasized that mate choice, host preference and clutch size are among the many maternal effects that must be accounted for, but are often ignored, in studies of local adaptation[12]. Philip Agnew (Université P. & M. Curie), Michael Hochberg and Michael Singer highlighted the importance of the effects of abiotic factors in host–parasite interactions. Matthew Parker (State University of New York, Binghamton, USA) showed how local adaptation in host–parasite interactions might be extended to mutualistic interactions[13]. William Boecklen (New Mexico State University, Las Cruces, NM, USA) and Sharon Strauss (University of California, Davis, USA) showed that local

adaptation occurs even within strongly interacting, complex communities. These results demonstrate that multiple species interactions do not necessitate the diffusion of coevolutionary relationships. Emphasizing the value of this community perspective, Allen Herre (Smithsonian Tropical Research Institute, Balboa, Panama) and Richard Lenski both showed that an understanding of host–parasite specificity can aid in understanding mechanisms that promote community diversity. Integrating this community-level perspective with the mechanistic, model-oriented view of much host–parasite research will lead to a broader understanding of local adaptation in host–parasite interactions.

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Establishing cryptic female choice in animals

In studies of paternity where females accept multiple mates, the explanatory mechanism responsible for variation in male reproductive success has generally been attributed to sperm competition. This is viewed as a process of competitive male–male interactions[1]. This emphasis is partly because of the technical difficulties of demonstrating a role for females in sperm usage, which requires direct observation of sperm movement inside females. Current convention, however, relies on postfertilization protocols to measure paternity. This is unfortunate because copulatory and postcopulatory mechanisms of so-called 'cryptic female choice' – such as females selecting how many sperm from each male are initially stored, survive during storage or are lost during remating – may be equally important factors that bias reproductive success towards certain males[2]. Although cryptic female choice has been extensively publicized, its importance is still debated[3,4].

Another reason for the emphasis on sperm competition is that there is arguably greater selection on males to ensure fertilization than there is on females to use sperm differentially from mates[5] (even though genetic benefits for offspring resulting from female mate choice are now widely accepted). Patterns of sperm usage are conventionally defined as the proportion of eggs fertilized by the second male when females are doubly mated (P_2). In support of selectional asymmetry, mathematical models that predict fertilization patterns based solely on rates and numbers of sperm transferred and displaced by males, without incorporating differential responses by females to individual males, have had considerable success in predicting P_2 in several species[4,6,7].

Qualitative arguments have been used to promote the view that females can influence the way in which a male's sperm is used[2]. For example, analysis of the functional morphology of female reproductive tracts clearly shows that they have the potential to preferentially store, transport or extrude sperm from successive mates. Ultimately, however, experimental evidence is required to show that variation among females, or variation in their responses to different classes of males, also accounts for variation in P_2. If female traits account for variation in P_2 then they become available for sexual selection. It is these quantitative, statistical data that have been in short supply, with examples coming from studies across species or genetic strains rather than from within single populations (Box 1).

A recent study by Nina Wilson *et al.*[8] provides compelling evidence that female bruchid beetles (*Callosobruchus maculatus*) influence P_2 values within a population. The experimental design used was similar to that of an earlier study on flour beetles (*Tribolium castaneum*), where a pair of males was mated to a series of females, with male mating order held constant[9]. By using several pairs, this approach allows for an estimate of the variation in P_2 attributable to differences among pairs of competing males. If variation among males is important, then a given pair of competing males should generate similar P_2 values when mated to each female. Statistically, this means that P_2 for male pairs is repeatable because there is greater variation among pairs than within them.

Wilson *et al.* perceptively extended this methodology by also allowing for an estimate of the variance in P_2 that could be attributed to female genotype. Their approach was as follows. Each replicate consisted of two unrelated males who were

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