Emeishan Gall Wasp Project
Expedition Report
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Introduction

Oak gall wasps (Hymenoptera: Cynipidae: Cynipini) are an intriguing group of insects that induce complex gall structures on trees of the Fagaceae family. An adult female gall wasp lays her eggs into the meristem tissue of a host plant, and the gall wasp larva then controls development of plant tissues around it. larva consume nutritive tissues within a specialised chamber, before pupating within the gall, and tunnelling out as adults (see Plate 1). Oak galls occur on various plant organs including buds, leaves, catkins, stems and roots, and may be single or multi-chambered. Most oak gall wasp species have two generations each year, with a sexual generation developing in galls during spring (March-May), and an asexual generation developing during late summer and autumn (July-October) (Stone et al. 2002). Although oak gall wasp species are formally described from the morphology and genotyping of adult insects, the morphology of galls, host tree, and location on the tree, are generally diagnostic of a particular generation of a single gall wasp species, and can be used to build a useful intermediate taxonomic framework (see Plate 2).

Plate 1. Oak Gall wasp life stages: adult female gall wasp (top left); gall wasp larva (top & middle right); gall wasp pupa (bottom right); gall with visible emergence hole (bottom left). Images courtesy of G. Stone.

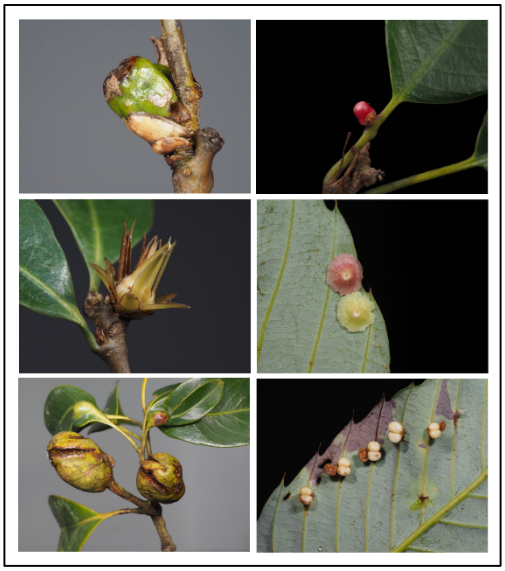

Plate 2. Examples of novel gall morphotypes collected from Mount Emei during the expedition, expected to represent the asexual generations of 6 previously undescribed species. Images by Chang-Ti Tang.

Once formed, oak galls are often colonised by inquiline wasps from the closely related Synergini tribe, whose phytophagous larvae can modify the tissue of existing galls but are unable to induce independently. They are also attacked by hymenopteran parasitoids, whose larva may feed on the larvae or pupae of the gall-formers, inquilines, or other parasitoids. Oak galls therefore encompass multi-trophic communities, with host trees as primary producers, gall wasps and inquiline herbivores as primary consumers, and parasitoids and hyper-parasitoids as secondary and tertiary consumers (see Figure 1). These communities are

relatively closed, in that individual species of oak gall wasp are specialised parasites of a limited range of host trees, and individual species of inquiline and parasitoid are generally specialised inhabitants of a limited range of oak galls (Askew 1961, 1980; Stone et al. 2002).

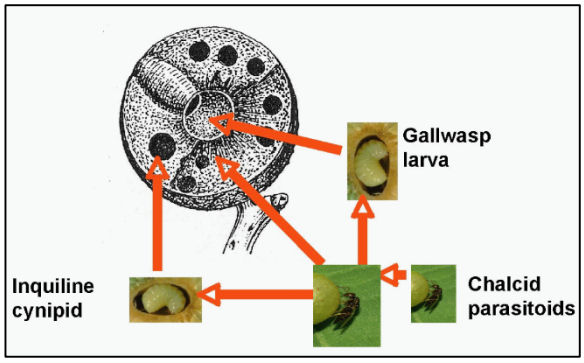

Figure 1. Illustration of a multi-trophic oak gall community, with the plant material (i.e. the gall) providing the primary resource, herbivorous gall wasp and inquiline larva feeding within chambers inside the gall, and parasitoids potentially targeting the gall wasp larva, the inquiline larva, or one another.

