International conference on Malaria and Related Haemosporidian Parasites of Wildlife

Dedicated to the memory of professor P. C. C. Garnham

Hosted by Nature Research Centre and Lithuanian Academy of Sciences

August 7th – 11th, Vilnius, Lithuania

Vilnius, 2013

Sponsors

The conference is funded by the European Social Fund under the Global Grant measure.

Malaria Research Coordination Network (http://malariarcn.org) has supported lectures and provided partial support for students and other researchers to attend this conference.

Scientific Organising Committee

Local Organising Committee (Nature Research Centre)

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Contents

Welcome to Vilnius

It is our great pleasure to welcome you to the international conference on ***Malaria and Related Haemosporidian Parasites of Wildlife***, hosted by Nature Research Centre and the Lithuanian Academy of Sciences, in Vilnius, Lithuania.

The conference was organised to strengthen professional links, and to exchange ideas and information among scientists dealing with similar parasitological problems in the field of wildlife malaria parasites (*Plasmodium*, Plasmodiidae) and related haemosporidians (Haemosporida). For decades, these parasites have been important models for the study of human malaria and, therefore, became objects of intensive investigation. Indeed, an early Nobel Prize winner, Sir Ronald Ross, gained many of his insights by studying *Plasmodium* species in sparrows. Malaria parasites and related haemosporidians infect all major groups of terrestrial vertebrates. Despite being nearly forgotten in human malaria research, these organisms remain an important source of ideas and information in evolutionary biology, and they certainly warrant more attention in conservation biology and veterinary medicine.

This conference is dedicated to the memory of eminent parasitologist professor Percy Cyril Claude Garnham (1901-1994), who was one of the rare breed of medical professionals curious and enthusiastic about wildlife haemosporidian research. His elegant studies on haemosporidian parasites of lizards, snakes, birds, bats, rodents, monkeys, apes, deer and even hippopotamus, as well as prominent taxonomic collections and the classic monograph *Malaria Parasites and Other Haemosporidia* (1966), remain important resources for current investigations on the diversity of wildlife malaria and related haemosporidians, with their extraordinary life cycles and beautiful structures.

The purpose of this international conference is to present and discuss scientific results and stimulate future research and education in the area of haemosporidian parasites of wildlife. The gathering of actively working scientists, environmental experts, and wildlife healthcare professionals will stimulate a robust dialogue regarding the main taxonomic, evolutionary, genomic, and conservation problems in this field of research. The conference will promote international exchange and enhance collaborations throughout the world for wildlife malaria research, and the conservation and health of terrestrial vertebrates. This meeting will provide an opportunity for extensive interdisciplinary discussion into current problems of wildlife haemosporidian investigation. Your participation will contribute to the success of this rare event in wildlife malariology and we welcome you to the conference.

Vilnius, the capital of Lithuania, is situated near the geographical centre of Europe. Most roads between East and West intersect here. Vilnius with its beautiful baroque-style Old Town and surrounding rivers, lakes and forests is a gateway into a rich and historic past, as well as a modern European capital. The various activities associated with the conference, and the charming summer setting, will make your journey an enjoyable and memorable experience.

In the beginning of August, the climate is pleasant, and there is daylight well into the evenings. We trust that the scientific and the social programmes, as well as the venue location, will provide an atmosphere of fruitful and friendly exchange of new and important knowledge, and provide opportunities for establishing new personal contacts.

We wish you such a pleasant stay in Lithuania that you will decide to come back soon!

The Scientific and Local Organising Committees

Programme notes

1. **Invited speakers.** The Organising Committee is pleased to be able to announce that the following experts will give lectures on various aspects of wildlife haemosporidian research:

Keynote lectures:

- Ulrike Böhme, The Wellcome Trust Sanger Institute, UK
- Robert Sinden, Imperial College London, UK
- Yoshio Tsuda, National Institute of Infectious Diseases, Japan

Plenary lectures:

- Robert Adlard, Queensland Museum & Science Centre, Australia
- Staffan Bensch, Lund University, Sweden
- Robert Fleischer, Smithsonian Institution, USA
- Santiago Merino, Museo Nacional de Ciencias Naturales, Spain
- Patricia Parker, University of Missouri-St. Louis, USA
- Susan Perkins, American Museum of Natural History, USA
- Robert Ricklefs, University of Missouri-St. Louis, USA
- Ravinder Sehgal, San Francisco State University, USA
- Gabriele Sorci, Université de Bourgogne, France
- Gediminas Valkiūnas, Nature Research Centre, Lithuania

2. **Venue.** The conference will take place in Vilnius at the *Lithuanian Academy of Sciences* (http://lma.lt/index.php?lang=en, Gedimino Ave. 3) from *August 7-11, 2013.* To see the location of the conference venue, hotels, and some other buildings where the scientific and social programmes will take place, please see the ***map***. *August 7, 2013* is scheduled for arrival of participants, registration and a 'Get-together Reception'; *August 11, 2013* is scheduled for departure of participants.

3. **Conference materials and registration.** All conference materials are handed out at the registration, which will take place **(a)** at the Hotel *AMBERTON* (see: http://www.ambertonhotels.com/en/, L. Stuokos-Gucevičiaus str.) on *Wednesday 7 August* between 19:00 and 21:00 and **(b)** at the Lithuanian Academy of Sciences (see (http://lma.lt/index.php?lang=en, Gedimino Ave. 3) on *Thursday 8 August* between 8:00 and 8:30.

4. **Presentations.** There will be one presentation session. The name of the presenter is underlined, followed by the title of the presentation and the affiliation of the presenter. All oral presentations should be in PowerPoint. A PC-based laptop and a projector will be available in the presentation room. You ***cannot*** present your talk from your own computer. ***If you use an Apple Macintosh, make sure that your presentation is readable via PC PowerPoint.***

We advise you to install your presentations into the conference laptop one day before your talk, but not later than 30 min before beginning of your session. You may also install your presentation during the *Get-together Reception* and *Registration* in the Hotel AMBERTON on *Wednesday 7 August* between 19:00 and 21:00. The responsible members of the Local Organising Committee will assist you with installation of your presentations.

The conference programme is very intense. We kindly ask all presenters to not exceed the time-limits of their oral presentations.

5. **Posters** (100 cm in height and 70 cm in width) should be placed on *Thursday 8 August* following the opening of the conference, and they should be on display during the entire meeting. The poster board will display numbers that correspond to the numbers in the conference programme. The members of the Local Organising Committee at the Registration Desk will be happy to assist you in assembling your posters.

Electronic versions of posters will be uploaded on the website of the MalariaRCN (http://malariarcn.org/) in July 2013 before the conference begins. They will be freely available to the scientific community worldwide. We encourage presenters of the posters to email pdf files of their presentations to the Conference Secretariat (malaria.vilnius@gmail.com) as soon as convenient, but before 30 July 2013.

6. **Poster session.** To facilitate the poster discussion, presenters are asked to stay by their posters on *Friday 9 August* between 14:00 and 15:30.

7. **Abstracts** of the keynote lectures, plenary lectures, oral presentations and posters will be printed and also will be freely available online on sites of Nature Research Centre (http://www.gamtostyrimai.lt/en), MalariaRCN (http://malariarcn.org/), and the Scandinavian Baltic Society for Parasitology (http://sbsp.eu/) in *June 2013.* All pre-registered participants will be given a copy of the conference abstracts when they register for the conference in Vilnius. To facilitate the use of the conference materials, all abstracts will be arranged in alphabetical order by presenting authors.

8**. Free internet facilities** will be available from your hotels and the Lithuanian Academy of Sciences (wireless internet). During the conference, all registered participants can use free wireless internet at Hotel AMBERTON (L. Stuokos-Gucevičiaus str. 1), where the Get-together Reception and the conference lunches will take place.

9. **Refreshments, meals, conference banquet, and excursion**. The Local Organising Committee will arrange a Get-together Reception, Coffee, Tea, Lunches, a Conference Banquet, and an Excursion for all registered participants who have pre-paid 150 EUR for these services.

Those who did not pay, but wish to join us during these events, should arrange payments as a bank transfer. ***It would be helpful for the Local Organising Committee if you arrange payments in advance before the conference using instructions provided in the Second Announcement (see http://malariarcn.org/conference2013)***. If you like to pay during the conference, please contact the Registration Desk during the Registration, which will take place **(a)** at *Hotel AMBERTON* (L. Stuokos-Gucevičiaus str. 1) on *Wendesday 7 August* between 19:00 and 21:00 and **(b)** at the Lithuanian Academy of Sciences (Gedimino Ave. 3) on *Thursday 8 August* between 8:00 and 8:30. The responsible members of the Local Organising Committee will assist you with these payments.

Please note that we shall not be able to help you with participation in these social events if the payment not processed until 8:30 on Thursday 8 August.

10. **The Conference Banquet** will take place in Trakai (http://en.wikipedia.org/wiki/Trakai) at the restaurant *Apvalaus Stalo Klubas* (see http://www.asklubas.lt/en.html, Karaimų str. 53A, Trakai) at 19:30. From the restaurant terrace, you will have an opportunity to enjoy one of the most glorious sites in Lithuania. Before the Conference Banquet, ***an excursion to the Trakai Castle*** will be arranged. All participants, ***who pre-paid for these social events,*** are invited to attend. Those wishing to join us for the Conference Banquet and the excursion, but did not pay, ***must pay by 8:30 on Thursday 8 August*** (for the payment details see paragraph 9)***.***

Trakai is a small ancient Lithuanian city located approximately 30 km from the conference venue; the single direction bus journey takes approximately 40 min. ***Buses to Trakai will depart from the Hotel AMBERTON at 16:30. From Trakai to Vilnius, the buses will depart from the restaurant Apvalaus Stalo Klubas (Karaimų str. 53A, Trakai) at 22:30.***

11. **Travel and transport.** Vilnius International Airport, Train Station and Bus Station are located relatively close to the conference venue (approximately, 8 km and 2 km, respectively). There are several options you can choose to get to your hotels from the Airport. For the location sites of the conference hotels, see the **map**.

(1) The easiest would be taking a taxi to your hotel. The whole trip should approximately cost around 40 Litas (LT).
(2) You can take a public bus number 2, which will take you to the city centre. Ticket can be purchased from the driver; the price for a bus ticket is 2.50 LT. The bus schedule and the nearest stops to your hotel can be found at http://stops.lt/vilnius/#bus/2/b-a/2613/en.
(3) You also can experience the mini train from the Airport to the Vilnius Train Station; the train ticket costs 2.50 LT. The mini train departs once per hour from the Airport; the train schedule is available on site http://www.litrail.lt/wps/portal [choose 'Passenger transportation', Departure station (oro uostas = airport), arrival station (Vilnius), and the date]. The Vilnius Train Station is within walking distance to the city centre. You can use the public transport to reach the city centre and your hotels from the Train Station. If you choose to do so, take the trolleybus number 5 or 6, or bus number 26 or 53. Tickets are purchased from the driver; the price for a ticket is 2.50 Lt.

12. **Currency and banking.** National currency is Litas (LT) in Lithuania. Exchange rate is 3.45 LT for 1 EUR. There are many places to exchange currency in Vilnius, including the Vilnius International Airport, Train Station and Bus Station, where cash machines are permanently available. Major credit cards are widely accepted. Most banks are open from 8:00 am to 5:00 pm.

13. **Climate.** Weather is usually mild and pleasant in Vilnius in August. Participants are advised to have a jacket for evenings. Umbrella sometimes may be handy.

14. **Insurance and disclaimer.** The hosts and organisers are not responsible for personal accidents, loss of private property, insurance and will not be liable for any claims.

15. **Assistance.** If you have questions, please do not hesitate to ask the members of the local Organising Committee (for names, see the second page). We will wear green badges and will be happy to assist you.

The Scientific and Local Organising Committees

Summary of the conference

WEDNESDAY, 7TH AUGUST

19:00 – 21:00 Get-together reception and Registration (Hotel *AMBERTON*, L. Stuokos-Gucevičiaus str. 1)

THURSDAY, 8TH AUGUST

8:00 – 8:30 Registration continuation (the Lithuanian Academy of Sciences, Gedimino Ave. 3)

8:30 – 8:45 ***OPENING SESSION***

Welcome by the Organising Committee
Gediminas Valkiūnas, Nature Research Centre, Lithuania

Welcome by the President of the Lithuanian Academy of Sciences
Valdemaras Razumas, Lithuanian Academy of Sciences

Welcome by the Malaria Research Coordination Network
Robert Ricklefs, University of Missouri-St. Louis, USA

SESSION 1

Chairman: Staffan Bensch
Lund University, Sweden

8:45 – 9:45 ***Keynote lecture***
R.E.Sinden. *The roles of model parasite systems in designing malaria transmission-blocking strategies.* University of Oxford, UK.

9:45 – 10:15 ***Plenary lecture***
S. L. Perkins. *The history of haemosporidia: Morphology, molecules, and moving on.* American Museum of Natural History, USA.

10:15 – 10:45 ***Plenary lecture***
G. Valkiūnas, T. A. Iezhova, V. Palinauskas, A. Križanauskienė, R. Kazlauskienė, S. Bensch & R. Bernotienė. *Haemosporidian co-infections: What happens during the sexual process?* Nature Research Centre, Lithuania.

10:45 – 11:00 P. Silveira, S. Y. M. Gomes, P. A. Moreira, B. B. Tocantins, G. A. Lacorte, T. A. Paixão, N. R. S. Martins & É. M. Braga. *Interactions of Plasmodium juxtanucleare and Chicken Anemia Virus: Establishing a model.* Universiadade Federal de Minas Gerais, Brazil.

11:00 – 11:30 COFFEE

SESSION 2

Chairman: Gabriele Sorci
Université de Bourgogne, France

11:30 – 12:00 ***Plenary lecture***
R. E. Ricklefs. *Observations on the diversity and distributions of avian haemosporidian parasites.* University of Missouri-St. Louis, USA.

12:00 – 12:15 A. Pérez-Rodríguez, I. De la Hera, S. Bensch, J. Pérez-Tris. *Evolution of patterns of seasonal transmission in avian blood parasites.* Complutense University of Madrid, Spain.

12:15 – 12:30 S. V. Drovetski, S. A. Aghayan, V. Mata & R. J. Lopes. *Does the niche-breadth or trade-off hypothesis explain the abundance-occupancy relationship in avian haemosporidia?* Tromsø University Museum, Norway.

12:30 – 12:45 A. Pérez-Rodríguez, Á. Ramírez, D. S. Richardson & J. Pérez-Tris. *Evolution of parasite island syndromes without long-term host population isolation: Avian haemosporidians infecting Macaronesian blackcaps Sylvia atricapilla.* Universidad Complutense de Madrid, Spain.

12:45 – 13:00 V. A. Ellis, M. R. Kunkel & R. E. Ricklefs. *Disentangling the ecological and evolutionary components of host immune responses to avian haemosporidian infection.* University of Missouri - St. Louis, USA.

13:00 – 13:15 S. C. Galen & C. C. Witt. *Diverse avian malaria in Andean house wrens: Evidence for co-diversification despite lability in host breadth and climatic niche.* University of New Mexico, USA.

13:15 – 14:30 LUNCH (Hotel *AMBERTON*, L. Stuokos-Gucevičiaus str. 1)

SESSION 3

Chairman: Patricia Parker
University of Missouri-St. Louis, USA

14:30 – 15:00 ***Plenary lecture***
R. C. Fleischer. *Patterns of host infection by avian malaria lineages across space and time.* Smithsonian Conservation Biology Institute, National Zoological Park, USA.

15:00 – 15:15 J. P. M. C. Maia, D. J. Harris, S. Carranza & E. Gómez-Díaz. *Infection estimates of apicomplexan hemoparasites in reptiles: A comparison of multiple quantification methods.* Universidade do Porto, Portugal.

15:15 – 15:30 L. Gutiérrez Jiménez, R. E. Ricklefs & A.Guillén Servent. *Multiple haemoparasite co-infections in bats from Southeast Asia and Central America*. University of Missouri-St. Louis, USA.

15:30 – 15:45 J. Schaer, K. Matuschewski & S. L. Perkins. *Diversity of African chiropteran haemosporidian parasites and close affiliation with rodent Plasmodium species.* Max Planck Institute for Infection Biology, Germany.

15:45 – 16:00 B. Penman, G. Faust, B. Bia, F. Rangkuti, F. Piel, N. Rose, A. Smith, A. Dobson & S. Gupta. *Malaria selection in long-tailed macaques (Macaca fascicularis).* University of Oxford, UK.

16:00 – 16:15 H. M. De Nys, S. Calvignac-Spencer, U. Thiesen, Ch. Boesch, R. M. Wittig, R. Mundry & F. H. Leendertz. *Age-related effects on malaria parasite infection in wild chimpanzees.* Robert Koch-Institut, Germany.

16:15 – 16:45 COFFEE

SESSION 4

Chairman: Santiago Merino
Museo Nacional de Ciencias Naturales, Spain

16:45 – 17:15 ***Plenary lecture***
R. N. M. Sehgal. *Manifold habitat effects on the prevalence and diversity of avian hematozoa.* San Francisco State University, USA.

17:15 – 17:30 N. E. Matta, I. A. Lotta, A. D. Gonzalez, L. I. Moncada & O. A. Rodriguez. *Lessons learned from Leucocytozoon spp. in a Neotropical country.* Universidad Nacional de Colombia, Colombia.

17:30 – 17:45 T. Jenkins, J. Delhaye & P. Christe. *Malaria infection of both parents can affect an ecosystem service.* University of Lausanne, Switzerland.

17:45 – 18:00 B. G. Daly, R. E. Ricklefs, O. Hellgren, Ch. L. Merkord, J. E. Jankowski, N. Seddon & J. A. Tobias. *Testing the environmental and ecological predictors of avian malaria prevalence.* Edward Grey Institute, University of Oxford, UK.

18:00 – 18:15 P. Zehtindjiev, M. Ilieva, D. Dimitrov, A. Bobeva, M. Marinov, S. Hahn, V. Amrhein, A. Križanauskienė, F. Liechti & S. Bensch. *Transmission regions of haemosporidian parasites of three common nightingale populations with different wintering grounds in Africa.* Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Bulgaria.

18:15 – 18:30 D. Dimitrov, M. Ilieva, A. Bobeva, S. Bensch, M. Marinov, S. Hahn & P. Zehtindjiev. *The real prevalence of haemosporidian (Apicomplexa, Haemosporida) parasites in the Spanish Sparrow (Passer hispaniolensis): What we can estimate?* Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Bulgaria, and Nature Research Centre, Lithuania.

18:30 – 18:45 P. Shurulinkov, C. Barboutis, G. Daskalova, L. Spasov & N. Chakarov. *Does blood parasite composition and load change in advance of spring migration in long distance migratory birds?* National Museum of Natural History, Bulgaria.

18:45 – 19:00 J.C. Dunn, S. J. Goodman, T. G. Benton & K. C. Hamer. *Active blood parasite infection outside the breeding season in a declining population.* University of Leeds, UK.

19:00 – 19:15 J. Rivero-de Aguilar, H. Westerdahl, J. Martínez-de la Puente, G. Tomás J. Martínez & S. Merino. *MHC class I genes are related with high intensity of Leucocytozoon infections in blue tits (Cyanistes caeruleus).* Museo Nacional de Ciencias Naturales, Spain.

19:15 – 19:30 J. Vézilier, A. Nicot, R. Pigeault, N. Barthes, B. Buatois, A. Rivero & S. Gandon. *Deciphering the impact of Plasmodium parasites on bird odorant profile: What makes infected birds more attractive to mosquitoes?* CEFE Montpellier, France.

FRIDAY, 9TH AUGUST

SESSION 5

Chairman: Robert Ricklefs
University of Missouri-St. Louis, USA

8:30 – 9:30 ***Keynote lecture***
U. Böhme. *Comparative genomics in Plasmodium.* Wellcome Trust Sanger Institute, UK.

9:30 – 9:45 V. Palinauskas, A. Križanauskienė, T. A. Iezhova, S. Bensch & G. Valkiūnas. *How to obtain purified template for genomic studies of haemosporidians inhabiting nucleated red blood cells?* Nature Research Centre, Lithuania.

9:45 – 10:15 ***Plenary lecture***
S. Bensch, B. Canbäck, O. Hellgren, T. Johansson, V. Palinauskas & G. Valkiūnas. *Preliminary reports from the genome of Haemoproteus tartakovskyi.* Lund University, Sweden.

10:15 – 10:30 O. Hellgren. *Merozoite surface protein 1 (MSP1), a candidate gene for a better understanding of the epidemiology of Plasmodium relictum*? Lund University, Sweden.

10:30 – 10:45 N. Chakarov. *How much vertical transmission is there in avian blood parasites? More than most would think*. Bielefeld University, Germany.

10:45 – 11:00 F. A. Reed. *Applying evolution: Transformation of a population using underdominance principles.* University of Hawaii at Manoa, USA.

11:00 – 11:30 COFFEE

SESSION 6

Chairman: Robert Adlard
Queensland Museum & Science Centre, Australia

11:30 – 12:00 ***Plenary lecture***
P. Parker. *Galapagos endemic birds and their parasites: Does understanding their arrival help predict their future?* University of Missouri-St. Louis, USA.

12:00 – 12:15 J. Cornuault**,** B. H. Warren, B. Milá, C. Thébaud & P. Heeb. *Timing and number of colonizations but not diversification rates affect diversity patterns in hemosporidian lineages on a remote oceanic archipelago.* University of Toulouse, France.

12:15 – 12:30 F. Witsenburg, L. Clément, L. Dutoit, A. Lòpez Baucells, J. Palmeirim, I. Pavlinić, D. Scaravelli, M. Ševčík, A. Brelsford, J. Goudet & P. Christe. *How malaria gets around: The genetic structure of a parasite, vector and host compared.* University of Lausanne, Switzerland.

12:30 – 12:45 L. García-Longoria, L. Z. Garamszegi & A. P. Møller. *Escape behaviour of hosts and blood parasites infections.* University of Extremadura, Spain.

12:45 – 13:00 E. Schoener, I. Castro, L. Howe, K. Parker & D. Tompkins. *Avian Malaria in New Zealand.* Massey University, New Zealand.

13:00 – 13:15 I. A. Lotta, A. D. González, G. Valkiūnas, O. A. Rodríguez, L. I. Moncada & N. E. Matta. *Distribution of blood parasites in hummingbirds, with discovery of a new Leucocytozoon sp. infection.* Universidad Nacional de Colombia, Colombia.

13:15 – 13:30 M. M. Marinov, P. Zehtindjiev & C. Marchetti. *Prevalence of avian blood parasites in birds of different personalities: Comparative research in Certain European bird species.* Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Bulgaria.

13:30 – 14:30 LUNCH (Hotel *AMBERTON*, L. Stuokos-Gucevičiaus str. 1)

14:30 – 16:00 **Poster session**

16:30 – 22:30 The excursion to the Trakai Castle and the Conference Dinner, which will take place in Trakai.
Buses will depart for Trakai from the Hotel *AMBERTON* (L. Stuokos-Gucevičiaus str. 1, Vilnius) at 16:30.

SATURDAY, 10th AUGUST

SESSION 7

Chairman: Susan Perkins
American Museum of Natural History, USA

8:30 – 9:30 ***Keynote lecture***
Y. Tsuda. *Multiple transmission cycles and "incomplete transmission" of avian Plasmodium parasites in wild bird communities: Implications of entomological studies in Japan.* National Institute of Infectious Diseases, Japan.

9:30 – 9:45 L. I. Moncada, M. Rojas, G. E. Hernández & M. L. Quiñones. *Biting activity and seasonal abundance of Culex quinquefasciatus in Bogotá.* Universidad Nacional de Colombia, Colombia.

