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Conference report

The Second International Congress on Phthiraptera (Lice), held 8–12 July 2002 at the University of Queensland, Brisbane, Australia

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Lice (Phthiraptera) are insects of major medical and veterinary importance as parasites, pests, and vectors of animal and human diseases. Almost 5000 species are recorded from all groups of birds and many mammal species. While many lice are a seemingly benign presence on their host, this belittles their economic, medical, and evolutionary significance. The Second International Congress on Phthiraptera (ICP2) brought together many of the world's leading authorities on lice and louse-borne disease to present the latest developments in the control, treatment, and evolution of these parasites.

Despite a gap of nearly three decades since the first international congress devoted to lice, almost 100 delegates from at least 20 countries attended the 5-day meeting. In the interim, the medical and biological community has undergone a revolution in their approach to understanding infectious disease. In particular, evolutionary biology is yielding dramatic insights for health scientists, and this was evident from the diverse mix of participants including representatives from the medical and veterinary community, alongside systematists, evolutionary biologists and comparative ecologists. This conference was an attempt to integrate some of the principles of evolutionary biology and ecology with those from medical and veterinary science, providing a rare chance for scientists from these diverse disciplines to interact.

When good parasites go bad

It is often advocated that obligate parasites evolve towards benignness, and lice are taken to epitomise an evolutionary stable association between host and parasite. This assertion was challenged by Dale Clayton's group (University of Utah, USA) through a series of elegant experiments that document the reciprocal selective forces between lice and their hosts. Virtually all species of bird and many species of mammal are host to one or more species of louse, and evidence of cospeciation in many host-lice assemblages is taken to indicate the antiquity of this association. With a series of experimental manipulations, Clayton and his colleagues measured the reciprocal fitness effects that lice and their hosts exert on each other. Specifically, he showed that louse infestation in many bird species is a selective force acting on bill morphology, such that their form represents a compromise between the optimal shapes for feeding and for preening. Similarly louse morphologies are selected that facilitate their escape from preening. Thus, hosts and their lice are involved in a continuing "arms race" between host defence and parasite escape.

Lice and public health: policy and practice

Over the last century, rising living standards and improved hygiene levels have led to a marked fall in the prevalence of human body lice, and in the developed world they are principally confined to the underprivileged in society. In contrast, head louse infection, particularly among school children, crosses all social barriers, and in many countries has reached epidemic proportions. While it is not clear whether the global incidence of head louse infection is truly rising, rapid growth in the sale of anti-lice treatments (pediculicides) has recently been widely reported, and the best method of head louse control is the subject of intense debate. These issues were considered in two special symposia at the ICP2 conference.

The recent Cochrane Review (Dodd 2000) on interventions for treating head lice was criticised by Hilde Lapeere (Ghent University, Belgium).

She described the study as flawed, due to the inclusion of trials that have been described as "unsound" in other systematic reviews of head louse treatments, and in the reviews' omission of studies that provide evidence of alternative diagnostic techniques and treatments. Ian Burgess (Insect Research & Development Ltd, UK) responded on behalf of the report's author, Ciara Dodd, stating that many available studies on head louse treatments were sub-standard and that much of the clinical evidence on all methods of head louse eradication are of poor quality. This theme was echoed by several other studies reported at ICP2, detailing practical difficulties in evaluating the incidence of head louse infection before and after clinical trials. María Picollo (CITEFA, Argentina) outlined a laboratory protocol for evaluating the biological effects of pediculicides. Her work suggests that the addition of aliphatic alcohols enhanced the activity of several commercial insecticides, perhaps by enhancing their absorption through the chitinous exoskeleton of the louse.

Several health charities worldwide advocate the use of combs as the first line of defence against head lice. Joanna Ibarra (Community Hygiene Concern [CHC], UK) presented data on the "bug busting" approach for louse detection and removal. This mechanical method of control developed by CHC has been exported from the UK to Belgium and Denmark. It has subsequently received backing from the UK Department of Health as an alternative to insecticide treatment. Ian Burgess presented data on the management of head louse infections in the UK. He raised concern on the increasing reliance on unregulated procedures and alternative treatments that may be less effective and/or more toxic than modern insecticides available for managing head lice.

The use of nit combs has been a major cultural component of louse control for centuries. Kosta Mumcuoglu (Hebrew University, Israel) provided details on the archaeological evidence of louse infestation among ancient inhabitants of the Middle East dating back 9000 years. He showed a surprised audience that the act of "delousing" is even the principal subject in many major artworks, including 24 paintings by Flemish and Dutch masters during the 17th century. In a modern clinical study Mumcuoglu showed that use of a nit comb provides a four-fold improvement in the detection of head lice, compared with direct visual examination. Hilde Lapeere showed that the diagnostic utility of combing was further improved if hair is wetted

(preferably with addition of a hair conditioner) before combing. The design of combs plays a major role in their effectiveness at removing nits and lice. Kim Larsen (KSL Consulting, Denmark) highlighted design flaws in several modern combs, allowing many nits to pass through without even touching the comb's teeth.