Oak gall wasps are parasites, and have a negative effect on their host plant through consuming nutrients and causing structural damage to plant organs. The impact of oak gall wasps on their host plant is usually minor relative to other types of herbivory and damage, but one species – *Dryocosmus kuriphilus* – has become a major economic pest of chestnut trees (*Castanea spp.*). Having lost its sexual generation, the asexual generation of *D. kuriphilus* induces large multi-chambered galls on young shoots during late spring (see Plate 2). Infestation can reduce chestnut production by 60-80%, and major successive attacks can kill the tree. It is native to China, and spread to Japan in 1941, North America in 1974, and Europe in 2002. In its invaded range, the impact of *D. kuriphilus* is being countered by releasing a natural enemy from its native range - the parasitoid *Torymus sinensis*. Little is known about the ecology of *D. kuriphilus* within its Chinese native range, but it is not considered to be a major pest there, probably due to natural control by *Torymus sinensis* and other parasitoids.

Plate 3. *Dryocosmus kuriphilus* galls on *Castanea sp.* Galls on current growth are pinkish green. The darker gall on the right is from the previous year. Image courtesy of G. Stone.

Oak galls are a useful model system for community ecology, and over the past 25 years, research centred at the University of Edinburgh has established *Gallbase* - a large database of ecological and molecular data that has aided studies of community genetics (Sinclair et al. 2015), community phylogeography (Hayward and Stone 2006; Stone et al. 2012), and biological invasions (Schönrogge, Stone, and Crawley 1995, Schönrogge et al. 2012). Efforts have been most extensive in the Western Palearctic and North America, and oak gall communities remain relatively under sampled in East Asia. Recent studies from Japan, Taiwan, and China, have described numerous new species associated with the Fagaceae genera *Quercus, Castanopsis, Lithocarpus,* and *Cyclobalanopsis**, the latter three of which are found only in Asia (see Figure 2 & Table 1). Based on this rapid rate of recent species discovery, and the currently limited extent of geographic sampling, it is expected that there are still many undiscovered species, especially in Southern and Western China where the diversity of potential host plants is at its greatest. Additional sampling of in this region is necessary for understanding the full diversity of the group, and would facilitate further exploration of ecological questions. Of particular interest are: (i) how are parasitoid communities structured between *D. kuriphilus* and other oak galls within its native range? (ii) what are the relative roles of hostplant-tracking and hostplant-switching in driving diversification of oak gall wasps? (iii) does low hostplant diversity promote higher structural diversity of gall morphologies through competition for enemy free space?

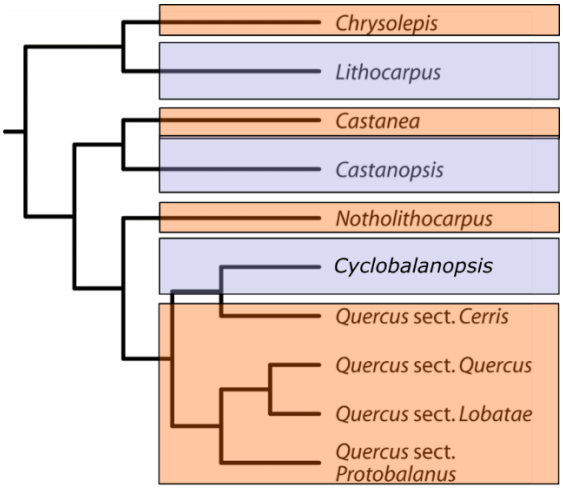

Figure 2. Evolutionary relationships of *Fagaceae* genera associated with oak gall wasps (adapted from (Oh and Manos 2008). The three genera highlighted in blue are found only in Asia.