9:45 – 10:00 S. Cornet, A. Nicot, A. Rivero & S. Gandon. *Plasticity of avian malaria transmission following exposure to mosquito bites.* Centre d'Ecologie Fonctionnelle et Evolutive, and CNRS-Montpellier, France.

10:00 – 10:15 J. S. Carlson, E. Walther, R. Trout-Fryxell, S. Staley, L. A. Tell, R. N.M. Sehgal, G. Lanzaro & A. J. Cornel. *The role of mosquitoes in the transmission of avian malaria in Central California.* University of California at Davis, USA.

10:15 – 10:30 K. S. Kim & Y. Tsuda. *Microscopic observation of oocysts and sporozoites and subsequent PCR for identification of genetic lineages of avian Plasmodium spp. in natural vector, Culex pipiens pallens.* Tottori University, Japan.

10:30 – 10:45 L. Freed & R. Cann. *Vector movement underlies avian malaria at upper elevations in Hawaii.* University of Hawaii at Manoa, USA.

10:45 – 11:00 R. Kazlauskienė, R. Bernotienė, V. Palinauskas, T. A. Iezhova & G. Valkiūnas. *Long-lasting survival of Haemoproteus parasites (Haemosporida, Haemoproteidae) in resistant blood-sucking insects, with perspectives on haemosporidian vector research.* Nature Research Centre, Lithuania.

11:00 – 11:30 COFFEE

SESSION 8

Chairman: Ravinder Sehgal
San Francisco State University, USA

11:30 – 12:00 ***Plenary lecture***
G. Sorci. *Immunity, resistance and tolerance to avian malaria.* Université de Bourgogne, France.

12:00 – 12:15 H. Westerdahl, M. Asghar, D. Hasselquist, M. Stjernman, L. Råberg, J. Nilsson & S. Bensch. *MHC-I influences infection intensity and infection status of avian haemosporidian parasites within populations - but patterns differ across populations and host species.* Lund University, Sweden.

12:15 – 12:30 V. Palinauskas, V. Armalis, C. V. Bolshakov & G. Valkiūnas. *Virulence of different isolates of Plasmodium relictum: Implications for bird conservation projects.* Nature Research Centre, Lithuania.

12:30 – 12:45 K. Krend, L. Freed & R. Cann. *Experimental Challenge of wild-caught Oahu amakihi to Plasmodium relictum parasites.* University of Hawaii at Manoa, USA.

12:45 – 13:00 E. H. R. Sari, V. A. Ellis, L. Rois & P. G. Parker. *Immune responses to haemosporidian and other parasites in Myiarchus tyrannulus from Costa Rica.* University of Missouri-St. Louis, USA.

13:00 – 13:15 E. Arriero, J. Pérez-Tris, C. Remacha & A. Ramirez. *Experimental reduction in parasite intensity in the context of disease tolerance.* University Complutense of Madrid, Spain.

13:15 – 13:30 A. Marzal, J. M. C. Callirgos & R. N. M. Sehgal. *Prevalence, genetic diversity, deforestation and invasive avian malaria in Peru.* University of Extremadura, Spain.

13:30 – 14:30 **LUNCH** (Hotel *AMBERTON*, L. Stuokos-Gucevičiaus str. 1)

SESSION 9

Chairman: Robert Fleischer
Smithsonian Institution, USA

14:30 – 15:00 ***Plenary lecture***
S. Merino, J. Martínez-de la Puente, J. Rivero-de Aguilar & J. Martínez. *Effects of interactions between haemoparasites, vectors and wild birds.* Museo Nacional de Ciencias Naturales-CSIC, Spain.

15:00 – 15:15 F. Zélé, O. Duron & A. Rivero. *Plasmodium-Wolbachia interactions in Culex pipiens mosquitoes*. CNRS-Montpellier, France.

15:15 – 15:30 F. Lalubin, A. Delédevant, O. Glaizot & P. Christe. *Nutritional stress mediates malaria-infection costs in naturally infected mosquitoes.* University of Lausanne, Switzerland.

15:30 – 15:45 S. Larcombe, M. Gauthier-Clerc & B. Sheldon. *The effects of mosquito control on vector dynamics and prevalence and diversity of avian malaria in Camargue sparrows.* University of Oxford, UK, and Tour du Valat, France.

15:45 – 16:00 R. Bernotienė, R. Kazlauskienė, V. Palinauskas, T. Iezhova & G. Valkiūnas. *Molecular detection of Haemoproteus species (Haemosporida, Haemoproteidae) as a tool for the determination of ornithophilic blood-sucking insects.* Nature Research Centre, Lithuania.

16:00 – 16:30 COFFEE

SESSION 10

Chairman: Érika Braga
Universiadade Federal de Minas Gerais, Brazil

16:30 – 17:00 ***Plenary lecture***
R. Adlard. *The International Reference Centre for Avian Haematozoa: A resource for current research.* Queensland Museum & Science Centre, Australia.

17:00 – 17:15 V. Palinauskas, M. Ilgūnas, R. Kazlauskienė, T. Iezhova & G. Valkiūnas. *Experimental data on the development of Plasmodium sp. (lineage pCOLL4) in avian hosts and mosquitoes.* Nature Research Centre, Lithuania.

17:15 – 17:30 M. Moens & J. Pérez-Tris. *Evolution of a generalist assemblage of blood parasites in a megadiverse community of tropical birds.* Complutense University of Madrid, Spain.

17:30 – 17:45 A. Bobeva, D. Dimitrov, M. Marinov, M. Ilieva, P. Zehtindjev & S. Bensch. *A survey of Haemoproteus* spp*. vectors with respect to their host preferences and transmission of avian haemosporidians.* Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Bulgaria.

17:45 – 18:00 J. Martínez-de la Puente, M. Ferraguti, S. Ruiz, R. Soriguer & J. Figuerola. *Diversity of avian malaria and malaria-like parasites from the potential vectors biting midge Culicoides and wild birds from SW Spain.* Estación Biológica de Doñana, Spain.

18:00 – 18:15 J. Figuerola, C. Marfil, J. Muñoz, J. Martínez-de la Puente, E. Cuevas & R. Soriguer. *Telomere shortening and survival probability in relation to avian malaria infection status: A long term study on Western Jackdaws (Corvus monedula).* Estación Biológica de Doñana, Spain.

18:15 – 18:30 M. Asghar, D. Hasselquist, B. Hansson, P. Zehtindjiev, H. Westerdahl & S. Bensch. *Malaria infection reduces telomere length, lifespan and offspring quality in a songbird.* Lund University, Sweden.

18:30 – 18:45 C. Remacha, E. Arriero, A. Ramírez & J. Pérez-Tris. *Exploratory behaviour and avian malaria infection in juvenile blackcaps (Sylvia atricapilla).* Complutense University of Madrid, Spain.

18:45 – 19:00 S. McNew & D. Clayton. *Assessing the cost of haemosporidian infection through flight performance in Rock Pigeons (Columba livia).* University of Utah, USA.

19:00 – 19:30 ***CLOSING SESSION***
R. Ricklefs. *Malaria Research Coordination Network: Update of activities.* University of Missouri-St. Louis, USA.

Discussions

SUNDAY, 11TH AUGUST

DEPARTURE

Poster presentations

P1. S. A. Aghayan & S. V. Drovetski. *Avian haemosporidia differ in their ability to use long-distance migrants to colonize new areas.* Scientific Centre of Zoology and Hydroecology of NAS RA, and Yerevan State University, Armenia.

P2. L. Berthová, M. Ružić, D. Rajković & E. Špitalská. *Occurrence of haemosporidian parasites in Long-eared owls (Asio otus) in winter roost in Vojvodina province, Northern Serbia.* Institute of Virology, Slovak Academy of Sciences, Slovakia.

P3. J. Borner, C. Pick, I. Bruchhaus & T. Burmester. *A phylogenomic approach to the evolution of Haemosporida.* University of Hamburg, Germany.

P4. R. Kazlauskienė, D. Bukauskaitė, R. Bernotienė, V. Palinauskas, T. A. Iezhova & G. Valkiūnas. *Complete sporogony of two Plasmodium relictum lineages (pSGS1 and pGRW11) in mosquitoes Culex pipiens form molestus.* Nature Research Centre, Lithuania.

P5. A. Castillo, J. Pérez-Emán & L. Herrera. *Avian haemosporidians from mountain regions of Venezuela.* Arizona State University, USA.

P6. C. R. F. Chagas, G. Valkiunas, J. M. M. Tolentino, P. C. Henrique, L. O. Guimarães, E. F. Monteiro, F. J. V. Guida & K. Kirchgatter. *Host sharing of malarial parasites in Sao Paulo Zoo, Brazil.* Zoological Park Foundation, Brazil.

P7. A. Yildirim, A. Ciloglu, O. Duzlu, Z. Onder, A. Inci & Z. Dogan. *Molecular detection and characterization of two Leucocytozoon lineages from a Long-legged Buzzard (Buteo rufinus) and a Common Buzzard (Buteo buteo) from Kayseri Province of Turkey.* Erciyes University, Turkey.

P8. N. Clark & S. Clegg. *Temporal variation of haemosporidian infections in an island population of silvereyes (Zosterops lateralis chlorocephalus).* Griffith University, Australia.

P9. S. Clegg, N. Clark, S. Olsson-Pons & F. Ishtiaq. *Avian malaria diversity in southern Melanesian bird communities.* Griffith University, Australia.

P10. A.T. Constance. *Prevalence of avian blood parasites in some protected areas in Ghana.* University of Ghana, Ghana.

P11. J. Delhaye, T. Jenkins & P. Christe. *Oxidative stress in breeding Great tit, Parus major, infected by Plasmodium spp.* University of Lausanne, Switzerland.

P12. A. Dubiec, E. Podmokła, M. Zagalska-Neubauer & L. Gustafsson. *Malaria parasites do not affect reproductive success in the great tit.* Museum and Institute of Zoology, Poland.

P13. M. Ferraguti, J. Martínez-de la Puente, J. Muñoz, D. Roiz, S. Ruiz,

R. Soriguer & J. Figuerola. *Avian Plasmodium in Culex and Ochlerotatus mosquito species from southern Spain: Effects of season and host-feeding source on parasite dynamics.* Estación Biológica de Doñana, Spain.

P14. Jr. F. C. Ferreira, M. V. R. Marques, G. A. Lacorte, G. M. F. Félix, É. M. Braga & N. R. S. Martins. *Molecular characterization of heamosporidians in toucans and aracaris (Piciformes: Ramphastidae) from Brazil.* Federal University of Minas Gerais, Brazil.

P15. I. Góźdź, A. Dubiec & T. D. Mazgajski. *How costly is nest building in terms of infection with blood parasites?* Museum and Institute of Zoology, Polish Academy of Sciences, Poland.

P16. A. Biruksew Hordofa. *In Vivo antiplasmodial activities of Echnops kebericho Mesfin and Zingibir officinale Roscoe.* Jimma University, Ethiopia.

P17. T. A. Iezhova, A. Križanauskienė, V. Palinauskas, R. Kazlauskienė, R. Bernotienė & G. Valkiūnas. *How different morphologically are the reproductive cells and ookinetes of haemosporidian parasites?* Nature Research Centre, Lithuania.

P18. T. Imura, S. Sato, Y. Sato, T. Isobe, K. Sasaki, K. Murata, T. Holder & M. Yukawa. *Isolation and genetic analysis of Leucocytozoon caulleryi.* Nihon University, Japan.

P19. K. Ivanova, P. Zehtindjiev, J. Mariaux & B. B. Georgiev. *New data on the genetic diversity of avian haemosporidians in Eastern Asia: Cytochrome b lineages of the genera Plasmodium and Haemoproteus (Haemosporida) from China and Malaysia.* Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Bulgaria.

P20. G. Karadjian & I. Landau. *Morphological study of Haemoproteus syrnii (Mayer, 1910) in Strix aluco and in a hippoboscid fly.* MNHN, France.

P21. R. J. Lopes, O. Gonçalves, S. Reis & P. Rodrigues. *Azores archipelago has low haemosporidian diversity and high host specificity in forest passerines.* University of Porto, Portugal.

P22. V. A. Mata, L. P. da Silva, R. J. Lopes, S. V. Drovetski. *A comparison of avian haemosporidian parasite communities across the strait of Gibraltar.* CIBIO, Portugal

P23. H. L. Lutz, W. M. Hochachka, J. I. Engel, J. D. Weckstein, J. Mertes, V. Tkach & S. J. Hackett. *Ecological determinants of haemosporidian prevalence in tropical African birds.* Cornell University and Field Museum of Natural History, USA.

P24. J. S. Mantilla, A. D. González, S. R. Hernández, L. J. Madroñero, I. A. Lotta, L. I. Moncada & N. E. Matta. *Biodiversity of avian haemoparasites in a high altitude city of Colombia.* Universidad Nacional de Colombia, Colombia.

P25. A. Marzal, M. Asghar, L. Rodríguez, M. Reviriego, I. G. Hermosell, J. Balbontín, L. Garcia-Longoria, F. de Lope & S. Bensch. *Co-infections by malaria parasites decrease feather growth but not feather quality in house martin.* University of Extremadura, Spain

P26. N. E. Matta, J. S. Mantilla, A. A. Escalante, A. M. Pachecho & L. I. Moncada. *Plasmodium* (*Haemamoeba*) *lutzi in Colombia*. Universidad Nacional de Colombia, Colombia.

P27. R. Megía-Palma, J. Martínez & S. Merino. *Molecular detection of haemosporidian parasites infecting both red and white blood cells in a Mabuya skink (Reptilia: Squamata).* Museo Nacional de Ciencias Naturales-CSIC, Spain.

P28. P. A. Moreira, G. M. F. Félix, L. O. Leite & É. M. Braga. *Avian malaria parasites in a brazilian seasonally dry tropical forest.* Universidade Federal do Rio Grande do Norte, Brazil.

P29. P. Munclinger, P. Synek, R. Reifová, M. Jandová & P. Procházka. *Migration divide and haemosporidian parasite spectra in the Reed Warbler Acrocephalus scirpaceus.* Charles University in Prague, Czech Republic.

P30. O. Orkun, Z. Karaer, A. Cakmak & S. Nalbantoglu. *Leucocytozoonosis in Long-legged Buzzards (Buteo rufinus), Turkey.* Ankara University, Turkey.

P31. S. Peev, P. Zehtindjiev, M. Sanchez, J. A. Amat, C. Ramo, N. Varo & A. J. Green. *Systematic survey for haemosporidian parasites in the Black-necked Grebe (Podiceps nigricollis).* Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Bulgaria.

P32. E. Platonova, V. Palinauskas, I. Vakoluk & A. Mukhin. *Behavioural and physiological changes in Siskins* (*Spinus spinus*) *infected by Plasmodium ashfordi.* Immanuel Kant Baltic federal university, Russia.

P33. E. Podmokla, A. Dubiec, S. M. Drobniak, A. Arct, L. Gustafsson & M. Cichon. *Paternity in the blue tit infected with avian malaria.* Jagiellonian University, Poland.

P34. C. Silverio & B. J. Stutchbury. *The effects of avian malaria on purple martins: Fitness, extra pair paternity and migration.* York University, Canada.

P35. J. Stockdale & J. Dunn. *Haemoproteus and Plasmodium spp. prevalence in a declining population of European Turtle Doves (Streptopelia turtur).* Cardiff University, UK.

P36. P. Synek, T. Albrecht, E. Garcia-del-Rey, J. T. Lifjeld & P. Munclinger. *Haemosporidian parasites in Canarian birds: Low lineage diversity and rare lineage expansion.* Charles University in Prague, Czech Republic.

P37. A. Inci, A. Yildirim, O. Duzlu, P. H. Adler, Z. Onder, A. Ciloglu, H. Yesiloz & A. Demircioglu. *Bloodmeal Identification and Detection of a Leucocytozoon lineage from blackflies (Diptera: Simuliidae) collected from Kizilirmak River in Nevsehir province of Turkey.* Erciyes University, Turkey.

Abstracts

Keynote lectures

Comparative genomics in *Plasmodium*

U. Böhme[1], T. D. Otto[1], M. Hunt[1], M. Sanders[1], C. Newbold[2] & M. Berriman[1]

[1]*Wellcome Trust Sanger Institute, UK*
[2]*University of Oxford, UK*

Next generation sequencing technology has had a major impact on biomedical research. At the Parasite Genomics group we are using this technology to generate high quality reference genomes of malaria parasites. Genomes that have been recently sequenced are *P. reichenowi* and *P. gallinaceum*. *P. gallinaceum* is the first avian malaria parasite to be sequenced and will serve as an outgroup to already existing malaria genomes. A first analysis of the *P. gallinaceum* genome showed an unusual PIR (plasmodium interspersed repeat) gene family and the presence of P. falciparum specific genes. In order to perform comparative genomics it is important to have well curated reference genomes. One of the genomes that have been undergoing re-sequencing and re-annotation is *P. falciparum* 3D7. The original version of the *P. falciparum* 3D7 genome was published in 2002 with limited updates over the years. In 2007 we started to re-sequence and re-annotate the genome. Using different sequencing techniques and an in-house developed automated sequence correction tool we are now close to producing a base-perfect genome. The entire genome has been manually re-inspected resulting in structural changes of 20% of gene models, over 1000 genes had changes in functional annotation. The data is available online through GeneDB (http://www.genedb.org). Data is being exchanged at regular intervals with PlasmoDB (http://www.plasmodb.org).

The roles of model parasite systems in designing malaria transmission-blocking strategies

R. E. Sinden

University of Oxford, UK

The MEG and MalERA analyses of global malaria research recognized the essential need to reduce the number of new infections in endemic populations if we are to achieve effective programmes for malaria elimination, and potentially eradication, i.e. we must reduce R_0/R_c to below one. These campaigns will benefit from biological targeting, and highlight the importance of hitting transmission to and from the mosquito as core components of any effective and sustainable long term strategy. The first of these transitions is totally dependent upon the completion of the sexual phase of parasite development. Studies on the rodent and avian malarial parasites have played central roles in the discovery and development of transmission-blocking measures designed to inhibit sexual development and mosquito infection.

Cell biology

A brief recapitulation of studies in the rodent parasites *Plasmodium berghei,* and *P. yoelii* describing the stunning, yet contrasting, cellular processes of male/female gamete formation and function will be used in an attempt to understand new tools that might contribute to envisaged elimination/eradication programmes.

Assay development

Current assays to identify drugs targeted to the gametocyte stages of development will be analysed highlighting both their strengths and weaknesses for the identification of new interventions that might be used in endemic settings. Assays to measure transmission by infection of the mosquito currently describe outputs such as oocyst infection or prevalence. The talk will illustrate how a deeper understanding of the structure of parasite populations in the mosquito is essential to the useful comparison of experiments.

Population studies

A new rodent model transmission assay, the Population Transmission Assay (Blagborough et al, *Nature Communications* 2013), overcomes many of the theoretical constraints of current assays, and permits important variables to be controlled. The talk will illustrate the utility of this assay in providing decision makers with direct evidence of the impact of any intervention on malaria transmission in populations, and offers the critical data required to undertake effective field trials**.** Past campaigns shows that such campaigns might benefit from consideration of spatial, temporal and biological targeting.

Multiple transmission cycles and "incomplete transmission" of avian *Plasmodium* parasites in wild bird communities: Implications of entomological studies in Japan

Y. Tsuda

National Institute of Infectious Diseases, Japan

In vector-borne disease systems the efficiency of transmission largely depends on biological and ecological characteristics of vectors, and ecological studies of vector populations are important to understand the prevalence of vector-borne pathogens in wild animal communities. Ecological methods and results of entomological studies on avian malaria conducted in Japan are reviewed, and implications for ecological invasion and establishment of new transmission cycles of mosquito-borne pathogens are presented.

Molecular ecological studies on mosquito vectors of avian *Plasmodium* parasites in Japan indicated the presence of multiple transmission cycles and "incomplete transmission" of avian *Plasmodium* parasites in a single wild bird community. In spot surveys of mosquitoes in Sakata wetland, central Japan during 2007 to 2010, seven *Plasmodium* lineages were detected from two dominant mosquito species, *Culex pipiens pallens* and *Culex inatomii*. Four of the lineages were found frequently in both mosquito species indicating the presence of *C. pipiens pallens*-borne cycle and *C. inatomii*-borne cycle. One lineage was novel and detected almost exclusively from *C. inatomii* suggested a specialized avian *Plasmodium* lineage to *C. inatomii*. The remaining two lineages were detected only once.

In Rinshi-no-mori park, Tokyo, eleven *Plasmodium* lineages were detected from *C. pipiens pallens* in 2007 and 2012. Three lineages were detected from both abdomens and thoraxes of mosquitoes with high incidence indicating the presence of a local transmission cycle. The remaining 8 lineages were found mainly in abdomens of blood-fed mosquitoes with low incidence. The blood-meals were probably taken from infected migrating birds, but parasites were unable to develop in the mosquitoes because of biological barriers, and the transmission process was incomplete at present. These genetic lineages are considered as indicators of the early stage of ecological invasion and adaptive genetic changes that allow "vector shift" and "host switch" of parasites are required for successful invasion and establishment of new transmission cycles.

Plenary lectures

The International Reference Centre for Avian Haematozoa: A resource for current research

R. Adlard

Queensland Museum & Science Centre, Australia

Origin of the IRCAH

The systematic collection of avian blood films began in Malaysia in 1959 by H. Elliot McClure, then examined by Dr Marshall Laird with support from the U.S. Army Medical Research Unit at Kuala Lumpur. These collections were expanded in 1963 after the formation of the Migratory Animal Pathological Service under the auspices of the U.S. Army Research & Development Command (Tokyo, Japan) and the Walter Reed Army Institute of Research (Washington, D.C.).

In 1968, the World Health Organization established the International Reference Centre for Avian Malarial Parasites at Memorial University, St John's, Newfoundland, Canada. Avian blood films were sent from southern Asia to this Centre for verification of parasite identity, and to be lodged as reference specimens in the now rapidly expanding collection. In 1975, the collection was reorganized as the International Reference Centre for Avian Haematozoa, under the direction of Professor Gordon F. Bennett. In 1996, after Gordon Bennett's retirement, the IRCAH was transferred to the Queensland Museum in Brisbane, and Dr Robert Adlard was appointed as curator.

The Collection in 2013

The collection comprises over 61,500 registrations of avian blood parasites in stained, thin blood films on microscope slides, of which over 1,500 have been added since its arrival in Australia. These include 658 type specimens plus reference and voucher specimens representing approximately 45,000 individual infected birds. Samples from over 4,000 species of bird in 150 bird families from 63 countries are represented in the collection. This collection is the largest of its kind globally, with such material (as for all museum collections) underpinning the link between morphotypic and molecular data. These specimens are available now for comparative research into morphology and genetics either by loan from the museum, or to scientists visiting who are welcome to use the facilities of the Queensland Museum.