Pediculicide resistance

The speed with which head lice acquired resistance to many common pediculicides has raised alarm within the medical community. Nigel Hill and colleagues at the London School of Hygiene & Tropical Medicine highlighted the factors responsible for the rapid onset of resistance in the UK head louse population. He proposed that prophylactic use of head louse treatments be prohibited, and stressed the need for stricter regulation in the application of new compounds to extend their effective life.

The two major mechanisms by which insects acquire biochemical resistance to insecticides are target-site resistance, whereby insecticides no longer bind to their target, and detoxification-based resistance, through which enhanced enzymatic activity prevents the insecticides from reaching their site of action. Both have been recorded in lice. Kosta Mumcuoglu and colleagues at the Liverpool School of Tropical Medicine described their work on the voltage gate sodium channel, the target site for pyrethroids and DDT. They located several point mutations in the para-orthologous sodium channel α -subunit of lice. These were incorporated into a simple PCR based diagnostic assay that could be used to monitor the frequency of louse pyrethroid resistance. William Brogdon (Centers for Disease Control [CDC], USA) described the role of increased cytochrome P450 activity in insecticide detoxification. Using fluorescence PCR analysis of oxidase expression in lice, he showed how the level of insecticide resistance could be monitored.

A possible solution to the problem of resistance would be the production of a louse vaccine. Astrid Kollien (Ruhr University, Germany) presented data on the mechanisms controlling production and secretion of digestive enzymes in the human head louse (*Pediculus humanus*) with this long-term goal in mind. Blocking enzymatic activity within lice by inoculating the host with antibodies specific to louse digestive enzymes would prevent lice from feeding. Using a cDNA library from *P. humanus*, Kollien identified and expressed two genes involved in the synthesis of louse digestive enzymes. Ongoing

research is attempting to purify and characterise these recombinant proteins, which might be used in production of a vaccine.

Lice and bacteria

Despite the crushing impact that epidemic typhus, relapsing fever, and trench fever have had on humans, little is understood about the interactions between the bacterial agents of these diseases and their louse hosts. Gregory Dasch (CDC, USA) described how the application of automated sequencing has improved our understanding of this unique interaction, yielding the possibility of new approaches for controlling louse infestation. The complete genomes for the etiological agents of epidemic typhus and relapsing fever are now available, alongside substantial data for *Borrelia* (the causative agent of trench fever), the human genome, and genetic data for the human body louse. Dasch showed how intensive work with DNA and protein arrays can permit identification of specific gene products essential for the replication of these infective agents in lice. These genetic tools could also provide a means of assessing the immunological responses of humans to lice.

With the exception of those bacteria associated with louse-borne disease, the bacterial flora of chewing and sucking lice is poorly known. Anna Murrell and colleagues at the University of Queensland surveyed bacterial diversity in lice using partial small subunit rRNA sequences. They found evidence of *Bacillus* and *Proteus*, which have potential use as biopesticides. However, as with most of the bacterial genera isolated from lice, their distribution is patchy. David Reed (University of Utah, USA) focused on tracing the coevolutionary history of lice and their endosymbiotic bacteria. Endosymbionts in other arthropods are known to produce essential amino acids necessary for host survival, aid digestion of complex foods, and manipulate host reproduction. However, their role in lice remains unknown. Reed used the presence or absence of common endosymbionts such as *Wigglesworthia* to speculate on their function in lice.

Gregory Dasch used a similar approach to understand the coevolutionary history of *Rickettsia prowazekii*, the etiological agent of louse borne typhus. Biochemical assays of *R. prowazekii* isolates were remarkably similar despite marked differences in their origin. This is consistent with the view that *R. prowazekii* is a highly conserved species. However, limited strain differences were observed that show some correlation with their geographic

origins. These differences may be useful in future epidemiological investigations.

Lice in livestock

Many of the insecticides used to control ectoparasites of human importance are spin-offs from agricultural research. Development of new compounds by pharmaceutical companies is usually focused on the most economically attractive markets, and with lice costing the domestic livestock industry many hundreds of millions of dollars each year, most attention has focused on the lice of veterinary significance. Kim Larsen described how human louse treatments are still dominated by well-established insecticides, many of which have limited use due to resistance or unacceptable side effects. In contrast, a raft of new compounds such as the macrocyclic lactones, arylpyrazoles, and insect growth regulators are being used with great success in the livestock industry. Larsen described how changing market conditions could lead to these new pediculicides becoming available for human treatment in the near future.