Preliminary reports from the genome of *Haemoproteus tartakovskyi*

S. Bensch[1], B. Canbäck[1], O. Hellgren[1], T. Johansson[1], V. Palinauskas[2] & G. Valkiūnas[2]

[1] *Lund University, Sweden*
[2] *Nature Research Centre, Lithuania*

Complete genome sequences are available from six species of *Plasmodium* parasites that infect primates and rodents but there is only one incomplete genome available (*P. gallinaceum*) for haemosporidians infecting birds. Obtaining material for genome sequencing of bird haemosporidian parasites is much more challenging than for mammalian parasites because bird erythrocytes are nucleated. The bird genome size of approximately 1300 Mb combined with the much smaller genomes of *Plasmodium* parasites (~25 Mb) would make random sequencing resulting in <2% from the parasite even if only infected erythrocytes were harvested. To overcome this difficulty we used parasite enriched DNA from isolated microgametes originating from siskins (*Carduelis spinus*) infected with *Haemoproteus tartakovskyi*. After whole genome amplification to increase the amount of template DNA, we generated 3 million sequence reads using a Roche 454 GS FLX Titanium platform including a 3kb paired-end library. BLAT searches of the raw reads against a merged genome of the zebra finch and *P. falciparum* gave about 35% significant matches against zebra finch and these were thus excluded in further analysis. The remaining 1.9 million reads were assembled into 2,243 scaffolds, built from 28,551 large contigs. This assembly gave a total length of 23.3 Mb. The overall GC content was 25%, thus slightly higher than in *P. falciparum*. Gene prediction carried out with GeneMark-ES resulted in 5,953 genes of which about 70% had significant hits to genes in the *P. falciparum* genome. The list of matching genes contains many of immediate interest for testing within species variation (e.g. several different merozoite surface proteins). These preliminary results show that we will soon have a draft genome of a bird haemosporidian parasite available for comparative analyses with the genomes of mammalian malaria, and a resource for constructing primers for phylogenetic and population studies of rapidly evolving nuclear loci. This study was partly supported by the European Social Fund under the Global Grant measure.

Patterns of host infection by avian malaria lineages across space and time

R. C. Fleischer

Smithsonian Conservation Biology Institute, National Zoological Park, USA

Avian malaria parasites show wide variation in the level of specificity across hosts, from some lineages that appear specific to particular bird families to ones that span the entire avian phylogeny. Based on phylogenetic trees, some clades contain avian malaria lineages that show low specificity and others high, so phylogenetic relatedness is not the only factor involved. However, most studies to date deal with a snapshot in time and space, and it is instructive to determine whether and how host use by malaria parasites varies temporally and spatially. I present analyses that illustrate the level of variability on both micro and macrogeographic scales, and also differences in host use across time (comparing, for example, lineages from museum specimen and modern samples). Also particularly effective for understanding the variation in host use among lineages is the assessment of patterns of malaria parasite infection in exotic captive avian collections embedded in a native bird matrix. Over all, our currently available data do not suggest that avian malaria lineage host diversity varies substantially over time or space.

Effects of interactions between haemoparasites, vectors and wild birds

S. Merino[1], J. Martínez-de la Puente[2], J. Rivero-de Aguilar[1] & J. Martínez[3]

[1] *Museo Nacional de Ciencias Naturales-CSIC, Spain*
[2] *Estación Biológica de Doñana-CSIC, Spain*
[3] *Universidad de Alcalá, Spain*

In several field experiments, we reduced through medication the intensity of infection by *Haemoproteus majoris* in blue tits (*Cyanistes caeruleus*), and demonstrated detrimental effects of natural levels of infection by this parasite species on host reproductive success, condition and survival. In addition, we found that those birds infected by more than one blood parasite genus were paler in colour than those parasitized just by one indicating that carotenoid-based colours are indicators of health status in blue tits.

On the other hand, although gametocyte sex ratios in haemosporidian parasites are usually female skewed, in some cases less female-biased and even male-biased sex ratios are encountered. The 'fertility insurance hypothesis' tries to explain these biases as an evolutionary strategy to facilitate gamete encounter. *Haemoproteus* Sex ratios became male skewed following the experimental medication treatment in agreement with the predictions of that hypothesis. Multiple infections (MIs), those by more than one parasite in the same erythrocyte, may also be a way to ensure fertility. However, our results do not support this possibility and MIs may be promoted by host immune system.

Moreover, to fully understand the blood parasite transmission networks, we used two different approaches. First, several *Haemoproteus* and *Plasmodium* haplotypes were isolated and sequenced from the potential vectors biting midges *Culicoides* and from 7 species of wild birds. Biting midge haplotypes from at least 7 different morphologically identified species showed both specific and generalist relationships with *Haemoproteus* haplotypes, but only generalist relationships with *Plasmodium* haplotypes. Several biting midge haplotypes established significant coevolutionary links with *Haemoproteus* haplotypes. Second, we experimentally tested the relationship between the abundance of potential vectors and bird susceptibility to infection. We have found a positive relationship between the number of biting insects and infection by trypanosomes in nestlings. In addition, broods infected by parasites show lower immune responses implying that these insects have a serious impact on birds' fitness.

Galapagos Endemic Birds and their Parasites: Does understanding their arrival help predict their future?

P. Parker

University of Missouri-St. Louis, USA

Since 2001, we have led a four-partner collaboration involving UMSL, the Saint Louis Zoo, the Charles Darwin Foundation, and the Galapagos National Park, to understand disease threats to Galapagos birds. Our motivation is to prevent Galapagos birds, which have to date suffered no extinctions, from following the sad fate of Hawaiian birds, where many endemic extinctions are attributed to the arrival of *Plasmodium* and avipox virus. We have repeatedly tested most major terrestrial and marine bird populations on all major islands and have identified several new *Haemoproteus* lineages using PCR, microscopy, and phylogenetic approaches. We have also identified four *Plasmodium* lineages, one of which is established on the archipelago, as it is recovered in multiple terrestrial and marine bird species, on multiple islands, over every sampling year. We have found only the earliest erythrocytic stages, suggesting abortive development in endemic vertebrate hosts (and the presence of an unidentified vertebrate reservoir). Tests of abundant sympatric reptiles have excluded them as reservoir candidates and we are now examining the two introduced bird species, which are common. ELISA tests of Galapagos penguins suggest widespread exposure and tolerance of the parasite, at least under benign environmental conditions. Tests of bobolinks, the only passerine migrant commonly seen in Galapagos, recovered exact matches for two of the four Galapagos *Plasmodium* lineages. This suggests that parasites are continually arriving through natural means, and that opportunities for control may be few, unless the primary vector is identified as one of the two introduced mosquitoes, and the primary reservoir is identified as one of the two introduced birds. These tests are underway. We have also characterized the avipox virus in Galapagos and estimated its arrival in the late 1800's. Together, these findings suggest that unless effective interventions can be erected, Galapagos birds may indeed follow those of Hawaii.

The history of haemosporidia: Morphology, molecules, and moving on

S. L. Perkins

American Museum of Natural History, USA

The order Haemosporida encompasses a diverse suite of sporozoan parasites that infect a range of vertebrate and dipteran hosts, including the well-studied species that cause the disease malaria in humans. The taxonomic organization of this order has been revised multiple times over the past century as various workers attempted to incorporate morphological, life history, and host characteristics into improved frameworks. Incorporation of molecular data, primarily from mitochondrial gene sequences challenged several of the previous constructs of families and genera, however. Developing new markers has been somewhat challenging for a variety of reasons, but progress through "omics" approaches is making some headway. In addition to a synthesis of this tumultuous taxonomic history, I will also show new analyses that attempt to better resolve the evolutionary history of the order, which suggest a polyphylyetic *Plasmodium* but which support many of the genera advocated for my Garnham and earlier workers. I will also comment on the potential for making significant progress towards a resolved "tree of life" for these diverse and important parasites.

Observations on the diversity and distributions of avian haemosporidian parasites

R. E. Ricklefs

University of Missouri-St. Louis, USA

My laboratory has been engaged in a DNA sequence analysis of the host and geographic distribution of avian haemosporidian parasites in North and South America, including the West Indies. These analyses, which will be summarized in this talk, have provided insights into the diversification of haemosporidians, host and pathogen specialization within communities, migratory connections between avian breeding and wintering grounds, and the dynamics of change in occurrence and host switching in parasite communities.

Manifold habitat effects on the prevalence and diversity of avian hematozoa

R. N. M. Sehgal

San Francisco State University, USA

The effects of deforestation and climate change on health are diverse and are becoming increasingly apparent with the highly publicized recent outbreaks of several diseases spread to humans by animals. Here we present data of the effects of anthropogenic change on the prevalence and diversity of blood parasites in birds ranging from the tropics to the arctic. Using complementary techniques of blood smear analysis and molecular biology, samples are assayed for species of *Plasmodium*, *Haemoproteus*, *Leucocytozoon* and *Trypanosoma*. We have obtained results regarding the host-specificity, prevalence and lineage diversity of these parasites in several communities of birds from Africa, Costa Rica, California and Alaska. We find that habitat degradation leads to altered patterns of parasite prevalence and disruptions in parasite species dominance. We also present data on the evolution of specialist vs. generalist strategies in avian malaria. Our work incorporates satellite imagery and bioclimatic data to quantify differences among the collection sites, and predict how habitat changes may affect the spread of infections. We have also initiated studies on genes involved in host specificity, with the characterization of an erythrocyte binding-like gene from the chicken parasite *P. gallinaceum*. With this multidisciplinary approach we have developed models to help predict how deforestation and climate change will influence future scenarios of host-parasite interactions.

Immunity, resistance and tolerance to avian malaria

G. Sorci

Université de Bourgogne, France

Interacting pathogens and hosts have evolved reciprocal adaptations whose function is to allow host exploitation (from the pathogen stand point) or minimize the cost of infection (from the host stand point). Once infected, two strategies are offered to the host: parasite clearing (resistance) or withstanding the infection while paying a low fitness cost (tolerance). In both cases, the immune system plays a central role. Interestingly, whatever the defence strategy adopted by the host, this is likely to have an effect on parasite evolution. Given their short generation time and large population size, parasites are expected to rapidly adapt to the environmental conditions provided by their hosts. The immune system can therefore represent a powerful engine of parasite evolution, with the direction of such evolutionary trajectory depending on, among other factors, i) the type of mechanism involved (resistance or tolerance), ii) the damage induced by overreacting immune defences. Here, I will discuss these different issues focusing on selected examples of recent work conducted on avian malaria parasites.

Haemosporidian co-infections: What happens during the sexual process?

G. Valkiūnas[1], T. A. Iezhova[1], V. Palinauskas[1], A. Križanauskienė[1], R. Kazlauskienė[1], S. Bensch[2] & R. Bernotienė[1]

[1]*Nature Research Centre, Lithuania*
[2] *Lund University, Sweden*

Various combinations of haemosporidian co-infections are predominant in wildlife. Additionally, multiple interrupted feeding has been reported in many species of blood-sucking dipterans. Accordingly, haemosporidian between-lineage interactions should be a common phenomenon in vectors after taking infected blood meals, but is insufficiently investigated. Knowledge on this issue is important for better understanding mechanisms of parasite speciation. Haemosporidians are a convenient model organisms for such studies, particularly because fertilization occurs extracellularly and, therefore, the development of gametes, zygotes, meiosis, and ookinetes can be initiated and analysed under controlled in vitro conditions.

Our in vitro experiments revealed several patterns of *Haemoproteus* spp. between-lineage interactions, which are related to the activity of reproductive cells (microgametes and macrogametes) and might prevent the mixing of lineages during the simultaneous sexual process of 2 different parasites: (1) the blockage of ookinete development of both parasites; (2) the development of ookinetes of one parasite and blockage of ookinete development of the other; (3) selective within-lineage mating resulting in ookinete development of both parent species and absence of hybrid organisms; (4) absence of selective within-lineage mating resulting in presence of ookinetes of both parents and also the development of hybrid organisms with unclear potential for further sporogony. Surprisingly, the simultaneous sexual process of two genetically distant lineages of *Haemoproteus* might increase the efficiency of reproductive cells, resulting in the development of a smaller number of anomalous ookinetes.

This study indicates that the simultaneous sexual process of different haemosporidian lineages is a complex process, which ranges from the complete blockage of the development of both species to an increase in the efficiency of reproductive cells. The outcome depends on the particular combination of lineages. Widespread avian *Haemoproteus* spp. are laboratory-friendly organisms for in vitro experimental research addressing between-lineage interaction in parasites during the sexual process. This study was funded by the European Social Fund under the Global Grant measure.

Oral presentations

Experimental reduction in parasite intensity in the context of disease tolerance

E. Arriero, J. Pérez-Tris, C. Remacha & A. Ramirez

University Complutense of Madrid, Spain

Understanding how organisms protect themselves from infectious diseases is a major question in Ecoimmunology and Evolutionary Parasitology. Besides the well-known role of the immune system protecting organisms from infections, the defence strategy may also rely on the organism's ability to tolerate the presence of the pathogen. This dichotomy between disease resistance and disease tolerance has been largely overlooked in animal studies and is becoming the focus of an increasing number of studies in recent years. We used naturally infected blackcaps *Sylvia atricapilla*, as a model system to identify physiological parameters involved in disease tolerance, and to test predictions about interactions between resistance and tolerance. We treated a group of birds with an anti-malaria drug (primaquine) to experimentally reduce intensity of infection and thus emulate the role of the immune system reducing parasite intensity. Our results indicate that the experimental treatment successfully reduced intensity of infection by *Haemoproteus* sp. We will present the results concerning variation in parameters likely involved in disease tolerance such as acute phase proteins (haptoglobin), and in parameters involved in resistance such as immunoglobulin levels and leukocyte profiles.

Malaria infection reduces telomere length, lifespan and offspring quality in a songbird

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Plasmodium malaria parasites constitute a major threat against human welfare. While acute malaria-related mortality is relatively well understood, little is known about the degree of, and mechanisms mediating, long-term fitness effects of malaria infections. Here we show compelling evidence that relatively benign malaria infection resulted in long-term effects on Darwinian fitness mediated by telomere shortening. In the great reed warbler, a migratory songbird, individuals experimentally infected with avian malaria had significantly higher rate of telomere shortening than non-infected individuals, and this was also confirmed in a long-term study of wild warblers with chronic malaria infections. Infected birds showed a positive correlation between parasite intensity and telomere loss rate. Among wild warblers, malaria infection status and early life telomere length were both predictors of lifespan. Moreover, there was a transgenerational cost of infection, because mothers with chronic malaria produced offspring with shorter telomeres than non-infected mothers. Our study demonstrates a causal link between a benign disease and telomere shortening, and how this translates into long-term adverse effects on Darwinian fitness. Our study implies that malaria infections may have cumulative and irreversible effects on the phenotype, thus contributing to variation in senescence and affect offspring quality. These results can have far-reaching implications for our general understanding of long-term effects of diseases also in other animals and humans.

Molecular detection of *Haemoproteus species* (Haemosporida, Haemoproteidae) as a tool for the determination of ornithophilic blood-sucking insects

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Data about the host preferences of haematophagous insects are important for vector and epidemiological research. Numerous PCR-based studies have detected host blood DNA in engorged haematophagous insects. This tool provides straightforward opportunities for investigation of preferences of blood-sucking insects and other arthropods; it is easy to detect and identify DNA of blood-source animals in engorged insects. However, the opportunities to use the host DNA markers are limited in such research because of rapid DNA degeneration in the digestive tract of insects. Usually, host DNA can be amplified only up to 3-4 days after a blood meal. Recent experimental studies show that haemosporidian parasites can survive for several weeks both in vectors and resistant insects; that provides opportunities to use parasite molecular markers for investigation feeding specialisation of blood-sucking insects.

We investigated natural infection of wild-caught *Ochlerotatus cantans* mosquitoes with *Haemoproteus* parasites using standard PCR-based methods. Three *Haemoproteus* lineages (hSISKIN1, hDELURB11, hSW1) were detected in 240 investigated mosquitoes on the Curonian Spit in the Baltic Sea. These parasites do not complete development in the mosquitoes, but persist over 2 weeks before complete abortion and their DNA degeneration. We found *H. hirundinis* (lineage hDELURB11), *H. tartakovskyi* (hSISKIN1) and *H*. sp. (hSW1) in *O. cantans*; this finding indicates that this mosquito naturally takes blood meals on the house martin *Delichon urbica* (strictly specific host of hDELURB1), siskin (*Spinus spinus*) or common crossbill (*Loxia curvirostra*) (natural hosts of hSISKIN1), and *Acrocephalus* sp. (common hosts of hSW1). All these birds were numerous at our study site.

We recommend using haemosporidian parasite molecular makers in determining possible links between blood-sucking insects and their blood-source animals. Many haemosporidian lineages are restricted to a few avian species; reports of such lineages indicate possible insect-bird associations. This study was funded by the European Social Fund under the Global Grant measure.

A survey of *Haemoproteus* vectors with respect to their host preferences and transmission of avian haemosporidians

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Pigment-forming haemosporidian parasites (Haemosporida) of the genera *Haemoproteus* and *Plasmodium* are globally widespread in birds. These pathogens are transmitted exclusively by blood-sucking dipteran insects, which for the majority of the avian *Haemoproteus* species are biting midges of the genus *Culicoides*. However, the degree of vector-parasite-host specialization remains largely unknown. The associations between parasites and hosts should in part depend on the feeding preferences of the vectors. This study is focused on the *Culicoides* biting midges in NE Bulgaria presenting results about their diversity, food preferences and presence of haemosporidian parasites in them. The identification of *Culicoides* spp. was carried out by morphological and molecular-genetic methods. Up to now, ten species of this genus were found in the studied area. *Culicoides alazanicus* was reported for first time for Bulgarian fauna. The feeding preferences of the biting midges were investigated with several pairs of primers followed by direct sequencing of the PCR products. The blood meal analyses showed a remarkable diversity of bird species indicating low degree of specialisation. The prevalence of haemosporidian parasites established in our study is presented for all investigated species of *Culicoides*. Vector-host associations, revealed in our study, shed light on the food preferences of the biting midges, but revealing the specificity of haemoproteids to their dipteran vectors needs further investigations.

Interactions of *Plasmodium juxtanucleare* and Chicken Anemia Virus: Establishing a model

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Plasmodium juxtanucleare and chicken anemia virus (CAV) are two pathogens easily transmitted and potentially harmful to chickens. In this study, we established an experimental model to investigate the effects of avian malaria caused by *P. juxtanucleare* in white leghorn specific pathogen free (SPF) chicks previously immunosuppressed with CAV. Parasitemia, hematological variables, clinical and pathological parameters were determined in four different experimental groups: chicks co-infected by CAV and *P. juxtanucleare* strain (co-infected group), chicks exclusively infected by CAV (CAV group) or *P. juxtanucleare* (malaria group) and uninfected ones (control group). Our data showed that *P. juxtanucleare* parasitemia was significantly higher in the co-infected group. Furthermore, hematological parameters, such as RBC count, hematocrit and hemoglobin were significantly reduced in those co-infected chicks. In agreement with the changes observed to hematological features, the mortality among co-infected chicks was higher compared to animals with single infections. Clinical analysis indicated moderate changes related to different organs size (bursa of Fabricius, heart and liver) in co-infected birds. The experimental co-infection of SPF chickens with *P. juxtanucleare* and CAV may represent a reproducible research tool for the study of avian malaria after CAV immunosuppression, enabling the magnification of malarial effects.

Experimental Challenge of wild-caught Oahu amakihi to *Plasmodium relictum* parasites

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The island of Oahu has no high elevation habitat that acts as refugia from vector borne disease. Oahu also has more introduced avian species than the other Hawaiian Islands. Therefore, throughout its entire range, a native Hawaiian honeycreeper, the Oahu amakihi (*Hemignathus flavus*) has been under strong selective pressure to respond to recently introduced malarial pathogens, especially *Plasmodium relictum*. Eight wild adult Oahu amakihi, scored as free of malaria by PCR and thin blood smear, were captured from two mountain ranges on Oahu and brought to the Honolulu Zoo, where they were housed in individual cages within a mosquito and predator proof aviary. A House finch (*Carpodacus mexicanus*) collected from the wild provided the parasites that were amplified in common canary (*Serinus canaria*) for experimental challenge. All four experimentally inoculated birds developed patent infections, sick with malaria and reaching peak parasitemia levels between 8-24% during days 14-18 post innoculation, declining by day 22. The other four amakihi served as controls for antibody (AMA, EBA, MSP-1 and MSP-2), hematocrit, PCR analysis, diet and behavior studies. All birds survived this challenge. The fact that no additional signs of illness commonly associated with birds sick with avian malaria were observed, points to only mild morbidity even during the pre-crisis and crisis stages. Tolerance and resistance have different ecological and evolutionary implications, and disentangling the two concepts has only recently begun to receive attention in the animal infectious disease literature. It is possible that lowland Hawaii amakihi and Oahu amakihi, two closely related congeners, have evolved different responses to the same selective pressure of avian malaria. Parasite prevalence is high in lowland Hawaii amakihi, and low in Oahu amakihi, providing additional support of tolerance in the former and resistance in the latter.

The role of mosquitoes in the transmission of avian malaria in Central California

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Much still remains to be understood about the transmission of avian malaria parasites in the wild, especially from the vector standpoint. An area of particular interest in our group, and that is currently poorly explored, is the role of vector competence in a multitude of mosquito species, and across several genera, in the transmission of avian malaria parasites. Here we present two years worth of avian malaria prevalence and diversity data from both avian and mosquito populations at our field site in Fresno County, California. Up to 18 lineages of *Plasmodium* were isolated with preliminary implications of both and host and vector specificity. *Culex tarsalis*, *C. stigmatosoma*, and *C. restuans* were implicated as major vectors based on salivary gland infection rates. We also discuss preliminary results from experimental infections of vector competence that are currently underway.

How much vertical transmission is there in avian blood parasites? More than most would think

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The advent of next-generation sequencing platforms finally allows us to approach population genetics of extremely defiant non-model organisms, such as avian blood parasites. In the end this should allow us to trace host-to-host transmission and obtain a very detailed insight into host-parasite interaction dynamics. I will report on a three-year battle to sequence noticable parts of the genome of *Leucocytozoon buteonis* and extract microsatellites for parasite fingerprinting. This parasite has several major advantages. It is a host specialist infecting a common but not superabundant host, allowing the complete sampling of all hosts in an area. It can also reach 50% prevalence in nestlings, thus providing sizable sample sizes for transmission path reconstruction. The main hypothesis for the high prevalence in nestlings is a quasi-vertical transmission, i.e. via a vector from parent to offspring. But are haemosporidian microsatellites suitable for such analyses and do they support this transmission mode? I will present first results.

Plasticity of avian malaria transmission following exposure to mosquito bites

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Malaria parasites have the ability to adjust their life history traits to changing environmental conditions to enhance their survival and transmission. Parasite relapses (here broadly defined as the reappearance of parasites in the blood from dormant stages or recrudescence from existing low infection) are expected to be part of such adaptive plastic strategies. Indeed, the launch of such relapses may be adaptive only if it coincides with the presence of mosquitoes.

Here, we experimentally tested the hypothesis that *Plasmodium* parasites can respond to the presence of vectors. We regularly exposed domestic canaries infected by the avian malaria parasite *Plasmodium relictum* to the bites of uninfected females of its natural mosquito vector *Culex pipiens*. We followed within-host parasite dynamics in the blood of exposed and unexposed birds. Both control birds and birds exposed to mosquitoes had detectable blood parasitaemia (by qPCR) and did not cure the infection over a 9-month period, indicating the importance of chronic infections in this system. We found that following mosquito biting, exposed birds had higher blood parasitaemia than control unexposed birds. Besides, we also found that the prevalence of infection in the mosquitoes increased with time after the first exposure.

This demonstrates the ability of *P. relictum* to adopt plastic life history strategies in response to variations in the abundance of insect vectors in the population.

Timing and number of colonizations but not diversification rates affect diversity patterns in hemosporidian lineages on a remote oceanic archipelago

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Parasite diversity on remote oceanic archipelagos is determined by the number and timing of colonizations, and in-situ diversification rate. In this study we compare intra-archipelago diversity of two hemosporidian parasite genera, *Plasmodium* and *Leucocytozoon*, infecting birds of the Mascarene archipelago. Despite the generally higher vagility of *Plasmodium* parasites, we report a much lower diversity of *Plasmodium* cytochrome-b haplotypes in the archipelago compared to *Leucocytozoon*. Using phylogenetic data, we find that this difference in diversity is consistent with differences in the timing and the number of colonizations, while rates of diversification do not vary significantly between the two genera. This study brings new insights into the dynamics of island colonization by potentially harmful blood parasites infecting island birds.

Testing the environmental and ecological predictors of avian malaria prevalence

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The distributions of pathogenic organisms among individuals and species are poorly understood. Hypotheses based on host abundance, immune response, vector abundance, and other environmental conditions have support although their overall importance is not well resolved. Here we report the incidence of avian haemosporidian (malaria) infections (*Plasmodium, Haemoproteus, Leucocytozoon*) in resident birds from a hyperdiverse elevation gradient and simultaneously test the roles of host and environmental characteristics in shaping observed patterns. We show that variation in infections among host species cannot be due simply to chance, but reflects aspects of host ecology and environmental characteristics. Notably, we show that overall haemosporidian prevalence and *Plasmodium* prevalence increase with individuals' centeredness within their species elevation range, and that intrinsic host characteristics such as body size and foraging strata are important determinants of infection. Body mass was positively correlated with the incidence of infections, and midstory birds were significantly less infected than canopy birds. *Haemoproteus* prevalence significantly declined with elevation, whilst *Leucocytozoon* prevalence significantly increased, and *Plasmodium* prevalence showed no variation. Our results reveal that an individual's position within a population and local environment lead to predicable variation in the prevalence of avian malaria, which has important consequences for understanding current and predicting future disease distribution.

Age-related effects on malaria parasite infection in wild chimpanzees

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Wild great apes are widely infected with a number of malaria parasites (*Plasmodium* spp.). Yet, nothing is known about the biology and pathogenesis of these infections in the wild. Determining which host related factors influence malaria infection in great apes will lead to better understanding of the dynamics of *Plasmodium* parasites in wild great ape populations and bring valuable information for the further study on the impact of malaria on these populations' fitness. Using fecal samples collected from wild chimpanzees, we investigated the effect of age on *Plasmodium* spp. detection rates. The data show a strong association between age and malaria parasite positivity, with significantly lower detection rates in adults. This suggests that, as in humans, individuals reaching adulthood have mounted an effective protective immunity against malaria parasites and also implies that immature individuals may possibly be victims of malaria infection in the wild.

The real prevalence of haemosporidian (Apicomplexa, Haemosporida) parasites in the Spanish Sparrow (*Passer hispaniolensis*): What we can estimate?

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Prevalence is one of the most widely reported quantitative descriptors of parasitic infection because it requires only detection of the presence of the parasite and not enumeration of the individuals present. The studies on avian malaria parasites and related haemosporidians are not an exception. We combined microscopic examination and nested PCR methods in a study of haemosporidian parasites in the Spanish sparrow (*Passer hispaniolensis*). In total 186 birds were mist-netted and sampled around Kalimok Biological Station (KBS) in North East Bulgaria between 1999 and 2011. According to microscopic examination 89 (47.8% prevalence) individuals were found infected. We screened 88 of the 186 birds with both the PCR and microscope methods. The prevalence was slightly different and we found 49 infected (55.7%) individuals according to microscopic examination and 45 (51.1%) according to PCR screening, and somewhat higher 61 (69.3%) when we considered both methods combined. We kept 55 randomly chosen birds in vector free aviaries during the entire winter between 2010 and 2011 at the KBS. In autumn 2010 we recorded 35 (63.6%) infected according to microscopic examination and 30 (54.5%) according to PCR amplification, but 41 (74.5%) when we consider all infected individuals. Among the 14 birds that were scored to be negative for haemosporidians in the autumn, we found 10 to be infected in the following spring. Therefore the real prevalence in all experimental birds was 92.7%, which is substantially higher than recorded by single sampling only. As a general comment, we note that although the value for each estimate can be determined accurately and unambiguously and its interpretation made with reference to the source population and time of sampling, the prevalence of haemosporidian parasites always introduces the element of uncertainty. This study was partly funded by “Postdoctoral Fellowship Implementation in Lithuania”.

Does the niche-breadth or trade-off hypothesis explain the abundance-occupancy relationship in avian haemosporidia?

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Two hypotheses have been proposed to explain evolutionary mechanisms behind abundance-occupancy relationship in parasitic animals. The niche-breadth hypothesis suggests that parasites that infect multiple hosts are more abundant and efficient at colonizing different host communities and thus acquire larger ranges than host-specific parasites. In contrast, the trade-off hypothesis (TOH) suggests that host-specific parasites adapt to a single host species and achieve high density across the entire host range, whereas host generalists parasitize hosts less efficiently due to the high cost of adaptation to diverse defence mechanisms used by hosts species. We test these hypotheses in a dataset containing 368 avian haemosporidian lineages identified using mtDNA Cytochrome-B sequences (1800 individual sequences) recovered from 2135 individual birds of 134 species sampled in 4 geographically and faunistically distinct regions: Northwest Africa (325 birds of 47 species), northwest Iberia (749/55), Greater Caucasus (216/30), and Transcaucasia (845/85). The number of regions occupied by parasite lineages was associated with their frequency. Only 4 of 356 lineages observed ≤ 30 times were found in all four regions, whereas 11 of 12 lineages observed > 30 times were found in all four regions and one was found in three regions. Eight of the 12 abundant and widespread lineages had a high prevalence in a single host species (37.7%±7.5%). Three were associated with multiple, distantly related host species and had a moderate prevalence (13.6%±4.8%) in each species. One, much more abundant than all other lineages (P_SGS1), had an intermediate host association - high prevalence in one species and moderate prevalence in several others, some of which are from different families. Our data support TOH predictions of higher host specialist prevalence than that of generalists. However, contrary to either hypothesis, both types of host specialists were successful in colonizing all study regions and achieving high frequency.

Active blood parasite infection outside the breeding season in a declining population

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Studies of the impacts of blood parasites on their avian hosts tend to focus on effects during the breeding season, and the potential for impacts during the non-breeding period tends to be overlooked. Here, we examine over-winter (December – April) infection in a population of yellowhammers *Emberiza citrinella* infected by two strains of *Haemoproteus* spp. We describe temporal dynamics of infection throughout the winter, which suggest a gradual decline in chronic infection, and immunological associations with infection, suggesting a continuing physiological cost of chronic infection. We also show a negative effect of infection on wing length during one, milder, winter and demonstrate that increased wing length confers a survival advantage in our population. We suggest both that the impact of blood parasites outside the breeding season may be greater than previously thought, and that such effects may be more pronounced in declining populations. We suggest that declining populations would make excellent model systems for further examination of such effects.

Disentangling the ecological and evolutionary components of host immune responses to avian haemosporidian infection

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Physiological responses to parasitism in the wild are often studied in the context of single host-parasite systems, precluding inference about the ecological and evolutionary dynamics of host-parasite interactions. Here we characterized immune system responses to infection by haemosporidian parasites in a sample of 424 individuals of 22 avian host species from the same local assemblage in the Missouri Ozarks. Two white blood cell types (heterophils and lymphocytes) were elevated in infected individuals across species, as was the acute-phase protein haptoglobin, which is typically associated with inflammatory responses. Linear discriminant analysis indicated that individuals infected by haemosporidians were shifted into a portion of the overall white blood cell multivariate space that was also occupied by uninfected individuals, suggesting that these latter individuals might have harbored other pathogens. DNA lineages of haemosporidian parasites were sparsely distributed across the assemblage of hosts, however in one well-sampled host species, the red-eyed vireo (*Vireo olivaceus*), heterophils were significantly elevated in individuals infected with one of two common parasite lineages. Another well sampled host, the yellow-breasted chat (*Icteria virens*), exhibited no differences in immune responses to different parasite lineages. These results emphasize the idiosyncratic nature of the ecological component of host-parasite interactions. We also tested for evidence of phylogenetic signal using both Pagel's λ and Abouheif's Cmean in host immune responses to infection and in infection prevalence itself to address the evolutionary component of host responses to infection. In keeping with previous findings of frequent host-switching in this system, we found little evidence of phylogenetic signal in immune responses among host species supporting the hypothesis that parasite-host interactions are evolutionarily labile.

Malaria selection in long-tailed macaques (*Macaca fascicularis*)

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Malaria-causing *Plasmodium* parasites infect a wide taxonomic range of hosts, but examples of malaria-resistance polymorphisms are so far limited to humans. In order to expand our knowledge of malaria-resistance mechanisms in non-human primates, we focus on the long-tailed macaque (*Macaca fascicularis*). *Macaca fascicularis* is host to five malaria parasites (*P. coatneyi, P. cynomolgi, P. fieldi, P. knowlesi*, and *P. inui*), and displays structural variation in its alpha globin. In humans, structural variation in beta globin and copy number variation in alpha globin have been unequivocally shown to offer protection against death from malaria. Using historical data on *M. fascicularis* hemoglobin phenotypes, we demonstrate that heterogeneous alpha-globin is significantly correlated with increasing malaria diversity. We provide multiple lines of evidence that the frequency of a particular *M. fascicularis* alpha globin phenotype is driven by malaria selection. We also use a population genetic model to relate these historical observations to new sequencing results. We propose that the mechanism of malaria protection in *M. fascicularis* is best explained by an altered environment for the malaria parasite within the macaque red blood cell due to the co-expression of two different hemoglobin variants. This study represents the first demonstration of selection from *Plasmodium* species on a non-human primate. *Macaca fascicularis* offers an important counterpoint to our knowledge of malaria resistance mechanisms in humans, and shows that further investigations into non-human malaria systems are likely to provide key insights into the coevolution of hosts and their parasites.

Telomere shortening and survival probability in relation to avian malaria infection status: a long term study on Western Jackdaws (*Corvus monedula*)

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The effects of avian malaria parasites on host health are still under an active debate. Here, we used a molecular approach to test for the effects of infection by avian malaria parasites (genus *Plasmodium*) and the related genera *Haemoproteus* and *Leucocytozoon* on cellular senescence (estimated by telomere length) and survival probability of wild Western Jackdaws. Birds were captured-recaptured between 2005 and 2012 in a locality close to Doñana National Park, in southern Spain. Birds were blood sampled, their infection status determined and the blood parasite lineages identified on the basis of a fragment of 478bp of the cytochrome *b* gene. The population showed a high prevalence of infection (75%) by blood parasites. Lineage diversity was also very high, but *Plasmodium* lineages Rinshi-1 (belonging to *P. relictum*) and pSPHUjJ showed the highest prevalence of infection. The effect of parasites on birds differed among parasite lineages with some of them showing an adverse effect on bird survival and/or telomere shortening. Overall, our results support the negative effects of avian malaria parasites on wild birds health.

Vector movement underlies avian malaria at upper elevations in Hawaii

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Avian malaria is considered the greatest long-term threat to Hawaiian forest birds because most are highly susceptible to the disease, which is expected to increase at upper elevations with climate warming. Our 1900 m site at Hakalau Forest National Wildlife is too cool for malarial sporozoites to develop on-site in the mosquito vector. Therefore transmission in resident species must come from mosquitoes that become infectious at warmer lower elevations and move to the 1900 m site. We develop a mosquito-movement-warming model that deals with mosquito movement and climate warming at lower elevations. This model is supported because we document several epizootics at 1900 m over a 14 year period, show that 10 of 14 native and introduced bird species tested positive, prove that transmission occurs at upper elevation, and identify a reservoir of malaria in one native species that spans the elevational range of native birds. These data indicate that warmer temperatures at lower elevations can increase the number of infectious mosquitoes that move to upper elevation. The model indicates further increases in malaria at upper elevation as warming sufficient to support sporogony progresses up the mountain.

Diverse avian malaria in Andean house wrens: Evidence for co-diversification despite lability in host breadth and climatic niche

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Recent research has revealed hundreds of cryptic genetic lineages of avian malaria parasites, but the extent to which this diversity may be associated with host population structure or environment is unclear. We surveyed haemosporidian and host mtDNA in an ecological generalist, the house wren (*Troglodytes aedon*), across the complex landscape of the Peruvian Andes. We detected deep genetic structure within the house wren across its range, represented by seven clades that were between 3.4-5.7% divergent. From 140 sampled house wrens we found an overall parasite prevalence of 0.41, comprising 23 divergent evolutionary lineages of haemosporidian mtDNA, of which ten were novel. We found no discernible co-phylogenetic structure between haemosporidians and house wrens, and divergence date estimates revealed that the majority of parasite diversity was present prior to the diversification of house wren populations. However, a clade of six *Haemoproteus* lineages appeared to be host-specific and to have diversified contemporaneously with house wren populations. Individual haemosporidian lineages varied widely with respect to host specificity, prevalence, and geographic distribution, with the most host-generalist lineages also being the most prevalent and widely distributed. *Haemoproteus* and *Leucocytozoon* included lineages with restricted ranges and high host specificity; however, all *Plasmodium* parasites were host-generalists with broad geographic distributions. Combined and genus-specific haemosporidian prevalence differed significantly across environments and elevation. We also observed spatial stratification of haemosporidians along the west slope of the Andes in central Peru where five lineages were restricted to non-overlapping elevational bands.

Escape behaviour of hosts and blood parasites infections

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Animal populations often consist of individuals that differ in their behavior by being consistently bold or shy, and such consistent individual variation in behavior across time and contexts is generally termed personality. Bold personality may more often bring animals into contact with predators, but also results in more frequent encounters with parasites or their infective stages and vectors. Therefore, we predicted that personality would correlate with risk of infection with parasites at the interspecific level, with bolder species having more parasites than shy species. Here we tested whether different species of birds with specific behavior also tended to show consistent differences in prevalence with blood parasites including malarial parasites. We investigated the relationship between escape behavior and prevalence of blood parasites. The intensity of escape behavior was positively related to prevalence with *Haemoproteus* and *Leucocytozoon*, while that was not the case for the more virulent *Plasmodium*. Species that were habitat generalists and hence encountered a greater diversity of habitats had higher prevalence of blood parasites than specialists. In addition, two aspects of escape behavior were correlated with habitat exploration, as reflected by the relative frequency of feeding innovations. Therefore behavioural response to an approaching predator and to the situation of being caught by a predator represents two independent axes of anti-predator behaviour that do not evolve in concert. These findings are consistent with the hypothesis that personality is related to risk of infection with blood parasites as partially mediated by the effect of diversity of breeding habitats.

Multiple haemoparasite co-infections in bats from Southeast Asia and Central America

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Phylogenetic analyses have been broadly used for a better understanding of coevolutionary processes between host and parasites. Such complex interactions among parasites, host species and vectors still remain unveiled. A finer scale picture accurately identifying blood parasites richness is fundamental to evaluate host-parasite-vector dynamics and interactions.

To assess the haemoparasite richness and prevalence we undertook a molecular characterization of vector-borne haemoparasites of insectivorous bats from Southeast Asia (n=17; genus *Rhinolophus, Aselliscus*, and *Hipposideros*) and frugivorous bats (n=10; genus *Balantiopteryx, Artibeus, Dermanura* and *Sturnina*) from Central America.

Tissue samples were screened through nested-PCR. *Hepatocystis* sp. parasites were isolated in four bats from the genus *Rhinolophus* and two from the genus *Hipposideros*. Phylogenetic analysis of the 663bp sequences of cytochrome *b* mitocondrial gene indicates these isolates cluster in a well-defined monophyletic clade within the mammalian *Plasmodium* parasites, which is basal and paraphyletic to the Asian and African primate *Hepatocystis* clades.

In addition, we screened for piroplasm and eubacteria parasites by RLB macroarray which allows remarkably high sensitivity and specificity, and therefore appeared as a very powerful tool to detect extremely low parasitemias rates and discriminate co-infections.

Bats from localities in Southeast Asia showed the following prevalence: *Babesia* sp. 23.5%, *B. microti* 5.9%, *B. rossi* 5.9%, *B. felis* 5.9%, *B. ovis* 29.4%, *B. vogeli* 17.6%, *Theileria* sp. 42.1%, *T. annulata* 58.8%, *T. parva* 5.9%, *T. equi* 5.9%, *Borrelia burgdorferi* ss 5.9%, *Francisella tularensis* 35.3%, *Rickettisia rickettsii* 17.6%, and *R. endosymbiont* 11.8%.

Bats from localities in Central America showed the following prevalence: *Babesia* sp. 20%, *B. rossi* 10%, *B. felis* 20%, *Theileria* sp. 40%, *T. annulata* 50%, *Borrelia burgdorferi* sl 10%, *B. burgdorferi* ss 10%, *B. lonestari* 10%, *Ehrlichia canis/ovis/muris* 20%, *Fransicella tularensis* 20%, *Rickettsia rickettsii* 10%, *R. endosymbiont* 20%.

Co-infections occurred with varying frequency, but single infections are significantly less common ($p<0.001$) than dual infections or multiple infections (up to 5). Correlation coefficient is significant ($p <0.05$) between co-infection rate and the infection by *Hepatocystis* sp., *Babesia* sp., *Theileria annulata, Borrelia burgdorferi* ss, and *Rickettsia rickettsii*. Only two of the screened bats (*Artibeus hirsutus* and *Sturnina lilium*) were free of haemoparasites.

Merozoite surface protein 1 (MSP1), a candidate gene for a better understanding of the epidemiology of *Plasmodium relictum*?

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Cyt *b* lineages belonging to the taxonomically defined species of *Plasmodium relictum* (e.g. SGS1, GRW4 and GRW11) are lineages that exhibit an extraordinary broad host range as well as large transmission areas. However, until recently we haven't been in possessions of molecular markers for understanding the epidemiology of these lineages neither across host species nor across their transmission areas. With the applications of new sequence techniques such markers are now available. Here I present one of such markers, the Merozoite Surface Protein 1 (MSP1). The MSP1 gene is one of the more variable genes in *P. falciparum* and has frequently been used for inferring population structures on human malaria parasites. Within *P. relictum* there is extensive variation in certain regions of the gene and by utilizing this genetic variation across samples of GRW4, SGS1 and GRW11 clear patterns of geographical structuring appear on the global scale. Thus, allowing us to get a better understanding of the spread and geographical limitations these *P. relictum* strains posses.

Experimental data on the development of *Plasmodium* sp. (lineage pCOLL4) in avian hosts and mosquitoes

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Recently, the lineage pCOLL4 of *Plasmodium* sp. was deposited in the MalAvi database. This parasite has been reported in South American and European birds belonging to 7 families. However, the life cycle of this parasite remains unknown. We investigated the sporogonic development of the lineage pCOLL4 in three mosquito species *Culex pipiens pipiens, C. pipiens* form *molestus* and *Aedes vexans* and its erythrocytic and exoerythrocytic development in experimentally infected domestic canaries *Serinus canaria*.

The lineage pCOLL4 was obtained from a naturally infected Red-Backed Shrike (*Lanius collurio*). Eleven canaries were infected experimentally with this parasite. Additionally, mosquitoes of 3 species were allowed to take infected blood meals, and were tested for the presence of sporogonic stages.

This study shows that the lineage pCOLL4 is virulent and may cause death to its host. Parasitemia of 70% has been recorded in some infected birds. Erythrocytic merogony is synchronized, with a periodicity of 24 hours. Phanerozoites were observed in numerous organs (brain, heart, liver, lungs, spleen and muscles) of the dead canaries. Numerous phanerozoites developed in the brain, indicating possible cerebral paralysis. This lineage developed numerous gametes and ookinetes, and a few oocysts in *C. p. pipiens, C. p.* form *molestus* and *A. vexans*. However, further sporogonic development is abortive; sporozoites do not develop. Because this parasite has been reported only in a few African migrants in Europe, it is likely that transmission takes place only in tropics and requires other mosquito species. This study was funded by the European Social Fund under the Global Grant measure.

Transmission regions of haemosporidian parasites of three common nightingale populations with different wintering grounds in Africa

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We evaluated the patterns of transmission of haemosporidian infections (*Plasmodium* and *Haemoproteus*) in the breeding and wintering grounds of common nightingales (*Luscinia megarynchos*) captured in France, Italy and Bulgaria. The lineages: LULU1 (*Haemoproteus balmorali*), ROBIN1 (*Haemoproteus attenuates*), SGS1 (*Plasmodium relictum*), GRW11 (*P. relictum*) and the *Plasmodium* spp. lineages LINN1 and PASDOM7 found in the nightingales have previously been found in many resident European bird species suggesting transmission in the breeding grounds. None of the lineages ACCTAC01, AFTRU5, GRW2, GRW9, PBPIP1, RTSR1, GRW10 and BT6, found in our study, have been registered in resident bird species in Europe. Additionally, for all of these lineages there is evidence of infection of many resident African bird species. Since the wintering areas of our nightingale populations were known by geolocation in the 2009/10 season, we could evaluate regional-specific transmission probabilities of the *Plasmodium* species. Wintering regions with high probability of the *Plasmodium* lineages ACCTAC1 and AFTRU5 transmission are Guinea, Ivory Coast and Ghana. The lineage BT6, found only in Italian nightingales, is transmitted with high probability in a region from Ghana to west Nigeria, but the findings of this lineage in Scandinavian bluethroats and in rosefinches suggests transmission also in southern Asia. The lineages GRW2 and RTSR1, infecting mainly Bulgarian nightingales, with high probability are related to wintering sites in the Central African Republic, Chad and Uganda, but there are records of these parasites in other bird species from Nigeria, Gabon, Zimbabwe, Namibia and Botswana. The lineage GRW9 found in all three populations can be transmitted with relatively equal probability in the whole wintering range. We could not find distinct transmission areas for all haemosporidian parasite lineages found in nightingales. However, our study highlights the potential role of *Plasmodium* lineages as regional-specific or habitat-specific markers applicable in ecological studies and monitoring programs.

Malaria infection of both parents can affect an ecosystem service

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Birds play a key role in providing important ecosystem services such as pest control in forests. They are also prone to infection by diverse groups of malarial parasites which may have effects on host fitness. There is an increasing realization that parasites can structure communities and play an often hidden, yet vital, role in food webs. However, it remains relatively unknown whether parasite infection might have effects at the ecosystem level. Here, we used a dataset gathered in May 2012 to link infection with malaria to provision of an ecosystem service. We presented nesting individuals of a population of great tits, *Parus major*, with a novel stimulus, in the form of ten plasticine caterpillars. This is a standard method to show the effect of birds on pest control, a key service in forest ecosystems. We also measured feeding rates and habitat quality, parental age and condition, gathered when the chicks were 14 days old. We found that fake caterpillars placed near nests where both parents were infected with *Plasmodium* spp. suffered much higher attack rates compared to nests where neither or only one parent was infected. These effects remained after controlling for habitat quality and feeding effort. Taken together, our results indicate that infection with malaria parasites can have strong effects, which cascade up to the ecosystem level.

Long-lasting survival of *Haemoproteus* parasites (Haemosporida, Haemoproteidae) in resistant blood-sucking insects, with perspectives on haemosporidian vector research

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Haemoproteus spp. are cosmopolitan vector-borne haemosporidian parasites, some species of which cause diseases in non adapted birds. Recent polymerase chain reaction (PCR)-based studies have detected mitochondrial cytochrome *b* gene lineages of these *Haemoproteus* parasites in blood-sucking mosquitoes and speculated about the possible involvement of these insects in transmission of avian haemoproteids. However, the development of *Haemoproteus* lineages has not been documented in mosquitoes.

We infected over 304 individuals of *Ochlerotatus cantans*, a widespread Eurasian mosquito, with *Haemoproteus tartakovskyi* (lineage hSISKIN1) and *Haemoproteus balmorali* (lineage hROBIN1). Mosquitoes were allowed to take non-infected and infected blood meals and maintained in the laboratory until 17 days post-infection (dpi) and tested for presence of sporogonic stages by microscopic and PCR-based methods. Microscopic examination revealed partial development of both parasites in the infected insects. Numerous ookinetes were seen in the gut area and adjacent tissues located in the head, thorax and abdomen of mosquitoes between 1 and 5 dpi. Numerous oocysts were seen in the midgut wall between 4 and 15 dpi; they were also present in the head and thorax of infected mosquitoes testifying to the active movement of ookinetes throughout the body. Oocysts degenerated between 11 and 17 dpi. In accordance with microscopy data, PCR and sequencing revealed presence of the lineages hSISKIN1 and hROBIN1 in experimental mosquitoes as long as 15 and 17 dpi, respectively, demonstrating relatively long survival of *Haemoproteus* parasites in the resistant insects without DNA degeneration.