Peter Bates (Veterinary Laboratories Agency, UK) described the role lice play as an indicator of underlying welfare problems within domestic livestock. Using sheep lice as an example, he showed how the lifting of compulsory dipping for sheep scab mite (*Psoroptes ovis*), and the replacement with endocide injections as their principal means of control, caused a surge in UK sheep louse infestations. Although sheep with a pre-existing scab infection are very prone to louse infections, sheep with a pre-existing louse infection are very difficult to infect with the sheep scab mite. This raises the possibility that low louse infestations, which have a minimal economic impact on sheep, could be used as a control against the more dangerous sheep scab mite.

Cophylogeny, cospeciation, and coevolution

Mark Hafner (Louisiana State University, USA) described his pioneering work on cospeciation between pocket gophers and their chewing lice. This "model system" has dominated recent methodological and empirical debates on cospeciation, and remains the paradigm example of host parasite cophylogeny. Hafner and colleagues showed how the genetic structure within pocket gopher lice was highly correlated with the genetic structure of their host populations—a relationship that stretches back to the deepest branches of the gopher's phylogenetic tree. Experimental transfers showed

that host specificity is maintained by the diameter of the host hair shaft. Pocket gopher lice are able to survive and reproduce on non-native hosts when their host's hair diameter is smaller. Hosts with larger hair shafts cannot be colonised as the louse morphology prevents them from gripping the oversized hair shaft. In conjunction with the isolated, fossorial lifestyle of pocket gopher populations, this has allowed the host-parasite association to remain intact for several million years.

Micro- and macroevolutionary patterns in host louse assemblages were also explored by Kevin Johnson (Illinois Natural History Survey). The dispersal ability of lice across host populations and species impacts the genetic structure of the louse population and subsequently louse speciation. Thus, microevolutionary processes can influence macroevolutionary events such as cospeciation. Across various bird louse systems Johnson showed a general correspondence between the dispersal ability of lice and their degree of cospeciation. Lice with lower dispersal generally exhibit more cospeciation with their hosts.

The arrangement of the mitochondrial genome in insects has long been held to be highly conserved. Work presented by Renfu Shao and colleagues (University of Queensland, Australia) challenged this assumption, showing that thus far, lice are the exception to this rule. The complete mitochondrial genome of the wallaby louse was sequenced and compared to complete and partial genome arrangements for over 400 other insect species. Relative to the ancestral gene arrangement of insects, all 22 tRNA genes are rearranged, and of the 15 protein coding genes, all but six have been translocated and/or inverted. The largest non-coding region in the genome was just 73 bp long, and is among the shortest non-coding regions observed thus far in animals. Work by Cath Covacin and colleagues (University of Queensland, Australia) highlighted the phylogenetic utility of mitochondrial gene order in other louse groups, which show rearrangements even within selected louse families. These may explain the elevated rates of mitochondrial substitution and hypervariable secondary structures of rRNA genes that were reported in several other studies presented at ICP2.

Species concepts

Arguably, the debate over what constitutes a species is at an impasse, and such a discussion might seem out of place at a conference on lice. However, defining the boundaries for gene flow within taxa are

of critical importance if we are to better understand the epidemiology of parasites and the diseases they transmit. To this end, lice are no exception. Perhaps the best example of this is with human head and body lice. These taxa exhibit startlingly different ecological and behavioural characteristics, and only body lice are responsible for the natural transmission of major louse-borne disease in humans. They have been traditionally defined as different species; however, Natalie Leo and colleagues (University of Queensland, Australia) presented evidence suggesting that human head and body lice are conspecific. Based on data from the mitochondrial COI gene, Leo showed that variation in haplotype frequencies could be better explained by geography, than by differences between head and body lice. This has important implications for the study of louse borne disease, and offers the prospect of studying parallels between gene flow in lice and their human hosts.

Different concepts over what constitutes a louse species have plagued louse taxonomy for centuries. Some researchers consider lice to be passed down like genetic heirlooms from one host generation to the next, and thus host species limits provide the deciding factor when evaluating the taxonomy of lice. To others, host associations are of secondary importance, and need to be examined in conjunction with independent data. This issue was the subject of a special debate chaired by Ricardo Palma (Museum of New Zealand Te Papa Tongarewa), during which consensus was sought over the criteria used to define species limits for lice.

Genetic data are playing an increasingly important role in defining species limits. Noah Whiteman (University of Missouri-St. Louis, USA) presented data from his study on the population genetics, coevolution, and ecology of lice from the Galapagos hawk. Examining prevalence, intensity, and the genetic diversity of louse infections, Whiteman showed how these correlate with ecological and behavioural characters of their hosts. Galapagos hawks in breeding groups have fewer lice than non-breeding birds. Hawk louse populations from different islands show more genetic variation and population structure than their hosts. This pioneering study is one of the first to examine the population genetics and host correlates of an avian louse species in detail, and offers the prospect of resolving determinants of louse population structure.

The meeting concluded with a decision to convene the next Congress in 2006, and to accept the invitation of María Picollo to meet in Buenos Aires,

Argentina, hosted by the Centro de Investigaciones de Plagas e Insecticidas.

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