The present study shows that PCR-based diagnostics should be carefully used in vector studies of haemosporidians because it detects parasites in insects for several weeks after initial infection, but does not distinguish abortive parasite development. Demonstration of infective sporozoites in insects is essential for definitively demonstrating that certain insect species are vectors. This study was funded by the European Social Fund under the Global Grant measure.

Microscopic observation of oocysts and sporozoites and subsequent PCR for identification of genetic lineages of avian *Plasmodium* spp. in natural vector, *Culex pipiens pallens*

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Culex pipiens pallens have been considered a main natural vector for avian malaria parasites (*Plasmodium*) in Japan, based on PCR-based analysis for detection of *Plasmodium* DNA and blood-meal identification. More than ten genetically distinct *Plasmodium* lineages have been previously found from *C. pipiens pallens* collected at a park in urban Tokyo. The purpose of this study is to confirm development of oocysts and sporozoites of *Plasmodium* lineages in the potential natural vector by microscopy and subsequent PCR-analysis of field collected mosquitoes. Mosquitoes were collected at the same park as the previous study using a sweeping net between May and September, 2012. A total of 491 *C. pipiens pallens* (including both unfed and blood-fed) were dissected under microscopy to examine oocysts and sporozoites in the midguts and the salivary glands, respectively. Oocyst or oocyst-like structures were observed on the outer wall of midguts for 33 (6.7%) mosquitoes, while, motile sporozoites were observed in the salivary glands for 9 (1.8%). Subsequent PCR targeting the 478 bp of cytochrome *b* gene of *Plasmodium* parasites were applied for 31 of the 33 specimens that had been considered as oocyst-positive by microscopy. Positive amplification was obtained in 14 specimens, 9 of which had sporozoites in the salivary glands. The remaining 17 specimens were negative for the PCR assay, which might be due to misidentification of oocysts upon microscopic examination, failure in either DNA extraction or PCR. The PCR-products were sequenced and identical to CXPIP09 (n=6), SGS1 (n=7), and GRW4 (n=1). The former two *Plasmodium* lineages were dominant in the previous study, while GRW4 was detected for the first time in the study park. The present results demonstrate that at least the three *Plasmodium* lineages develop into sporozoites within *C. pipiens pallens* under natural condition.

How to obtain purified template for genomic studies of haemosporidians inhabiting nucleated red blood cells?

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The first decade of the XXI century yielded breakthrough in whole genome sequencing from an increasing number of organisms. Currently, the completely sequenced genomes provide the most comprehensive data for studies of genetic variation in various organisms. However, there is still no data about whole genomes of malaria or related haemosporidian parasites infecting birds and lizards. The majority of molecular studies on these parasites are still based on mitochondrial gene sequences. Moreover, the lack of template for genomic studies of haemosporidians is an obstacle for the construction of primers for the amplification of rapidly evolving DNA regions. These challenges are determined mainly by the obligate intracellular development of haemosporidian parasites and the structure of avian red blood cells. Unlike mammals, red blood cells of birds and reptiles possess nuclei, resulting in a predominant amount of host DNA in each blood sample. This complicates the preparation of purified DNA template from avian haemosporidians, and markedly limits genomic studies of these parasites.

We developed a simple method that generates large amounts of purified avian haemosporidian DNA. The method is based on a biological feature of haemosporidians: in vitro exflagellation leading to the development of numerous microgametes, which can be easily separated from host blood cells by simple centrifugation.

We used siskins (*Carduelis spinus*) naturally infected with *Haemoproteus tartakovskyi*. Blood was taken using heparinized microcapillaries, placed immediately in a microtube containing sodium citrate solution (3.7%), gently mixed, and exposed to air. After 4 min, the sample was centrifuged for 5 min at 7000 rpm. Approximately 20-50 µl of supernatant (blood plasma) was stored in SET-buffer and used for further processing. Our results reveal that this simple method provides opportunities to collect pure parasite template for DNA studies, which can be used for various genetic analyses, including whole genome sequencing. This study was funded by the European Social Fund under the Global Grant measure.

Nutritional stress mediates malaria-infection costs in naturally infected mosquitoes

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Many studies have investigated the effect of avian malaria parasites on bird host phenotypical traits. In comparison, few studies have determined their effects on the mosquito vectors. These studies have often relied on unnatural vector-parasite associations due to the lack of available knowledge on natural vectors and have mostly ignored environmental variability. Here, we used naturally infected or uninfected *Culex pipiens* mosquitoes that were field caught throughout one season (April-August) 2011 and individually maintained in laboratory conditions. We experimentally manipulated an environmental parameter, the food quality, and measured the effect of the parasite in both poor and rich food qualities. We found that *Plasmodium* parasites affect vectors' survival under nutritionally stressed conditions only. Parasitized mosquitoes maintained similar reproductive outputs as the unparasitized ones and we did not find evidence that the reduction in survival was mediated by a re-allocation of resources towards reproduction. The environment-dependent effect of *Plasmodium* on the vectors' life history traits was constant throughout the season, although the vectors' fecundity and tolerance to nutritional stress both varied across periods of capture. Our results highlight the importance of environmental conditions on the costs imposed by parasites on their hosts. This may have major implications for disease transmission in wild conditions.

The effects of mosquito control on vector dynamics and prevalence and diversity of avian malaria in Camargue sparrows

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Despite the great importance of vectors in the transmission of avian malaria, it is still relatively poorly understood how vector abundance and diversity affects the diversity, prevalence and virulence of avian malaria infection. The Camargue, the Rhone river delta in southern France, offers an excellent opportunity to study vector and host dynamics in avian malaria. There is a large abundance of potential vectors, principally mosquitoes, active for a long part of each year. There is also a large diversity of hosts given the Camargue's high biodiversity, particularly of migratory and wading birds. We also found prevalence of malaria was high in a range of resident and migratory passerines. Finally, mosquitoes have been controlled using pesticides and other agents in some areas of the Camargue for decades, while a protected natural zone has remained uncontrolled during this time. A comparison of avian malaria between natural and controlled populations allows an assessment of the role of mosquito reduction in determining prevalence and diversity of avian malaria. We studied house sparrows and tree sparrows in several locations of both natural and controlled regions in the Camargue. We also collected mosquitoes at these locations. We found that mosquito abundance varied significantly from site to site. *Culex pipiens* was found to carry different lineages of *Plasmodium* in the Camargue. Prevalence of *Plasmodium* was higher in house sparrows than tree sparrows, and neither host species appeared to carry either Haemoproteus or *Leucocytozoon* infection. We found no effect of mosquito control, or of mosquito abundance, on prevalence or diversity of *Plasmodium* infection in sparrows. The impact of *Plasmodium* infection in wild male and female Camargue sparrows on fitness-related traits, and the role of vector control in determining infection risk in wild birds will be discussed.

Distribution of blood parasites in hummingbirds, with discovery of a new *Leucocytozoon* sp. infection

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Hummingbirds (Trochilidae) are the New World birds strongly associated with Neotropical areas. Colombia has a record of 162 species of the Trochilidae; 78 of them have been reported in the Andes Mountains. In studies carried out along an altitudinal gradient in this country, 289 hummingbirds belonging to 35 species have been sampled; only eight individuals belonging to eight different species were found infected with *Leucocytozoon* spp. (the prevalence is 1.3%), *Haemoproteus* sp. (1%) and microfilaria (0.3%). The prevalence of these infections in hummingbirds is consistently low, as has been reported previously in other hummingbirds. That can be explained by the dilution effect related to the huge biodiversity of the Neotropical birds, which represent a great variety of alternatives of blood-meal for potential vectors. On the other hand, hummingbirds sampled are tiny (size between 7 and 12 cm). The small size and also the particular flight behaviour can make these birds less attractive to vectors or hindering the vector-host interaction.

Leucocytozoon sp. was first reported in hummingbirds in 1944, but it has never been described before. We detected undescribed morphological diversity of *Leucocytozoon* parasites and their host cells in hummingbirds. This parasite develops in roundish host cells, which nuclei are consistently comma-like in shape. Cyt b lineage (478 bp) of this parasite is similar (0.2% genetic distance) to a lineage found in the Trochilidae bird *Sephanoides sephaniodes* of Chile. All previous reports of *Leucocytozoon* spp. in hummingbirds came from high altitudes. It is probable that the transmission occurs mainly at high altitudes in Central and South America; that can be associated with the distribution of susceptible vectors.

Infection estimates of apicomplexan hemoparasites in reptiles: A comparison of multiple quantification methods

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Hemogregarines are intraerythrocytic apicomplexan parasites and the most common hemoparasites of reptiles. Among these, the genus *Hepatozoon* is the most abundant, yet little is known about its prevalence, infection intensity and distribution in natural reptile populations. Microscopy has been traditionally used in parasite screening but conventional and quantitative PCR (qPCR) have been shown to be more sensitive and are increasingly being used in parasitology. Using as a model system two lacertid lizard species living in sympatry, *Podarcis hispanica* and *Podarcis bocagei*, from a single location in Portugal, we wanted to compare how hemogregarine intensity levels varied between closely related species living in sympatry. These two species were chosen because they have been previously shown to have high *Hepatozoon* prevalence. In addition, we compare the accuracy of different methods, namely microscopy, PCR and qPCR, for the detection and quantification of hemoparasites in reptiles. Our results show that qPCR was much more accurate and sensitive than microscopy and conventional PCR, especially at low intensity levels. The comparison of various extraction methods suggest that blood samples provide higher parasite detection success (prevalence) and sensitivity (intensity) compared to tissue samples. Our qPCR assay was also able to detect mixed infections of hemogregarines and putative coccidia infections. In relation to our biological system, intensity of hemogregarine infection varied significantly among host species, sexes and snout-vent length. Males had higher intensity levels, also demonstrated in other studies, which is often attributed to testosterone immunosuppressive effects and to frequent engagements in aggressive encounters that make them more exposed to parasites. Also, bigger animals of the same species had higher intensities, however higher intensity levels were found in *P. hispanica*, which is smaller than *P. bocagei*. Thus, these differences may be associated with hormonal and behavioural aspects of host species and sexes and should be further studied.

Prevalence of avian blood parasites in birds of different personalities: Comparative research in Certain European bird species

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The concept of animal personality is a relatively new field in behavioural ecology and evolutionary biology. In the last two decades there has been an increasing interest in the relationship between individual behavioural differences and other ecological aspects such as parasite-host relationships. We formulated two hypotheses according to which the evolution of animal personality might have been heavily influenced by parasites. The first hypothesis suggests that some personality types are more susceptible to parasites than others. The second suggests a direct influence of parasites on the host behaviour, in the long run causing personality differences.

As a first step to answering these questions we conducted a study aimed at finding a relationship between the prevalence of haemosporidian parasites and the behavioural types in a number of relatively common passerine bird species. The personality of each individual was estimated by standard behavioural tests. Blood from the same birds was scanned both by PCR and microscope for haemosporidian parasites of three genera: *Plasmodium, Haemoproteus* and *Leucocytozoon*. We analysed the distribution of personality types in non-parasitized and parasitized birds. On a reactive-proactive axis, more proactive individuals were more heavily parasitized. Infected birds also take more risks in approaching a new object. Finally, we discuss the adaptive value of these differences and we make suggestions for future research projects.

Diversity of avian malaria and malaria-like parasites from the potential vectors biting midge *Culicoides* and wild birds from SW Spain

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Blood-sucking insects play a key role in the transmission of vector-borne parasites. However, there is a lack of information regarding the avian malaria parasites infecting insect vectors. We used a molecular approach to identify the *Haemoproteus* and *Plasmodium* lineages in 97 parous the ornithophilic biting midge *Culicoides circumscriptus* trapped in the Doñana National Park and surroundings areas. Also, to identify the potential hosts of these parasite lineages, samples from 123 birds of 11 species were screened for the presence of parasite lineages. We identified six *Haemoproteus* and two *Plasmodium* lineages infecting 13 midges. Comparison of this parasite lineages with those deposited in public databases and those isolated in our laboratory, allowed us to identify some potential transmission networks. One *Haemoproteus* lineage was identical to a previous described lineage GAGLA03 isolated from *Garrulus glandarius* in Bulgaria and another *Haemoproteus* (undescribed lineage) was identical to that isolated from *Corvus monedula* in Spain. Moreover, a new described lineage showed a 99% similarity with the *Haemoproteus* lineage hAPPAjS isolated in Japan from *Aptenodytes patagonicus*. The two *Plasmodium* lineages isolated were identical to previously described sequences corresponding to Rinshi-1 (from *P. relictum*) and pSPHUjJ. Also, six *Plasmodium* and one *Haemoproteus* lineages were isolated from 10 infected birds. The *Haemoproteus* lineage was not isolated from biting midges. Four *Plasmodium* lineages isolated from birds completely matched to previously described lineages: GAL-2012, Delurb5, P15 and Rinshi-1. Altogether, our results add valuable information of the blood parasites harboured by the potential *Haemoproteus* vector *C. circumscriptus* and highlight the necessity to identify the transmission networks and blood parasite diversity infecting vertebrate and invertebrate hosts in the wild.

Prevalence, genetic diversity, deforestation and invasive avian malaria in Peru

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Habitat degradation can pose a direct threat to many species, but the effects of deforestation on the spread of pathogens are largely unknown. Avian malaria and related parasites are abundant and distributed worldwide, making them excellent models for exploring the factors contributing to the emergence of infectious diseases (EIDs). Some recent works have examined the relationship between the distribution of haemosporidian infections and deforestation in birds, but results are mixed. In addition, the fauna of haemosporidians in some geographical regions has been studied irregularly and the distribution of haemosporidians remains unknown for many bird species of the world. With these aims we conducted a molecular survey to explore the prevalence and genetic diversity of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* spp. in birds of Peru. We collected 207 blood samples from 70 bird species in 4 different localities: Lima (Pacific coast), Huanuco (eastern slopes of the Andes), Tarapoto (highly deforested area of Peruvian Amazonia) and Iquitos (less deforested area of Peruvian Amazonia).

We found an overall prevalence of 30%, with a local prevalence ranging from 18% (Iquitos) to 35% (Huanuco). We identified 18 *Plasmodium* and 13 *Haemoproteus* lineages, with 55% of them reported for the first time. Prevalence of *Plasmodium* infection was higher (57%) than prevalence of *Haemoproteus* infection (36%). The host range of *Haemoproteus* lineages varied from 1 to 3 host species, whereas *Plasmodium* lineages presented a broader range, varying from 1 to 8 host species. *Plasmodium relictum* SGS1 was widespread and the most prevalent parasite found in our study, infecting 13 individuals from 8 host species in 2 localities (Lima and Huanuco). To the best of our knowledge, this is the first report of this invasive pathogen in the mainland Americas. The presence of this exotic *Plasmodium* lineage in birds of South America may represent a threaten to avifauna, because naive host populations usually lacks protective immunity. This system in Peru allows us to test how the arrival of an introduced *Plasmodium* species affects birds that have already been exposed to other endemic malaria parasites.

We also observed that overall prevalence was higher in highly deforested areas of Amazonia (33%) than in less deforested areas (18%). In addition, genetic diversity of haemosporidians was higher in highly deforested areas (9 *Haemoproteus* lineages + 10 *Plasmodium* lineages) than in less disturbed areas from Amazonia (2 *Haemoproteus* lineages + 5 *Plasmodium* lineages). Further studies exploring the disease ecology of Amazonia deforestation and the patterns of the transmission of invasive avian malaria in America will contribute to better understanding of the consequences of the spread of EIDs, and provide data for policy decisions on biodiversity conservation. We are grateful to the NSF sponsored MalariaRCN for the support that allowed the training of students in the participation of this project.

Lessons learned from *Leucocytozoon* spp. in a Neotropical country

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In different works, 2183 birds were scanned for avian hemoparasites in Colombia. *Leucocytozoon* spp. showed preferential distribution at highlands where 1313 birds were analyzed, 96 of them were found infected, in altitudes from 2400- until 4000 masl. *Leucocytozoon* spp. were the only hemoparasites found at 4000 masl. Orders Passeriformes and Apodiformes were found infected, which also were the most frequently sampled; however another 33 birds belonging to 9 different orders were also analyzed, without detectable infection. The predominant morphotypes found in this study were *L. fringillinarum, L. dubreuili*, and *L. majoris*. Molecular lineages of parasite Cyt*b* fragments (478bp) were obtained, and a phylogenetic reconstruction was done using Bayesian Inference in MrBayes. Lineages corresponding to *L. fringillinarum* morphotypes were distributed along different clades, with great sequence divergence and present in several hosts at different altitudes, suggesting a case of cryptic speciation for this parasite. Further studies increasing the number of molecular markers to unscramble those relationships and to determine the potential cryptic speciation of this parasite are desirable. The Andes Mountains are characterized by species endemism and one of them is *Gigantodax* spp., a subgenus of the Simuliidae family. In the study areas where *Leucocytozoon* is distributed, 24 and 11 *Simulium* and *Gigantodax* species have been identified respectively. Since in Colombia and other Neotropical countries *Leucocytozoon* has not been found in resident birds in the lowlands, and based on the abundance of insects in the sampled localities, we hypothesized that *Gigantodax* spp, and some *Simulium* species only found at high altitudes, might be the vectors of *Leucocytozoon* in Colombia and in other highland ecosystems of South America.

Assessing the cost of haemosporidian infection through flight performance in Rock Pigeons (*Columba livia*)

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Studies worldwide have found that avian haematozoa are ubiquitous and diverse, yet much remains unknown about their effect on the host. While the pathogenicity of some *Plasmodium* parasites is well documented, the impact of *Haemoproteus* infection on host fitness may be subtler and previous research attempting to quantify this effect has produced incongruent results. We are using homing ability in Rock Pigeons (*Columba livia*) to evaluate the cost of *Haemoproteus columbae* infection to the host. Our preliminary data show that Rock Pigeons have the ability to return to their roosting/nesting site from over 100km away. Homing is an energetically demanding activity that may be affected by blood parasite infection even when body condition and survival are not. By using flight performance as a measure of host performance we hope to better understand the impact of avian haematozoa on the host and the potential role of chronic parasitic infections in shaping host ecology.

Evolution of a generalist assemblage of blood parasites in a megadiverse community of tropical birds

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Studies on host specificity and its underlying mechanisms are fundamental to understand the architecture of biodiversity and to predict the risks of host-switching for conservation issues. Here we test the hypothesis that *Plasmodium* and *Haemoproteus* blood parasites tend to be generalists when they face a mega diverse host environment, which is expected from the enhanced costs of finding a specific host. We analyzed if *Haemoproteus* blood parasites, which are normally more host specific than *Plasmodium* blood parasites, are more generalist in a bird community in Southern Ecuador. Our results were put in a global context by comparing with similar samplings across temperate and tropical areas worldwide. In a diverse sample of 345 wild birds, belonging to 89 species and 21 families, a prevalence of 16.2% was found. From the 21 parasite lineages detected, 18 lineages appeared to be new and had a wide phylogenetic distribution in the known lineage diversity worldwide. The level of host generalization was high for *Plasmodium* and *Haemoproteus* and the host range of *Haemoproteus* in this tropical bird community was shown to be the highest ever documented. These findings suggest that the evolution of generalist *Haemoproteus* and *Plasmodium* parasites could be favored in megadiverse bird communities. In these environments, generalist parasites may accrue benefits from the amplification effect associated with the exploitation of various host species, while parasite specialization in single host species may be penalized by dramatically reduced host availability.

Biting activity and seasonal abundance of *Culex quinquefasciatus* in Bogotá

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Culex quinquefasciatus is a nuisance for humans and animals in Bogota, due to its high abundance. The aim of this study was to characterize the abundance of these mosquitoes monthly, and their biting activity. Adults were collected using a Shannon trap, one night in each month, from January to June 2008 in two places located near mining excavation pits. These pits were flooded with water of the Tunjuelito River in 2002. To measure the variation of abundance, 15 CDC traps were installed in three houses in five different neighborhoods around of the main breeding places, from 17 pm to 8 am. 18,891 mosquitoes were collected, 99.93% were *Culex quinquefasciatus* and 0.06% *Culex* spp. These mosquitoes were most abundant from March to May, the highest rainfall months. Two patterns of nocturnal activity in one of them a unique peak in the darkest hours was found, in an area of active mining. In the other area, located nearest to houses, mosquitoes showed two activity peaks. The first one in the sunset hour and the other one varies from 23 pm to 3 am, but between this hours there was activity. The difference in these two behavioral patterns could be explained by micro-environmental conditions such as light and the intensity of mining activity, but it could not be associated with the presence of rain. An association between abundance of mosquitoes and the distance to the breeding places was found out.

Virulence of different isolates of *Plasmodium relictum*: Implications for bird conservation projects

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Avian malaria parasites (*Plasmodium*) are prevalent worldwide, but information about their impact on birds, especially during primary infections is insufficient. Recently, using molecular and traditional methods, it was shown that many morphospecies contain several distinctive mitochondrial cytochrome b gene lineages. The information about development and virulence of different lineages of the same *Plasmodium* species in avian hosts is lacking.

One common crossbill *Loxia curvirostra* and one house sparrow *Passer domesticus* naturally infected with the pSGS1 and pGRW11 lineages of *Plasmodium relictum*, respectively, were used as donors of the parasites to infect experimental birds. Eighteen juvenile common crossbills were used for experimental infections in 2010, and 18 birds in 2011. The development of parasitemia, body weight, and value of haematocrit were measured in all experimental and control birds for 30 days. The obtained results were compared with our former study, in which the same bird species was infected with the pSGS1 lineage isolated from a naturally infected reed warbler *Acrocephalus scirpaceus*.

The present study shows that the closely related lineages pSGS1 and GRW11 of *P. relictum* and the two isolates of the same pSGS1 lineage demonstrate different patterns of development and virulence in the same avian host. The mean peak of parasitemia reached 15% in crossbills infected with the reed warbler pSGS1 isolate and all birds survived. In contrast, the intensity of parasitemia of the common crossbill pSGS1 isolate reached 90% and the mortality of infected birds was 50%. The house sparrow lineage pGRW11 showed similar virulence: parasitemia reached 50% in the majority of birds and 39% of them died. In conclusion, different isolates of the same *Plasmodium* morphospecies and even of the same lineage might be of markedly different virulence to birds; that should be taken in consideration in conservation projects. This study was supported by the European Social Fund under the Global Grant measure.

Evolution of parasite island syndromes without long-term host population isolation: Avian haemosporidians infecting Macaronesian blackcaps *Sylvia atricapilla*

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The study of parasite biogeography on islands is important for our understanding of both the processes involved in the evolution of parasite assemblages worldwide, and the ecology and conservation of insular communities. By studying the haemosporidian blood parasites of a bird that has recently colonised a number of oceanic islands, we were able to test hypotheses relating to the processes involved in parasite colonization and community assembly prior to the permanent isolation of host species on islands. We determined the prevalence and richness of parasites of avian haemosporidians in blackcaps, *Sylvia atricapilla*, a widespread passerine which colonized the Atlantic archipelagos of Madeira and Canary Islands during the Last Glacial Maximum. We compared insular blackcap parasite assemblages with those observed in blackcap populations sampled on mainland Europe. The insular parasite assemblage was impoverished, containing ca.10% of the parasites found on the continent.

None of the parasites observed on the islands were blackcap specific. Some of the observed parasites appear to have switched from blackcaps to other Macaronesian host species, while others were of Afrotropical origin and were acquired after blackcaps colonised the islands. The prevalence of parasites in the island populations of blackcaps was lower than in mainland blackcap populations and parasite richness decreased with increasing island distance to the continent. Macaronesian blackcaps do not face the strong parasite load encountered by their mainland counterparts despite the fact that blackcap migration from the continent may directly transport mainland blackcap parasites to the islands. These results support the idea that normal mainland host-parasite associations are compromised on islands, and that parasite island syndromes (low richness, frequent host-switching, and reduced specialization) evolve even before insular host populations become completely isolated from their mainland counterparts.

Evolution of patterns of seasonal transmission in avian blood parasites

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In temperate regions, many vector-borne parasites maximize their transmission prospects by adjusting themselves to seasonal cycles of host susceptibility and vector availability. Nevertheless, in regions where environmental conditions are favourable during the whole year, parasites could benefit from a switch to a non-seasonal transmission strategy. We analysed how different transmission strategies (summer transmission, seasonal transmission extended into host non-breeding periods, and strict year-round transmission) have evolved throughout the phylogeny of a diverse clade of avian blood parasites shared by three sister species of passerine hosts. Our results indicate that, coming from an ancestral summer transmitted parasite of blackcaps (*Sylvia atricapilla*), year round transmission and switches to the other host species (*S. abyssinica* and *S. borin*) evolved recently several independent times. However, parasites that evolved longer periods of transmission did not diversify as much as summer transmitted parasites. This result suggests that, although non-seasonal transmission may be ecologically successful at the present-time, seasonal transmission may be more stable over evolutionary time. Switches from seasonal to non-seasonal transmission strategies could have ecological consequences if they promote the spread of parasites among distantly located regions assisted by migrating birds. Therefore, a deep knowledge of transmission strategies of parasites affecting birds in temperate areas is essential for understanding disease emergence risks.

Applying evolution: Transformation of a population using underdominance principles

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In theory, genetic modifications that are linked to underdominance (heterozygotes are less fit than either homozygote) can be introduced and maintained by natural selection in a wild population, even if they come at some fitness cost. This is stable in the sense that the effector gene is maintained indefinitely without any further intervention; however, in a different sense the system is completely reversible back to a wild-type state if desired (underdominance results in an evolutionary bi-stable switch). There is also the added beneficial property of geographic stability—the genetic modifications "stick where you put them." Early attempts at engineering underdominant systems with translocations (in the 1970s and \'80s) were not successful. We have revisited the problem and engineered single-locus underdominance in the highly tractable model organism Drosophila melanogaster as proof of principle. This system has a very robust fitness configuration and is designed to be portable to a wide range of species. Finally, a discussion of a possible species conservation application focused on non-native *Culex quinquefasciatus* mosquitoes that vector avian malaria (*Plasmodium relictum*) in Hawaii will be presented.

Exploratory behaviour and avian malaria infection in juvenile blackcaps (*Sylvia atricapilla*)

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Exploratory behavior, measured as latency to explore novel objects, is a trait with a wide variability between individuals. Fast-exploring individuals may benefit from increased access to food resources and mating opportunities, although they may also increase risk-taking. Therefore, the optimal behavior will depend on environmental context and challenges individuals face along their life. Parasitism may be one such element that may shape the balance between costs and benefits of exploratory behavior. There could be a link between parasite infection status and host behavior with fitness consequences, either because infection promotes costly behaviors that impact on body condition, or because exploration increases infection risk. Nevertheless, this association has received little attention, especially in birds. We analyzed the relationship between exploratory behavior and haemosporidian infection in young male blackcaps. To this end, we kept naturally infected and non-infected individuals in captivity, and treated individuals of each group with primaquine (an antimalarial drug) or water. One month post-treatment individual behaviors were recorded during five minutes in a set-up representing a novel environment for the bird. Infected individuals had higher exploratory activity, and took less time to visit different elements in the set-up, than non-infected ones. Fast-exploring birds lost more weight during the behavioural test, but the treatment had no effect on exploration, possibly because parasitemia had relapsed at the time of the test in most infected individuals treated with primaquine. Whether exploration increases infection probability or infection status alters exploratory behavior, infected birds were fast-explorers and this behavior caused a short-term decrease in body condition, revealing a physiological cost that may have fitness consequences. Our results add up to growing evidence of the links between bird behavior and malaria infection, and open new questions on the role of host behavioral syndromes in host-parasite coevolution.

Plasmodium-Wolbachia interactions in *Culex pipiens* mosquitoes

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In recent years, there has been a shift in the one host one parasite paradigm with the realization that, in the field, most hosts are co-infected with multiple parasites. Co-infections are particularly relevant when the host is a mosquito vector, because multiple infections can have drastic consequences for parasite transmission at both the ecological and evolutionary time scales. In the South of France, the mosquito *Culex pipiens* is the natural vector of the avian malaria parasite *Plasmodium relictum* and is also naturally infected by *Wolbachia*, a maternally inherited endosymbiotic bacterium. I shall present the results of several experiments showing that *Wolbachia* can modulate different components (longevity, parasite prevalence, parasite intensity) of the vectorial capacity of *Culex pipiens* mosquitoes.

MHC class I genes are related with high intensity of *Leucocytozoon* infections in blue tits (*Cyanistes caeruleus*)

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The MHC complex includes genes involved in parasite antigen recognition by effectors cells from immune system. Antigens from intracellular avian blood parasites are mainly presented by MHC class I molecules. If the antigen is recognised to be parasite derived, the adaptive immune response is activated. We investigated associations among MHC alleles and resistance/susceptibility to avian blood parasites in a blue tit population breeding in central Spain. An elevated MHC diversity was related to higher intensity of infection by *Leucocytozoon* parasites. Also specific MHC alleles were related with a higher intensity of infection by this parasite genus. Associations were sex or age dependent, with females and young individuals being infected with higher intensity. No significant associations between MHC and either prevalence or intensity of infection were observed for other blood parasites, neither significant relationships among MHC genes and parasite richness. Our results point out a susceptibility to *Leucocytozoon* parasites depending on MHC alleles or genes linked to these MHC alleles.

Immune responses to haemosporidian and other parasites in *Myiarchus tyrannulus* from Costa Rica

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Parasites are often detrimental for their hosts' health condition and fitness, eliciting a variety of immune and physiological responses, but the extent of these responses to specific parasites is widely unknown in wild birds. In this work we analyzed the health condition and immune responses of *Myiarchus tyrannulus* (Passeriformes: Tyrannidae) to their Haemosporidian parasites, feather mites, chiggers, and lice. We captured 74 birds in Costa Rica, from which we collected blood samples and ectoparasites. Haemosporidian parasites were identified through molecular analyses and ectoparasites were identified according to their morphology. We estimated the health of birds by measuring the following parameters: body condition index (BCI), packed red blood cell volume (PCV), and white blood cell counts (WBC) and differentials. We ran generalized linear models to test if prevalence of parasites could explain variation in these health parameters. We found that *Plasmodium* was the best predictor of variation in monocytes, whereas chiggers best explained the variation in the largest number of health parameters of their hosts: BCI, PCV, and WBC. Variation in BCI of birds could also be predicted by parasitism from lice and mites. We conclude that interpretation of health parameters is not simple, due to the fact that most immune responses are specific to the interaction between a particular host and parasite and cannot be easily generalized outside of that context.

Diversity of African chiropteran haemosporidian parasites and close affiliation with rodent *Plasmodium* species

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Eight different haemosporidian genera are described from bats, with all but two genera restricted to this diverse mammalian host group.

We report the genera *Plasmodium*, *Hepatocystis*, *Nycteria* and *Polychromophilus* from bats sampled in West Africa and South Sudan. The majority of parasites in the bats belong to *Hepatocystis* spp. with high prevalences in the epauletted fruit bats. The first molecular investigation of the genus *Nycteria* shows that the parasites form a very distinct phylogenetic group outside the mammalian *Plasmodium/Hepatocystis* clade.

Despite the high diversity of detected haemosporidian parasites in the African bats just two species of *Plasmodium*, namely *P. voltaicum* and *P. cyclopsi* were found in two different bat species. Phylogenetically, *Plasmodium* species from both chiropteran hosts group tightly with the rodent malaria parasites *Plasmodium yoelii* and *Plasmodium berghei* that naturally infect African thicket rats (Muridae: *Thamnomys, Grammomys*) in West and Central Africa. This unexpectedly tight relationship suggests multiple host switches of chiropteran *Plasmodium* species with rodent *Plasmodium* species. Latter are widely used model organisms to study *Plasmodium* biology and disease progression as well as pre-clinical murine infection models for novel malaria intervention strategies.

Avian Malaria in New Zealand

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Avian malaria parasites of the genus *Plasmodium* have the ability to cause extreme morbidity and mortality in naïve hosts, and their impact on the native biodiversity is potentially serious. The most famous example of the possible impact of avian malaria on naïve hosts is the extinction crisis on Hawaii after the introduction of the exotic mosquito vector *Culex quinquefasciatus* which permitted the establishment and spread of avian malaria amongst the endemic birds. So far, avian malaria parasites have been found in 32 different bird species in New Zealand. Despite the common asymptomatic nature of the infection, deaths in NZ birds caused by *Plasmodium* spp. have been recorded in South Island saddleback, yellow- eyed penguins, mohua (yellowhead), hihi and great spotted kiwi. Recent outbreaks of avian malaria in endangered New Zealand birds causing fatalities include an outbreak in captive New Zealand dotterel chicks in 1996, an outbreak in yellowhead / mohua in 2004 and mortality in a brown kiwi at a ONE (operation nest egg) facility in 2010/2011.

Does blood parasite composition and load change in advance of spring migration in long distance migratory birds?

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Migration is one of the most energy-intensive events in the life of birds. Resources needed for immune defense may then be drained and leave an opportunity for blood parasites to establish or relapse. Along a migration route, an increase in infection diversity, prevalence and intensity may hence be expected. Alternatively, infected migrants might be particularly selected against, leading to a decrease of diversity and prevalence down the migration route. To test this, we sampled blood parasites of long-distance migratory passerines simultaneously at six stop-over sites (five in Bulgaria and on Anthykethyra Island, Greece) during spring migration of 2012 and 2013. Parasite species and lineages were matched using microscopy and PCR in parallel and infection intensity was determined microscopically. Our main target hosts were warblers of the genus *Sylvia* (*S. atricapilla, S. communis*) and nightingales (*Luscinia megarhynchos*). Infection prevalence decreased from south to north (Greek to Bulgarian sites) in *Sylvia* species, but remained unchanged in nightingales. Down the migration route, infection intensity increased in *S. atricapilla* but not in *S. communis*.

Late migrants are expected to be in worse condition, which may also interact with their parasitation. Therefore, we sampled five additional species on the Anthykethyra Island over the whole spring migration season: *S. borin, Oriolus oriolus, Streptopelia turtur, Lanius senator* and *Saxicola rubetra*. Indeed, blood parasite prevalence increased from April to May in *L. senator* and *S. communis* but it decreased in *Str. turtur*. High lineage diversity was present in *L. senator, S. atricapilla, O. oriolus* and *S. communis* and we will present data on its development over the migration season.

Deciphering the impact of *Plasmodium* parasites on bird odorant profile: What makes infected birds more attractive to mosquitoes?

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It was recently demonstrated, using the avian malaria system (*Plasmodium relictum* SGS1 lineage), that infected birds are more attractive than non-infected birds to host-seeking *Culex pipiens* mosquitoes (Cornet et al. 2013 Ecology Letters). These results suggest that malaria parasites might manipulate bird traits that are used by mosquitoes to locate their host. This previous experiment eliminated visual or behavioural cues as potential signals, leaving olfactory cues as the most likely mechanism responsible for this enhanced attractiveness. To elucidate the proximal mechanisms of this fascinating manipulation, we characterised bird odorant profiles before and after a *Plasmodium* infection. The volatile organic compounds (VOC) emitted by hosts were captured on a solid phase (odour trap) using an innovative experimental setup. Gas chromatography was used for identification (mass spectrometry) and quantification (flame ionization detector) of VOCs. *Culex pipiens* mosquito feeding preference (between infected and non infected birds) was simultaneously recorded following the protocol described in Cornet et al. Here we correlate bird odour profiles and mosquito choice measurements, in order to identify VOC candidates susceptible to manipulation by *Plasmodium* parasites to render their hosts more attractive to mosquitoes.

MHC-I influences infection intensity and infection status of avian haemosporidian parasites within populations - but patterns differ across populations and host species

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Host resistance against parasites depends on three aspects: the ability to prevent, control and clear infections. In vertebrates the immune system consists of innate and adaptive immunity. Innate immunity is particularly important for preventing infection and eradicating established infections at an early stage while adaptive immunity is slow, but powerful, and essential for controlling infection intensities and eventually in clearing infections. Major Histocompatibility Complex (MHC) molecules are central in adaptive immunity, and studies on parasite resistance and MHC in wild animals have found effects on both infection intensity (parasite load) and infection status (infected or not). These data imply that MHC genotype can affect both the ability to control infection intensities and the ability to clear infections. However, these two aspects have rarely been considered simultaneously, and their relative importance in natural populations is therefore unclear. Furthermore, their relative importance may differ for different types of infections. Here we investigate if MHC-I genotype affects infection intensity and status of *Haemoproteus majoris*, an avian haemosporidian parasite with high prevalence, in a natural population of blue tits *Cyanistes caeruleus*. We also make a summary of results obtained so far regarding disease resistance to haemosporidian parasites and associations with MHC genotypes in passerines. We found a significant negative association between an MHC allele and infection intensity in blue tits, but no association with infection status. Blue tits that carry this MHC allele seem able to suppress H. majoris infection intensity, while we have no evidence that this allele also clear H. majoris infection. These results are in contrast with some previous studies of MHC-I and haemosporidian parasites and so far there seem to be limited consistency across studies for MHC mediated disease resistance in passerines. This could be explained by that the clearance rate differs between avian haemosporidian lineages and/or between avian hosts.

How malaria gets around: The genetic structure of a parasite, vector and host compared

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Parasites with complex life cycles have two host species shaping their genetic structure, but the traditional view posits that the parasite's structure will be mainly determined by the most mobile host species. Malarial parasites are a prime example of parasites with a complex life cycle, needing both a dipteran and vertebrate host to complete their life cycle. In both hosts they impose selection pressures. Yet, how vertebrate and dipteran host populations shape the parasite's genetics has been little studied. The relative contribution of each host to the parasite's population structure has therefore never been satisfyingly determined. Here we will compare the genetic structure of all three actors in a parasite-vector-host system: the vertebrate host, the long-fingered bat (*Miniopterus schreibersii*); the vector/dipteran host, the wingless bat fly *Nycteribia schmidlii*, and the malaria parasite *Polychromophilus melanipherus*. Being the most mobile host, we predict that *M. schreibersii* will show the most similarities with the structure of *P. melanipherus*.

Traditional microsatellite markers, cytb DNA sequencing and a SNP library obtained by ddRAD sequencing reveal relatively high structure in the bats. In contrast, the haplotype distribution of *P. melanipherus* shows little geographic differentiation. Using coalescence methods we investigate the role of the dipteran host in generating the observed patterns and conclude that the vertebrate host alone is not affecting the genetic structure of the haemosporidian parasite, but that it is probably the high dispersal of the vector that shapes the geographical structure of the parasite populations.

Poster presentations

Avian haemosporidia differ in their ability to use long-distance migrants to colonize new areas

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When avian communities consist of resident species (RS) and single flyway migrants (SFM), it is difficult to determine parasite transmission frequency on wintering versus breeding grounds. A study of pathogens infecting cross-flyway migrants (CFM) and their sympatrically breeding closely related RS and SFM can elucidate which parasites are more efficient in colonizing new areas using long distance migrants. Parasites found in CFMs and their sympatrically breeding RS and/or SFMs are transmitted on breeding grounds. Conversely, parasites found in CFMs and co-occurring during winter RS and/or SFMs are transmitted on wintering grounds. Only parasites found in CFMs and RS/SFMs within both migratory flyways, are efficient colonizers using CFMs to move between areas (hitchhikers). To test which avian haemosporidians are better hitchhikers, we analyzed 238 samples from 13 species of Philloscopidae (four) and Fringilidae (nine) from Armenia and northwest Caucasus. Each family has one CFM breeding within the African-Eurasian Flyway (AEF) and wintering within the East Asian-Australasian Flyway (EAAF) whereas other species reside or migrate within AEF. We also utilized GenBank, MalAvi, and our own data, to determine parasite lineage occurrence in different flyways. Haemosporidian lineages were identified using mtDNA cytochrome *b* sequences. We found 46 unique haplotypes: 28 *Haemoproteus*, nine *Leucocytozoon*, and nine *Plasmodium*. One bird was infected by three lineages, 19 birds were infected by 2, and 82 birds by a single lineage. Among lineages found in ≥2 birds, *Haemoproteus* and *Leucocytozoon* were more common in RS than *Plasmodium* (Fisher's exact p=0.01 and p=0.02). Both *Leucocytozoon* and 9 of 14 *Haemoproteus* lineages infected hosts on breeding grounds. Three *Haemoproteus* lineages had winter transmission, and only two were hitchhikers. In contrast, three of five *Plasmodium* lineages were hitchhikers, and two had winter transmission. The proportion of hitchhikers was marginally significantly larger among *Plasmodium* than *Haemoproteus* and *Leucocytozoon* lineages (Fisher's exact p=0.06).

Occurrence of haemosporidian parasites in Long-eared owls (*Asio otus*) in winter roost in Vojvodina province, Northern Serbia

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The Long-eared Owl (*Asio otus*) is a regular breeding and wintering species across Serbia. The largest densities of breeding pairs and wintering individuals are found within the Panonian part of the country (Vojvodina Province). Many wintering roosts include more than 100 birds. The origin of such high numbers of wintering birds is not known yet, although most likely the majority of them are local. Information about haemosporidian parasites in the blood of Long-eared Owl is scanty. The aim of this study was to determine prevalence of haemosporidian parasites in the blood of Long-eared Owls. In total, 65 individuals were caught and sampled in this area during winter 2011/2012. Presence of haemosporidian parasites according to PCR (cytochrome *b* gene) was found in the blood of 34 individuals (26 individuals were infected with *Haemoproteus* spp. and *Plasmodium* spp. or both/; 23 were infected with *Leucocytozoon* spp.; and 15 had mixed infections). Positive samples were sequenced and compared to the world database of haemosporidian parasites sequences. The presence of bacteria *Rickettsia* spp. was not found in the owls.
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A phylogenomic approach to the evolution of Haemosporida

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Haemosporidian blood parasites infect a great variety of vertebrate hosts and at least five species of the genus *Plasmodium* have independently acquired the ability to infect humans. As agents of human malaria, plasmodia are of great medical importance. Thus, large scale genome sequencing efforts have so far focused exclusively on members of this genus, while other key taxa for the understanding of haemosporidian evolution are scarcely represented in public databases. For this reason, deep-level phylogenetic relationships among major haemosporidian lineages are still poorly understood. Most phylogenetic studies to date have been limited to a small number of gene fragments, whereas studies using data from genome projects have much broader gene coverage but suffer from severely limited taxon sampling. In order to strike a balance between gene coverage and taxon sampling, we have developed an automated primer design pipeline capable of designing degenerated primer oligonucleotides for nested PCR on nuclear genomic DNA. These primers were used to obtain the sequences of about 20 gene fragments from parasites belonging to the genera *Leucocytozoon, Haemoproteus, Plasmodium* and *Polychromophilus*, which have been identified by screening blood samples from birds, reptiles and bats. These sequences were used in conjunction with data from genome projects in a phylogenetic analysis that comprises nearly all major haemosporidian lineages.

Complete sporogony of two *Plasmodium relictum* lineages (pSGS1 and pGRW11) in mosquitoes *Culex pipiens* form molestus

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Haemosporidians are a well-defined taxonomic group of haematozoa inhabiting many species of higher vertebrates nearly worldwide. These parasites are particularly widespread in birds. *Plasmodium* spp. are well studied parasites because some their species are the agents of virulent human malaria. Mosquitoes (Diptera: Culicidae) are the main vectors of *Plasmodium* parasites. Avian malaria is often caused by *Plasmodium relictum*, a cosmopolitan parasite, numerous lineages of which are insufficiently investigated, particularly in the stage of sporogony. It remains unclear if all lineages of this parasite can complete sporogony in the same mosquito vector.

The aim of this study was to investigate if two widespread *P. relictum* lineages (pSGS1 and pGRW11) complete sporogony in the mosquitoes *Culex pipiens* form *molestus.* The mosquitoes were cultivated in the laboratory and were allowed to take blood meals from *P. relictum*-infected canaries. The experimentally infected insects were maintained in the laboratory (22 ± 1°C, 50-60% relative humidity, the light-dark photoperiod 12:12 h) and dissected; preparations of midguts and salivary glands were prepared using traditional methods in order to detect ookinetes, oocyst and sporozoites. Ookinetes and oocysts of both parasite lineages were seen in the midgut. The presence of sporozoites in mosquito salivary glands (14-15 days post infection) indicated complete sporogony in *C. p.* form *molestus* mosquitoes. Presence of parasites in experimental insects was also confirmed by PCR based methods using the primers HaemNFI and HaemNR3, and HAEMF and HAEMR2. The study shows that the *P. relictum* lineages pSGS1 and pGRW11 successfully complete sporogony in this mosquito, which is a good experimental vector of these lineages of malaria parasites. We recommend the *Culex pipiens* form *molestus* mosquito for experimental sporogony studies of *P. relictum*, particularly because this mosquito is easy to cultivate and maintain under laboratory conditions.
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Avian haemosporidians from mountain regions of Venezuela

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Avian haemosporidians represent a diverse and cosmopolitan group of vector-borne parasites. Parasitic prevalence and composition varies among biogeographic regions and has been characterized as being relatively lower in the Neotropics in comparison to others regions, even though prevalence values over 30% have recently been described in certain locations. Nonetheless, little is known about the distribution and prevalence of Venezuelan bird haemosporidians. Blood samples from a total of 304 individuals (20 families and 78 species) were obtained from sporadic sampling (wet and dry seasons) using mist nets in 12 different mountain locations of Venezuela between February 2005 and February 2008. Blood smears prepared in the field were stained with Giemsa in the laboratory. A total of 100 random field/smears were examined under a light microscope at 1000x. The number of red blood cells and observed haemosporidians were recorded. These values were used to calculate prevalence and intensity of infection (parasitemia). We found 85 individuals (28%) infected with haemosporidians parasites. The genus most commonly found was *Haemoproteus* (22%), followed by *Plasmodium* (3.6%) and microfilariae (2.9%). Additionally, two bird families, Thraupidae and Emberizidae, specifically the species *Tangara cyanicollis*, *Thraupis cyanocephala* and *Zonotrichia capensis*, showed the highest prevalence and parasitemia. Furthermore, three new parasite-host associations were found (*Arremon brunneinucha, Pipreola formosa* and *T. cyanocephala*). Parasite prevalence was not associated with host geographical distribution, suggesting the potential importance of taxonomic composition of bird assemblages on the distribution of these parasites. A similar pattern has been found in the arid zones of northern Venezuela. Parasite prevalence reported in this study may be underestimated as microscopic based identification methods can be sensitive to low peripheral parasitemia and potentially produce false negatives when exhaustive blood smear examination is not performed. PCR based methods can also be affected by this factor, hence identification using both methods is recommended.

Host sharing of malarial parasites in Sao Paulo Zoo, Brazil

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Sao Paulo Zoo is located in a vast area of original Atlantic forest, where mosquitoes of the genera *Aedes* and *Culex*, the vectors of avian malaria parasites, are common. The Zoo contains three lakes in which the space destined for local birds is shared by migratory species, as well as opportunistic species that live freely in the area of the park. This environment favors many infections. Moreover, in captivity, infections can develop as asymptomatic disease and thus, the parasites could be transmitted to different hosts allowing the maintenance of lineages circulating for long periods and further spreading the parasite.

Plasmodium spp. infections were detected, by light microscopy, in two Black Swans (*Cygnus atratus*) and one King Vulture (*Sarcoramphus papa*). The parasites' morphological characteristics provided evidence of the subgenus *Haemamoeba*. Erythrocytic meronts contained plentiful cytoplasm and their size exceeded the erythrocytic nuclei size. Fully grown gametocytes were roundish, oval or irregular, and their size markedly exceeded the erythrocyte nuclei. PCR confirmed these *Plasmodium* infections and amplified the same ~1kb cytb sequence in all three birds. A phylogenetic tree is in agreement with our morphological identification since the reported sequence clustered with other *Haemamoeba* species, forming a well-supported clade with high posterior probability. Final microscopic identification is being carried out. This cytb sequence may assist in identification (barcoding) of this *Plasmodium* species in other birds. In a search in MalAvi database, we found a sequence from *Plasmodium* sp. (lineage PESA01), with 99% of identity in 466 bp with our sequence. According to MalAvi, the lineage PESA01 was recorded in two birds: Pectoral Sandpiper (*Calidris melanotos*) and White-tipped Dove (*Leptotila verreauxi*) from Alaska and Uruguay, respectively. These findings provide evidence for possible broad host specificity of this parasite species.

Molecular detection and characterization of two *Leucocytozoon* lineages from a Long-legged Buzzard (*Buteo rufinus*) and a Common Buzzard (*Buteo buteo*) from Kayseri Province of Turkey

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This study describes the first molecular findings of leucocytozoonosis from two buzzard species in Turkey. Two Long-legged Buzzards (*Buteo rufinus*) and three Common Buzzards (*Buteo buteo*) with broken wings were brought to the animal hospital of the Veterinary Faculty of Erciyes University in different periods in 2013. Before surgical operations, blood samples were taken by brachial venipuncture for investigating presence of haemosporidian parasites. DNA was extracted from the avian blood samples and were analyzed by nested PCR using the mt-cyt*b* gene and primers specific to *Leucocytozoon* and *Plasmodium/ Haemoproteus* species. One Long-legged Buzzard and one Common Buzzard were found to be infected with *Leucocytozoon* species while no *Plasmodium* and/or *Haemoproteus* infections were detected in any samples. The obtained *Leucocytozoon* mt-cyt*b* gene amplicons from two buzzards were gel purified and sequenced in both directions with the nested primers. The sequences from two *Leucocytozoon* lineages were deposited in GenBank with the accession numbers KC962151 for *Buteo rufinus* (isolate eruvetpar1) and KC962152 for *Buteo buteo* (isolate eruvetpar2). Phylogenetic analyses of the amplified sequences confirmed that the buzzards were infected with *Leucocytozoon* spp. Pairwise alignment of the sequences from eruvetpar1 and eruvetpar2 showed 100% identity between these two lineages. According to the phylogenetic comparisons these *Leucocytozoon* lineages showed 100% identity with the isolates obtained from an *Accipiter virgatus* in Philippines (GenBank JX418201), *Milvus* spp. in Spain (GenBank HF543631), *Circus aeruginosus* in Germany (GenBank: EF607287) and *Alcedo atthis* in Spain (GenBank GQ371174).

Temporal variation of haemosporidian infections in an island population of silvereyes (*Zosterops lateralis chlorocephalus*)

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Island ecosystems are prone to stochastic variation in colonisation and extinction events, which can lead to temporal changes in community dynamics. For hosts and their parasites, it is important to address temporal fluctuations in the occurrence of infections to understand the dynamics of host-parasite co-evolution. Heron Island is a small, vegetated cay on the Great Barrier Reef, situated 60km east of mainland Australia. Although vagrant individuals of birds and flying arthropods are commonly carried to the island when winds arrive from the west, resident biting arthropods have not been observed and colonisation has only been achieved for one passerine species (silvereye; *Zosterops lateralis chlorocephalus*). Until recently, parasitologists have been unable to find evidence of haemosporidian blood infections in Heron Island birds, with researchers suggesting that the island's absence of infections is due to its apparent lack of suitable freshwater breeding areas for vectors. However, using molecular techniques, my colleagues and I have confirmed that infections belonging to the genera *Haemoproteus* and *Plasmodium* do occur in Heron Island's resident silvereyes, suggesting that these vector-borne parasites may rely on opportunistic migration to the island through wind-swept hosts. Such stochastic variation in the exposure of island hosts to parasites may have important evolutionary implications for both the host and parasite. I will investigate temporal variation in the prevalence and diversity of haemosporidian infections in silvereyes on Heron Island, Australia. I will screen blood samples taken from silvereyes across four years for the presence of blood parasites belonging to the genera *Haemoproteus* and *Plasmodium*. This study will yield insights into both the temporal stability of haemosporidian communities on islands and the importance of opportunistic colonisation for vector-borne diseases on near shore islands.

Avian malaria diversity in southern Melanesian bird communities

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Host-pathogen interactions have the potential to influence broad scale ecological and evolutionary processes in host populations, influencing levels of endemism, divergence patterns and distributions. The development of localized co-adapted host-pathogen relationships could drive divergent selection and limit gene flow among populations of the same species or limit range expansions of closely related species. To examine the potential for pathogens to direct these types of processes in natural host populations, we explored the prevalence and phylogenetic diversity of avian malaria parasites, and the distribution of lineages across islands and host species, in southern Melanesian bird communities. We uncovered high avian malaria diversity, including many rare lineages, pointing to high estimates of undiscovered lineage richness in the region. General linear models of variation in prevalence in three well-sampled species (*Myzomela cardinalis, Zosterops flavifrons* and *Z. lateralis*) showed significant island, host species and interaction terms, however the host species and interaction effects were due to genera comparisons, as prevalence in *Zosterops* species varied across islands, but in a similar way for both species. Only a small number of lineages could be considered host species generalists, and more were *Haemoproteus* rather than *Plasmodium*. However, an analysis of molecular variance revealed that genetic variation in *Haemoproteus* lineages displayed significant structure at the host species and host family level, whereas *Plasmodium* lineages appeared less structured by host taxonomy and more by geography. The heterogeneity demonstrated in both the complement and prevalence of parasite lineages infecting local avian communities likely exposes species to a mosaic of disease-related selection pressures across their naturally fragmented distributions in southern Melanesia. The situation is complex, however, with the two genera of avian malaria parasites displaying different geographic and host-species patterns that require further exploration.

Prevalence of avian blood parasites in some protected areas in Ghana

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Parasites can have a great impact on their host populations and have the potential to reduce fitness, cause low reproductive output and, in severe cases, death in wild birds. This can also be a contributing factor to population decline and extinction of some species of wild birds. This study is important because Ghana has high diversity of bird species (over 750 species) including about eight threatened species that require conservation. This study aims to investigate avian haemosporidian parasites of the genera *Plasmodium* and *Haemoproteus* in two wildlife protected areas in Ghana. The results of this study will present the findings of prevalence of *Plasmodium* and *Haemoproteus* parasites among tropical rainforest birds compared to savanna birds, and the implication of habitat type on parasite prevalence.

Oxidative stress in breeding Great tit, *Parus major*, infected by *Plasmodium* spp.

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The physiological mechanisms involved in the cost of reproduction are poorly understood. In addition, the interaction between reproduction and infection with pathogens is still unresolved. Birds infected with pathogens may be more impacted by physiological mechanisms such as oxidative stress, because of the double physiological cost of reproduction and resistance to pathogens. In this case, an increased metabolism is expected and should lead to a physiological stress involving higher oxidative stress driven by an increase in oxidant production, a higher antioxidant recruitment and/or more oxidative damage to biomolecules. Here, we investigated the effect of reproductive investment and infectious status on parental oxidative stress measures in three wild populations of the great tit, *Parus major*, naturally infected by *Plasmodium* spp. Different physiological measures involved in both oxidant production and resistance to oxidative stress were taken from parents when chicks were 14 days old: erythrocyte superoxide production, a proxy of erythrocyte mitochondria quantity (cardiolipin content), and erythrocyte membrane resistance to oxidative attacks. We found a sex-dependent effect of reproductive effort on membrane resistance. We also found that parental infection with *Plasmodium* spp. was linked to an increased production of superoxides, which in turn indirectly decreased membrane resistance. This study suggests that breeding birds incur an additional physiological cost of reproduction linked to infection with malaria parasites.

Malaria parasites do not affect reproductive success in the great tit

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Haemosporidians causing avian malaria are one of the most common and widespread parasites among bird species. In wild populations, effects of malaria parasites on host fitness are generally difficult to detect, because the majority of individuals hold chronic infections. One way of overcoming this obstacle is experimental removal of parasites. During two breeding seasons we examined the effects of malaria parasites on parameters of reproductive success in the small hole-nesting passerine – the great tit (*Parus major*) through medication of females at the early stage of the nesting cycle. Females caught during the nest building stage were injected intraperitoneally either with an antimalarial drug, primaquine, or with a physiological salt. Reproductive performance of treated birds was characterized by clutch initiation date, clutch size, nestling body mass 2 days post-hatching, fledgling body mass and tarsus length. During the nest building stage 86.9% of females were infected with at least one parasite lineage (genera *Haemoproteus* and *Plasmodium*), while at the end of the nestling stage 96.4% were infected. Clutches laid by females from both groups did not differ in clutch initiation dates and size nor was there a difference in body size of nestlings. This data suggest a lack of observable fitness effects of malaria parasites on the reproductive fitness of great tits in this study population.

Molecular detection and characterization of a *Haemoproteus* lineage in a Tawny Owl (*Strix aluco*) in Turkey

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Avian blood parasites have been intensively studied using morphological methods with limited information on their host specificity and species taxonomic status. Now the analysis of gene sequences, especially the mitochondrial cytochrome *b* (mt-cyt*b*) gene of the avian haemosporidian species of *Plasmodium, Haemoproteus, Fallisia* and *Leucocytozoon*, offers a new tool to review the parasite specificity and status. The focus of this study was a Tawny Owl (*Strix aluco*) that was brought to an animal hospital with broken wings in Mugla province. A blood sample was collected by brachial venipuncture, from which genomic DNA was extracted and blood smears were prepared. Extracted genomic DNA was analyzed by nested polymerase chain reaction (PCR) for the amplification of partial avian haemosporidian mt-cytb gene. The final PCR product was gel purified and sequenced. The obtained isolate was deposited in GenBank International Nucleotide Sequence Database with the accession number JQ768232. Intraerythrocytic stages of *Haemoproteus* sp. were detected in the examination of the blood smears. The phylogenetic analyses of the amplified sequence confirmed that the owl was infected with *Haemoproteus* sp. According to the phylogenetic comparisons, the *Haemoproteus* lineage showed the highest identity (99.8%) with the “H-STAL2” lineage isolated from an owl (*Strix aluco*) in Germany among the *Haemoproteus* lineages available in GenBank. In conclusion, this study reports the first microscopic and molecular detection of *Haemoproteus* infection in an owl in Turkey. The lineage characteristics and phylogenetic relationships among several *Haemoproteus* lineages were also evaluated in this study.

Avian *Plasmodium* in *Culex* and *Ochlerotatus* mosquito species from southern Spain: effects of season and host-feeding source on parasite dynamics

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The heterogeneity in parasite transmission rates can have important consequences for the evolution of virulence of parasites. The study of interaction between avian malaria parasites-mosquitoes-birds is an excellent model to identify these evolutionary mechanisms. Although mosquitoes are crucial elements in the transmission of avian malaria parasites, little is known of their ecology as vectors. We identified the *Plasmodium* and *Haemoproteus* lineages in five mosquito species *Culex perexiguus*, *C. modestus, C. theileri, C. pipiens* and *Ochlerotatus caspius* and tested for the effect of vector species, season and host-feeding source on the transmission dynamics of those pathogens from an area near the Doñana National Park (southern Spain).

Using molecular methods, we analyzed the head-thorax of 166 blood-fed individual mosquitoes and 5,579 unfed mosquitoes grouped in 197 pools. Overall, 15 *Plasmodium* and two *Haemoproteus* lineages were identified on the basis of a fragment of 478 bp of the mitochondrial cyt b gene. Infection prevalence of blood parasites varied between species and seasons reaching the highest value in autumn. The feeding source was identified in 91 mosquitoes, and showed 78% of mosquitoes had fed on birds. Twelve (17.6%) and four (18.2%) head-thoraxes from blood-fed mosquitoes containing an avian or mammal-derived bloodmeal in their abdomen, respectively, harboured blood parasites.

In conclusion, we found that i) *Plasmodium* lineages are shared between mosquito species belonging to the genera *Culex* and *Ochlerotatus*; ii) mosquitoes harboured *Haemoproteus* parasites, although it is unclear if they can transmit these lineages, iii) in the studied area, unfed females of mostly ornithophilic *Culex* species had a higher prevalence of *Plasmodium / Haemoproteus* than those of *Culex* species feeding mainly on mammals; and finally, iv) the infection rate of mosquitoes varied among annual seasons, reaching its maximum in autumn. These results have important implications for the evolution and modelling of parasite transmission dynamics among wild bird populations.

Molecular characterization of haemosporidians in toucans and aracaris (Piciformes: Ramphastidae) from Brazil

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Toucans and aracaris are birds from Ramphastidae family, order Piciformes which are endemic in the Neotropical region. There are no studies on the prevalence and molecular diversity of haemosporidians in this group and the health impact, and distribution of this parasite is poorly understood among captive birds. We tested for haemosporidians in 143 captive individuals from 10 different species in Minas Gerais state, Brazil. PCR was conducted according to Fallon et al., 2003, followed by sequencing using primers for *Plasmodium/Haemoproteus* according to Hellgren et al., 2004. Results showed a prevalence of 40.5%. Blood smears from positive birds showed generally low parasitemia, ranging from two to eight parasites in 100 observed microscopic immersion fields. Sequencing of 20 samples yielded five different genetic lineages of *Plasmodium* spp. Two of these lineages are first described here: lineages RATOC1 and RAVIT1 were found in one *Ramphastos toco* and in two specimens of *R. vitellinus*, respectively. *Haemoproteus* was not found. Bayesian analyses were applied to infer the phylogenetic relationships between these five lineages and morphospecies with known cyt-*b* sequences present in MalAvi database. Our lineages were grouped in four different clades and demonstrated that at least four different morphospecies of *Plasmodium* parasitize this group of birds. The lineage DENPET03 was found in three species from the genera *Ramphastos* and a single species (*R. toco*) was parasitized by four different lineages. Two lineages were sequenced from four positive *R. vitellinus* (n=9) and sequencing of five samples from *R. dicolorus* showed one genetic lineage. *Plasmodium* spp. was shown to be pathogenic for *Ramphastids*, hence the distribution and diversity of these parasites in captive populations demonstrate the need for further molecular and morphological studies and adoption of management measures for controlling the infection, as some groups of birds have been reintroduced into the wild in Brazil.

How costly is nest building in terms of infection with blood parasites?

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The concept of the cost of reproduction is based on the assumption that every organism has at its disposal a certain amount of resources that can be used in a given moment either for self-maintenance or reproduction. Therefore, any increased investment in reproduction is expected to deplete resources available for functions associated with self-maintenance, e.g. immune function, which in turn should result in negative consequences such as increased susceptibility to parasites or accelerated senescence. A number of studies have shown that elevated reproductive effort translates into both impaired immune function and increased susceptibility to parasites. However, to date, most studies have focused on investments in the late stage of the nesting cycle, namely the nestling rearing period, while consequences of the magnitude of investment in earlier stages, such as nest building or egg laying are very poorly investigated. Here, the effect of the magnitude of investment in nest building on condition and health status of adult birds was investigated experimentally in the small hole-nesting passerine, the great tit, *Parus major*. In one subset of nests, the level of investment in nest building was experimentally increased, while in another subset of nests used as a control group, there was no manipulation . Condition and health status of adult birds, measured as infection status and parasitemia, was assessed when nestlings were 14 days old.

Preliminary data indicates that manipulation of nest building costs affected body condition neither in females nor in males. Adult great tits from the study population have been found to be infected with blood parasites from the genera *Haemoproteus* and *Plasmodium*. In general, 46% of birds were infected with malaria parasites and females were less heavily parasitized than males. There were no differences in the intensity of infection with parasites in both studied groups of females.

In vivo antiplasmodial activities of Echnops kebericho Mesfin and Zingibir officinale Roscoe

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Echnops kebericho Mesfin and Zingibir officinale Roscoe are traditionally used for the treatment of malaria and other ailments in Ethiopia. The aim was to evaluate in vivo antiplasmodial activities of 70% ethanol root extracts of these plants against *Plasmodium berghei* in adult albino mice. Oral acute toxicity of Echnops kebericho Mesfin was tested in mice by a single administration of the crude extract. The in vivo assays were done by administering mice infected with *P. berghei* with four consecutive daily doses of the extracts via the intra-gastric route, following Peters 4-Days' suppressive test. No toxicity effects were observed up to 5000mg/kg. Echnops kebericho Mesfin (1000mg/kg/day) showed antiplasmodial activity and suppressed parasitaemia by 49.53% and 34.66% at 500mg/kg/day. Zingibir officinale Roscoe (1000mg/kg/day) suppressed parasitaemia by 32.83%. This is the first report of the antiplasmodial activities of 70% ethanol root extracts of the plants given in a dose dependent manner.

How different morphologically are the reproductive cells and ookinetes of haemosporidian parasites?

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Species identification of avian haemosporidian parasites (Haemosporida) has traditionally been based on the morphology of their blood stages and limited information about their vertebrate host-specificity. Molecular identification of many morphospecies has recently been developed. It is less well known that some features of sporogonic stages can also be used for haemosporidian species identification. We designed this study to determine the differences in morphology of sporogonic stages of avian *Haemoproteus* parasites.

Eight species of *Haemoproteus* (Haemosporida, Haemoproteidae) were isolated from naturally infected passerine birds. They were identified to species based on morphology of their gametocytes and the sequences of a segment of the parasite's mitochondrial cytochrome *b* gene. Sexual process and ookinete development of *Haemoproteus* spp. were initiated in vitro by mixing blood containing mature gametocytes with a 3.7% solution of sodium citrate and exposing the mixture to air. The smears were prepared at set intervals of time after exposure to air (10, 30, 45 min and 1, 1.5, 3, 6, 12, 24, 48 hr); they were air-dried, fixed with methanol, stained with Giemsa, and examined microscopically.

This study shows that a combination of such characters as the length of microgametes, morphology of zygotes, the morphological peculiarities of ookinetes, and the rate of development of ookinetes in vitro are rather varied in different *Haemoproteus* spp. These characters can be used for identification of many species of haemoproteids without data regarding morphology of their gametocytes in the peripheral blood. This enables researchers to: (1) identify species of haemoproteids based on morphology of their sporogonic stages during in vitro development, particularly during between-lineage hybridization experiments, when the parasites of different species are present in a mixture; and (2) distinguish new morphological forms, which might be products of the experimental hybridization. This study was funded by the European Social Fund under the Global Grant measure.

Isolation and genetic analysis of *Leucocytozoon caulleryi*

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The genomics of *Leucocytozoon caulleryi,* a highly pathogenic bird haemosporidia that is important to veterinary medicine, , is not well understood in terms of its genetic and molecular biological features. *Leucocytozoon caulleryi* infects host chicken cells, and interference by the host genome results in difficulty to obtain isolated protozoa DNA for genetic analysis. We used flow cytometry analysis to separate *L. caulleryi* gametocytes from infected chicken blood. Infected blood cells stained with dye showed a specific area on 2-dimensional scattergrams compared to uninfected blood. The specific area was sorted, and approximately 99% of the sorted cells were identified as *L. caulleryi* gametocytes by microscopic observation. DNA was also extracted from the sorted fraction, and a clear increase of protozoa DNA in PCR was observed compared to infected blood without sorting. By using this purified protozoa DNA, we revealed the whole apicoplast genome DNA sequence of *L. caulleryi* for the first time. This unique extranuclear organelle has been recognized as a drug target for other apicomplexia protozoa such as *Plasmodium falciparum*. Our results showed that *L. caulleryi* has a similar apicoplast genome to known *Plasmodium* spp. in the order and the number of genes. These similarities suggest that this organelle of *L. caulleryi* could have a similar function as in *Plasmodium*. The apicoplast provides a platform for critical metabolism in *Plasmodium* and provides a drug target. Preceding knowledge about the apicoplast obtained in *Plasmodium* species can be applied to understand the dynamics of gene expression of *L. caulleryi* and to control *L. caulleryi* infection and transmission.

New data on the genetic diversity of avian haemosporidians in Eastern Asia: cytochrome *b* lineages of the genera *Plasmodium* and *Haemoproteus* (Haemosporida) from China and Malaysia

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Seventy-six individuals from China belonging to 23 bird species in 14 families and 80 individuals from Malaysia belonging to more than 30 bird species in 14 families were examined for the presence of *Plasmodium* spp. and *Haemoproteus* spp. Birds were trapped at four localities in Gansu Province, China, in June – July 2011 and at Gombak Field Station, Malaysia, in July – August 2010. DNA was isolated from blood samples and parasite detection and identification was based on PCR assays and sequences of 479 bp of the cyt *b* gene. The total prevalence of haemosporidians was 21.0% in samples from China and 32.5% in samples from Malaysia. *Haemoproteus* spp. were the most prevalent parasites at both sites, with prevalence reaching 18.4% in China and 23.7% in Malaysia. The lineage CYAPIC1 from *Cyanopica cyanus, Parus major, Passer montanus* and *Pyrrhocorax pyrrhocorax* from China was new; it is genetically distinct and probably represents a new species of the genus *Haemoproteus*. The lineages RBS5, WW1, YWT2 have not yet been identified to morphospecies. The rest of *Haemoproteus* spp. lineages found in China, i.e. RBS4, COLL2 and TURDUS2, have been identified to morphospecies. Only one bird from China was positive for *Plasmodium* (prevalence 1.4%): *Parus major* was infected with the lineage GRW4 of *Plasmodium relictum*. Fifteen lineages were found in samples from Malaysia. We identified 9 new *Haemoproteus* and 2 new *Plasmodium* lineages. Four of the lineages found in Malaysia are known from previous studies: NILSUN1 (*Plasmodium* sp.), YWT2, COLL2 and TURDUS2, the latter two being linked to *Haemoproteus* morphospecies. The prevalence of *Plasmodium* is 3.7%. We provide phylogenetic hypotheses and analyses of genetic distances of all *Plasmodium* and *Haemoproteus* spp. and lineages identified in birds from China and Malaysia.

Morphological study of *Haemoproteus syrnii* (Mayer, 1910) in *Strix aluco* and in a Hippoboscid fly

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Blood smears of Tawny owls, *Strix aluco*, from three different localities in France were examined and revealed the presence of *Haemoproteus syrnii*. The prevalence was different in adults (60%) and in juveniles (3%). In this study, we present additional morphological features of this species, in particular the type and the characteristics of the volutine granules. Moreover, a Hippoboscid fly was caught on one of the positive adult owls originating from the Cevennes, in the south of France, and in which only *H. syrnii* was observed but not *Plasmodium*, *Leucocytozoon* or another *Haemoproteus*. We found all the sporogony stages of *H. syrnii* in the blood meal of the fly, from the gametocyte (probably newly ingested) to the sporozoite stages. Although the fly was not specifically identified, this finding suggests that the classification of the subgenera *Haemoproteus*, in Columbiforms, and *Parahaemoproteus* in the other birds, might be revised.

Azores archipelago has low haemosporidian diversity and high host specificity in forest passserines

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Island archipelagos, such as the Azorean islands (north Atlantic), with geographically discrete units supporting a variety of habitats, environmental conditions and endemic species, have been providing suitable model systems in which to investigate the evolution of host-parasite interactions. We focused on the archipelago's forest passerine avian communities (five most abundant species). Two hundred and five avian samples were screened for haemosporidian lineages with a minimum of five samples per island/species).

The number of lineages/haplotypes was very small, including only two lineages of *Plasmodium* (LINN01 and SYAT05) and two lineages of *Leucocytozoon* (TUMER01 and TUMER02), while no *Haemoproteus* lineage was found. These lineages were found on all islands, but were mostly present in blackbirds, while the presence in the other species was residual.

The blackbird seems to be the main host for the parasite lineages due to its higher prevalence in this species, from which parasites may switch to other species. It is likely that the parasites came to Azores islands along with colonizing blackbirds since the lineages they carry are relatively common in European blackbirds and not common in the other species, according to an extensive sampling that was also performed at the same time in Iberia and Morocco. Additionally the absence of *Haemoproteus* is in concordance with the small diversity found in our sampling in southwest Europe rather than the absence of suitable vectors in Azores. Indeed, suitable vectors for all haemosporidian genera have been recorded on all islands.

The occurrence of this kind of host-parasite system, with a reduced number of lineages and sedentary hosts, could provide a suitable model to address specific hypotheses concerning the factors that promote or prevent infection of specific hosts and their survival.

A comparison of avian haemosporidian parasite communities across the strait of Gibraltar

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One of the major concerns of ongoing environmental global changes is the ability of parasites to shift distributions, hosts, and to increase in virulence. In order to understand the structure of host-parasite communities across the Mediterranean Sea we used forest avian haemosporidian parasite communities across the strait of Gibraltar as a model system. We sampled 321 birds of 43 species from NW Africa and 735 birds of 50 species from NW Iberia and tested them for the presence of infections using molecular tools (PCR). We identified a total of 200 unique haemosporidian lineages. We found 127 parasite lineages in NW Africa and 103 in NW Iberia, of which only 39 were shared between study areas. Overall prevalence was higher in NW Africa, where 79% of the birds carried haemosporidian infections compared to only 51% in NW Iberia. The number of hosts from which a parasite lineage was recovered varied from one to twenty nine. Parasite specificity varied among parasite genera with *Haemoproteus* being the most host-specific and *Plasmodium* the most host-generalist. The composition of haemosporidian communities differed between Maghreb and Iberia. *Haemoproteus* was more common in Maghreb, but *Plasmodium* dominated in Iberia. Nevertheless, the high proportion of infections with shared lineages and the lack of spatial structure in their phylogeny suggest that haemosporidian parasites are easily able to cross between Iberia and North Africa.

Ecological determinants of haemosporidian prevalence in tropical African birds

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Prevalence of Haemosporidian parasites in birds is highly variable within taxonomic groups and among geographic regions. The underlying causes of this variation in prevalence are unclear, though a combination of ecological factors and life history traits are suspected to play a role. Haemosporidian parasites are dependent on dipteran insects for reproduction and transmission, and exposure of avian hosts to infective dipteran vectors is necessary for parasite transmission. Therefore, factors that determine a host's exposure to vectors are likely to be important determinants of infection prevalence. Several characteristics, including nest height and type, social system, habitat, and foraging stratum, have been linked to vector exposure in Europe and North and South America. However, few studies have examined the extent to which these findings generalize to other regions and to the African tropics specifically. We tested the ability of these ecological and life history characteristics to predict the prevalence of Haemosporidian parasites (*Plasmodium, Haemoproteus*, and *Leucocytozoon*) observed in a broad range of Afrotropical bird species. We found that host nest type, nest height, and social system are significant predictors of infection prevalence, and suggest that these variables correlate with differential exposure of hosts to vectors, thus leading to the variation in parasite prevalence observed in Afrotropical birds.

Biodiversity of avian haemoparasites in a high altitude city of Colombia

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Due to fast and uncontrolled expansion of cities, ecosystems are affected by transformation, fragmentation and destruction of natural habitats, turning them into landscapes dominated by buildings, and changing ecological interactions in these places. The objective of this study was to analyze the diversity of avian blood parasites in an urban ecosystem in Bogotá, (campus of the Universidad Nacional de Colombia-CUN), located a 2560masl, with an average annual temperature of 15°C. Two hundred seventy-one birds belonging to 14 families and 42 species [22 of them resident (n=189) and 20 of them migratory (n=72)] were analyzed. The overall prevalence was 21%. The parasites found by light microscopy were*: Plasmodium* [*P.(Haemamoeba) lutzi* and two new species of *P.(Novyella):* sp1. and sp2.]; *Parahaemoproteus* [*H.(P.).coatneyi*, *H.(P.).vireonis* and *H.(P)* sp. nov.]; *Leucocytozoon majoris*, *Trypanosoma* and Filaroidea spp. Molecular lineages for haemosporidian parasites were obtained (Cyt*b* fragment-478bp). Only two resident avian species were found infected: *Turdus fuscater* (29.5%) and *Zonotrichia capensis* (67.6%) with *Plasmodium* and *Parahaemoproteus* spp., respectively, so they could be the key hosts that maintain the infections in this area. The possible explanations for the absence of these parasites in other resident birds, could be associated with good immunoresponse of the key host and that those parasite species could be lethal to the other birds, or produce abortive infections. Also, it is interesting that the high prevalence of *Plasmodium*. sp1 nov. (29.5%) was reported in *Turdus fuscater* in this altitude; this bird is the only host for this parasite and is resident and has a sedentary behaviour, so we suspect a possible effect of the city, such as an increase in temperature that allows the development and transmission of parasites. We are interested in analyzing this fact, in order to determine the impact of the haemosporidian species on birds of the area.

Co-infections by malaria parasites decrease feather growth but not feather quality in house martin

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During moult, stressors such as malaria and related haemosporidian parasites (e.g. *Plasmodium* and *Haemoproteus*) could affect the growth rate and quality of feathers, which in turn may compromise future reproduction and survival. Recent advances in molecular methods to study parasites have revealed that co-infections with multiple parasites are frequent in bird–malaria parasite systems. However, there is no study of the consequences of co-infections on the moult of birds. In house martins (*Delichon urbica*) captured and studied at a breeding site in Europe during 11 years, we measured the quality and the growth rate of tail feathers moulted in the African winter quarters in parallel with the infection status of blood parasites that are also transmitted on the wintering ground. Here we tested if the infection with two haemosporidian parasite lineages has more negative effects than a single lineage infection. We found that birds with haemosporidian infection had lower body condition. We also found that birds co-infected with two haemosporidian lineages had the lowest inferred growth rate of their tail feathers as compared with uninfected and single infected individuals, but co-infections had no effect on feather quality. In addition, feather quality was negatively correlated with feather growth rate, suggesting that these two traits are traded-off against each other. We encourage the study of haemosporidian parasite infection as potential mechanism driving this trade-off in wild populations of birds.

Plasmodium (*Haemamoeba*) *lutzi* in Colombia

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This study reports a broadening of the altitudinal range and new host for *Plasmodium (Haemamoeba) lutzi* in Colombia. The study was conducted in two localities: in the city of Bogotá at 2560 m above sea level (asl), and in Chingaza Natural National Park located in the Eastern Cordillera of Colombia at 3,100 m asl. Bogotá represents an urban ecosystem where the annual temperature average varies from 9 to15 oC, and Chingaza NNP is a protected area, the locality of sampled was mainly paramo ecosystem, where the annual temperature varies from 0 to 14 °C. In total, 567 specimens of birds belonging to 79 species and 22 families were captured using mist nets. The blood samples were collected through venipuncture and analyzed by light microscopy. *Plasmodium (H.) lutzi* was found in two individuals of *Turdus fuscater* (Great Thrush-GenBank KC138226) in Bogotá. mtDNA obtained from Great Thrush confirms that *P. lutzi* is a sister taxa of *Plasmodium relictum*, as previously proposed. In Chingaza NNP, three other individuals of species - *Diglossa* (*D. lafresnayi, D. cyanea, D. albilatera*)- showed gametocyte morphotypes quite similar to *P.lutzi*. Genetic distances between the cyt*b* fragments (478bp) were 0.4% - 0.6% when compared with the fragment of cyt*b* of KC138226 lineage. This parasite has previously been reported in *Aramides cajaneus* (Grey-Necked Wood Rail), in the lowlands of Brazil, Venezuela, and Colombia. This finding provides evidence for a broad host range for *P. lutzi* that include two different orders, Gruiformes and Passeriformes, and also altitudinal expansion of its known distribution.

Molecular detection of haemosporidian parasites infecting both red and white blood cells in a Mabuya skink (Reptilia: Squamata)

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A blood sample from *Mabuya* sp. (Squamata: Scincidae) was studied both microscopically and molecularly. The microscopic scanning of the samples showed a mixed infection of apparently two types of haemosporidian parasites. One of them corresponded to parasitic shapes infecting erythrocytes which morphology agrees with the genus *Plasmodium* Marchiafava and Celli, 1885 reported from reptiles. We also detected, in a lower number, parasites infecting leukocytes. The shape of these last ones corresponds with the genus *Saurocytozoon* Lainson and Shaw, 1969 previously reported to infect reptiles only.

However the molecular detection of these parasites by using several sets of primers for different genera of Apicomplexa parasites only revealed two *Plasmodium* haplotypes. Based in those results we can set out two possibilities. The first one is that the primers employed in the molecular detection of the parasites detected two cryptic *Plasmodium* species within the erythrocytes, remaining the parasite within the leukocytes undetected due to differences in its genetic sequence as compared to *Plasmodium*. The second one is that, lacking molecular information of the genus *Saurocytozoon*, this genus was in fact a species within the genus *Plasmodium* infecting white blood cells.

We highlight the importance of performing both morphologic and molecular descriptions of haemosporidian parasites in order to establish the correct identification of these parasites.

Avian Malaria Parasites in a Brazilian Seasonally Dry Tropical Forest

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Seasonally dry tropical forests (SDTFs) exhibit a disjunct distribution scattered throughout the Neotropics and the largest areas of SDTFs are found in South America, specifically in Brazil, where they are represented in 3.21% of its territory. Brazilian SDTFs are endangered because of human occupation, conversion of lands to agriculture and high deforestation rates in these fertile soils. In this way, SDTF can be considered the most threatened ecosystem in Brazil. Despite this, there is a lack of information about the diversity of avian haemosporidians in SDTFs. We used molecular markers to study the prevalence and genetic diversity of avian malaria parasite lineages in a Brazilian SDTF. From 2007 to 2012, birds of Mata Seca State Park were collected using mist nets during the dry and rainy seasons of each year. Blood was collected using microcapillary tubes following venipuncture of the brachial vein with a sterile syringe needle and stored in absolute alcohol until DNA extraction. A total of 944 samples were initially screened for the presence of *Plasmodium* and/or *Haemoproteus* infections by PCR using primers 343F and 496R. Approximately 20% (188 positive samples) of birds were infected with haemosporidian parasites. We amplified a 524 bp fragment of the mtDNA cytochrome *b* gene from 45 infected individuals by a nested-PCR using primers HaemNFI and HaemNR3 in a first amplification, and HaemF and HaemR2 in a second amplification. We detected 10 parasites' cyt-*b* lineages, eight of *Plasmodium* and 2 of *Haemoproteus*. One new lineage was identified for *Plasmodium* and *Haemoproteus*. Six *Plasmodium* lineages were previously identified in Brazilian SDTF and one lineage was described in Brazilian savannah. The *Haemoproteus* lineage was previously detected in Socorro Island (Mexico). The remaining positive samples are being sequenced to conduct phylogenetic analysis of avian haemosporidians.

Migration divide and haemosporidian parasite spectra in the Reed Warbler *Acrocephalus scirpaceus*

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Long-distance migrant bird species frequently use alternative wintering places and/or migration routes, which results in establishment of migration divide. Individuals showing different migration behaviour can be exposed to dissimilar parasite pressure, and hence parasite spectra of the host species are expected to show patterns coincident with the migration divide. However, data from nature show only mixed evidence to support this hypothesis. We investigated haemosporidian parasites in the reed warbler *Acrocephalus scirpaceus* using molecular detection methods. The Reed Warbler is a small passerine, breeding in the Western Palaearctic and wintering in sub-Saharan Africa. Ringing recoveries suggest existence of a migratory divide in Central Europe; however, there are only slight (if any) genetic differences between localities across the migratory divide. Our analyses show surprisingly similar parasite spectra in European Reed Warblers that are supposed to head southeast at the beginning of autumn migration (localities in Slovakia, Hungary, Bulgaria, Croatia). Moreover, the spectra differ from those found in birds supposed to use a southwest route (localities in the Czech Republic, Spain and Finland). Reed Warbler localities from the Eastern Mediterranean (Turkey, Jordan, and Cyprus) show varying pattern of parasite assemblages, which may suggest rather complex migration connectivity in this region.

Leucocytozoonosis in Long-legged Buzzards (*Buteo rufinus*), Turkey

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Leucocytozoon species can cause serious diseases in avian hosts, especially in young birds. There is very little information about this protozoon in Turkey, and this information is based only on morphological analyses. We have focused on leucocytozooids of buzzards, about which we also have little information worldwide. Previously, morphologically defined species *L. toddi* were accepted as unique species in falconiform birds, but mitochondrial DNA sequences of leucocytozooids obtained from different species of falconiform birds revealed that a significant difference is apparent between the *Leucocytozoon* spp. infecting the species of *Buteo* and *Accipiter*. Subsequently, *L. mathisi, L. buteonis* and *L. toddi* were gathered under the name of *L. toddi* species group. *L. buteonis* have been classified in *Buteo* spp. Also*, L. buteonis* can be readily distinguished morphologically from the others due to length of the cytoplasmic processes of their host cells and other features associated with length of the processes. In this study, blood samples belonging to 13 Long-legged Buzzards (*Buteo rufinus*) were examined for the presence of leucocytozooids. Three buzzards were found infected with *L. buteonis* by using morphological analysis and molecular analysis, which was based on the mitochondrial cytochrome *b* gene. In conclusion, this study contributes information about leucocytozooids of buzzards in this geographical area and new taxonomy of *Leucocytozoon* species in falconiform birds.

Systematic survey for haemosporidian parasites in the Black-necked Grebe (*Podiceps nigricollis*)

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Absence of blood parasites has commonly been attributed to the absence or scarcity of appropriate vectors or immunological capabilities. Some alternative explanations are low host density or insufficient time for the co-evolution. The latest proposed hypothesis for blood parasite absence was ecological, i e. competitive exclusion by ectoparasites. Population structure, age, sex, behaviour or habitat can also be reason for absence of blood parasites in some periods or regions. The Black-necked Grebe breeds in vegetated areas of freshwater lakes across Europe, Asia, Africa, northern South America and the southwest and western United States. In our study we have investigated 270 samples from molting in Odiel Saltpans, Andalusia, Spain Black-necked Grebe collected in 2009 and 2012. All samples were screened by nested PCR for *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. The investigated samples included birds from two different years and most probably different breeding populations with variable age and sex structures. The results clearly indicate absence of *Plasmodium, Haemoproteus* and *Leucocytozoon* infections in this host species, at least in the European part of its breeding range. The common habitats of these water birds are inhabited with abundant species of potential vectors. The immunological capability seems unlikely because the same species has been recorded as host of many helminths and ectoparasites. The ectoparasites (feather mites and chewing lice) of Black-necked Grebe have been investigated and numbers of species are described; therefore competitive exclusion can be one of the hypothetical reasons for absence of haemosporidian parasites in Black-necked Grebes.

Behavioural and physiological changes in Siskins (*Spinus spinus*) infected by *Plasmodium ashfordi*

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Parasitological data gathered during birds' trapping are biased, as a passive character of mistnetting has a tendency to get exceptionally active birds. Quiet and faint individuals during high parasitemia have a lower probability of being caught. Also, such underrepresentation of sick birds in trapping data can be a result of their quick elimination because of predation. In our study we test the hypothesis that infected birds may be more easily caught by birds of prey.

Forty-four subadult siskins *Carduelis spinus* were used for the experiment. Twenty-two birds were infected with *P. ashfordi* (gene lineage pGRW2) and 22 were used as controls. Experimental birds (and control in respective days) were subjected to simulated attacks from a plastic model of a merlin before infection, on the highest level and chronic phase of infections. Data on response speed, take-off velocity and angle were analyzed. There were no significant differences in these three parameters neither between stages nor between groups. However, we observed dramatic changes in the proportion of different blood cells in infected birds. Here we conclude that birds even in a severe stage of disease had enough capacity to escape from a predator for a short distance. However, this doesn't mean a successful rescue for a longer distance as it can happen in nature. More appropriate behavioural experiments are needed to check for birds' fitness during malaria disease. This study was partly funded by the European Social Fund under the Global Grant measure.

Paternity in the blue tit infected with avian malaria

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Extra-pair paternity comprises a common reproductive strategy among monogamous bird species. However, it remains unclear why females decide to mate with extra-pair males, risking costs such as abandonment by the social partner. Thus, it should be balanced by benefits, which may be either of direct or indirect origin. In the latter case, females may benefit from engaging in mating outside the pair bond if extra-pair males pass to the offspring genes of superior quality. One of the main determinants of individual quality is resistance to parasites. Therefore, if the social mate of the female is parasitized, she might be willing to seek resistance genes for her offspring through matings with other males. However, despite the strong theoretical prerequisites, robust empirical data verifying this prediction is lacking.

Here we test this hypothesis on a wild population of blue tits (*Cyanistes caeruleus*) breeding on the island of Gotland, Sweden in years 2009 - 2012. In this population, approximately 60% of individuals are infected with avian malaria parasites, mostly from genus *Plasmodium* (70% of infections) and there is a relatively high frequency of occurrence of extra-pair matings (40% of nests and 8% of offspring). We verified whether the presence of extra-pair offspring in the brood was related to infection, with avian malaria parasites of the social parents.

The Effects of Avian Malaria on Purple Martins: Fitness, Extra Pair Paternity and Migration

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Parasite resistance is an important mechanism for sexual selection. Parasitic infections are expected to negatively impact fitness as well as honest indicators of quality and thus influence social and extra-pair mate choice. This study investigates avian malaria parasite infections in purple martins (*Progne subis*) to test the predictions that birds with higher parasitemia have reduced annual survival probability, lower reproductive success in females and lower genetic mating success in males. Additionally, increased parasitemia was predicted to influence fall migration parameters including departure date, distance and pace. A medication experiment was also conducted to determine the effects of a reduced parasitemia on reproductive success and migration. This study was conducted at two purple martin colonies in northwestern Pennsylvania, U.S.A. over a seven year period (2006-2012). Specific migratory parameters were collected through the use of geolocators. Infection load was experimentally reduced using subcutaneous injections of the anti-malarial drug primaquine. Prevalence and parasitemia will be determined using molecular techniques including quantitative real-time PCR. Migration results from geolocation as well as preliminary prevalence data will be presented. This comprehensive study investigates the relationship between avian malaria parasites and both direct and compensated fitness costs using quantitative infection data and experimental manipulations.

Haemoproteus and *Plasmodium* spp. prevalence in a declining population of European Turtle Doves (*Streptopelia turtur*)

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Species of the Columbidae are thought to be substantial reservoirs for diseases, susceptible to co-infection by different parasite lineages, often belonging to different genera. However, little information is known about the prevalence of two different groups of blood parasites, *Haemoproteus* and *Plasmodium,* and the age of first infection within the European Turtle Dove *Streptopelia turtur*, on UK breeding grounds. We screened blood samples collected during the 2011 and 2012 breeding season in parallel by both microscopic examination of blood smears and nested PCR to determine blood parasite prevalence. Overall, we confirm the first known reported cases in the UK of *Haemoproteus* and *Plasmodium* spp. in Turtle Doves, with the prevalence of 71%. We found a haemosporidian parasite prevalence of 57% in Turtle Dove nestlings sampled at 7 – 9 days old. To our knowledge, this is one of the youngest ages at which haemosporidian infection has been detected in wild birds. Results are particularly concerning for the Turtle Dove, as the costs of parasite prevalence and immune responses could be contributing to the species' decline.

Haemosporidian parasites in Canarian birds: low lineage diversity and rare lineage expansion

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Islands represent interesting natural system for studying parasite host shifts due to exposure of naïve hosts to new pathogens brought from the mainland. We studied haemosporidian parasites of passerines from the Canary Islands using molecular (nested PCR) methods. We investigated 500 individuals of 11 species. The overall prevalence of haemosporidians was 19.2 % (*Plasmodium* lineages 16.8 %, *Haemoproteus* 0.2 %, and *Leucocytozoon* 6 %). Three and 0.4 % of birds harboured mixed infections of two and three lineages respectively. The total prevalence on islands varied from 2.2 % on El Hierro to 39.6 % on Gran Canaria. The largest lineage diversity was found in *Cyanistes teneriffae* (7 lineages). *Sylvia conspicillata* exhibited the highest prevalence (44.4 %). We have not detected any parasites in endemic species *Fringilla teydea* and *Regulus teneriffae*. Surprisingly, the largest lineage diversity was found in *Leucocytozoon* (11 lineages). In contrast, only one *Haemoproteus* lineage was detected (PARUS1 lineage in a single individual of *Cyanistes teneriffae* from El Hierro). *Plasmodium* LK6 was the most frequent haemosporidian lineage showing wide host breadth (9 host species), which is in sharp contrast to the situation outside the Canarian archipelago, where LK6 is a rare lineage restricted to *Falco naumanni*.

Bloodmeal Identification and Detection of a *Leucocytozoon* lineage from blackflies (Diptera: Simuliidae) collected from Kizilirmak River in Nevsehir province of Turkey

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This study was carried out on Simuliid (Diptera: Simuliidae) flies in the Central Kizilirmak Basin in Nevsehir province of Turkey and supported by the Scientific and Technological Research Council of Turkey with the project no 111 O 426. Larvae and adult blackfly samples were collected from several field stations in different periods of 2012 and 2013 in the part of Kizilirmak River. Species identifications of simuliid larvae specimens were done by morphological, chromosomal and molecular analyses. Two species *S. (Wilhelmia) lineatum* and *S. (Wilhelmia) balcanicum* were determined in the research area according to the larval identifications. The molecular identifications of bloodmeals were performed individually on a total of 90 adult black flies by using two pairs of primers specific to avian and mammalian species (Avian-3/ Avian-8 for avian; Mammalian-1/ Mammalian-2 for mammalian). The pathogens *Leucocytozoon* and *Onchocerca* species were investigated in the individual black fly specimens and also in 23 genomic DNA pools that consisted of 230 black fly specimens (10 black flies/pool) by using sybergreen based Real Time PCR. Five out of 90 individual simuliid specimens were found to be positive with the avian primer pairs whereas no positives were found with the mammalian primer pairs. Of the 90 individual simuliids screened for *Leucocytozoon* and *Onchocerca* species only one (also positive for avian bloodmeal) was positive for *Leucocytozoon* sp. Two out of 23 genomic DNA pools were also found positive for *Onchocerca* sp. according to the sybergreen qPCR assay. A *Leucocytozoon* positive simuliid specimen was identified as *S. lineatum* according to the sequence analyses of the partial mt-COI gene region. The obtained *Leucocytozoon* lineage from *S. lineatum* showed highest identity with the “SPOW30” isolate obtained from a *Strix occidentalis occidentalis* in the USA according to phylogenetic analyses of the partial mt-cytb gene sequences.

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CERTIFICATE OF ATTENDANCE

This Certificate is Awarded to

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in recognition of participation in the International Conference: ***Malaria and Related Haemosporidian Parasites of Wildlife,*** held at the Lithuanian Academy of Sciences in Vilnius, Lithuania, from August 7-11, 2013.

Chairman of the Organising Committee

Title *Signature* *Date*

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