Genus	Number of gall wasp species as of 2008	New species from Asia since 2008
Chrysolepis	1	NA
Lithocarpus	0	1
Castanea	1	1
Castanopsis	0	9
Notholithocarpus	1	NA
Cyclobalanopsis	1	30
Quercus	~1000	14

Table 1. Summary of global Gall wasp species by hostplant, and recent species discovery from Asia.

The Emeishan Gall wasp project was established in 2017 as a 3-year collaboration between Emeishan Biological Resources Research Station (EBRRS), Zhejiang University (ZU), and The University of Edinburgh (UoE). This collaboration brings together expertise in botany (EBRRS), gall wasp ecology, taxonomy, and field methodologies (UoE), and DNA sequencing technologies (ZU). The project aims to:

1. Survey oak gall wasps and associated communities on Emeishan Fagaceae, using gall rearing and DNA sequencing to identify and describe new species.

2. To obtain molecular data from collected samples in addition to taxonomic description and for incorporation into a comprehensive molecular phylogeny of the Cynipini that includes species associated with the various temperate and subtropical Fagaceae genera.

3. Incorporate data into *Gallbase* to facilitate further ecological analyses.

4. Investigate how parasitoid communities are structured between *Dryocosmus kuriphilus* on chestnut and galls on other host trees, so that we can better predict the consequences of releasing *Torymus sinensis* in other countries.

The first project expedition took place at Mount Emei during October-November 2017, with the objective of sampling galls from across the range of Fagaceae genera present in the region.

*Note: *Cyclobalanopsis* is often treated as a sub-genus of *Quercus*, but as the *Flora of China* classifies it as a distinct genus we shall adopt this treatment throughout the project.

Mount Emei Expedition

Study Area

The Mount Emei Scenic Area is situated in Sichuan Province, approximately 160km Southwest of the provincial capital Chengdu. The area covers approximately 115 square kilometres, with elevations ranging from 500 to 3099 meters. It is one of China's four sacred mountains, and has been designated as a Biological and Cultural World Heritage Site since 1996, together with nearby Leshan Giant Buddha Scenic Area. Biological research in the Scenic Area is conducted from the Emeishan Biological Resources Research Station, near to the Wannian Temple. The Area is known to contain 28 species of Fagaceae from the genera *Castanea* (3 species), *Castanopsis* (5 species), *Cyclobalanopsis* (5 species), *Fagus* (2 species), *Lithocarpus* (7 species), and *Quercus* (6 species).

Methods

Field sites were selected by Dr Zhi Qiang Fang, based on previous botanical surveys. The Fagaceae trees present at each site were identified by Dr Fang, and up to 10 trees of each species were selected for sampling. Five sections of foliage, approximately 1 meter in length, were removed from each sampled tree using extendable pole pruners. Foliage was searched in the field, and any galls were collected in zip-lock plastic bags. Some galls, particularly those of the asexual generations, can persist on a tree long after all resident wasps have emerged, while others fall or are excised from the tree once they have matured. Fieldwork was scheduled specifically to target maturing asexual generation galls, although older galls were also sampled where possible. In the laboratory, collected galls were separated into morphotypes based on host tree species and external gall morphology. A sample of each morphotype was dissected under a microscope to examine the internal structure and

determine whether it belonged to an oak gall wasp or another type of gall inducer (e.g. gall forming mites, midges etc.). Any larva or pupa encountered during dissection were preserved in ethanol. Oak gall morphotypes were either matched with existing records or nominated as novel gall-types. A photographic record of the external and internal structure of all gall-types was maintained. Undissected galls were placed in ventilated rearing containers, and were monitored at three-day intervals. All emerging insects were preserved in ethanol.

Preliminary Results

Twenty-one field sites in and around the Mount Emei Scenic Area were visited during October 12th-30th 2017 (see Figure 2), and 247 trees from 16 species of Fagaceae were sampled for galls (Table 3). Site elevation ranged from 522m to 2288m. A total of 100 gall morphotypes were collected, of which 68 were found to be induced by oak gall wasps (see Table 2). Only 12 of these could be matched with existing records, and the remaining 56 were nominated as novel gall morphotypes.

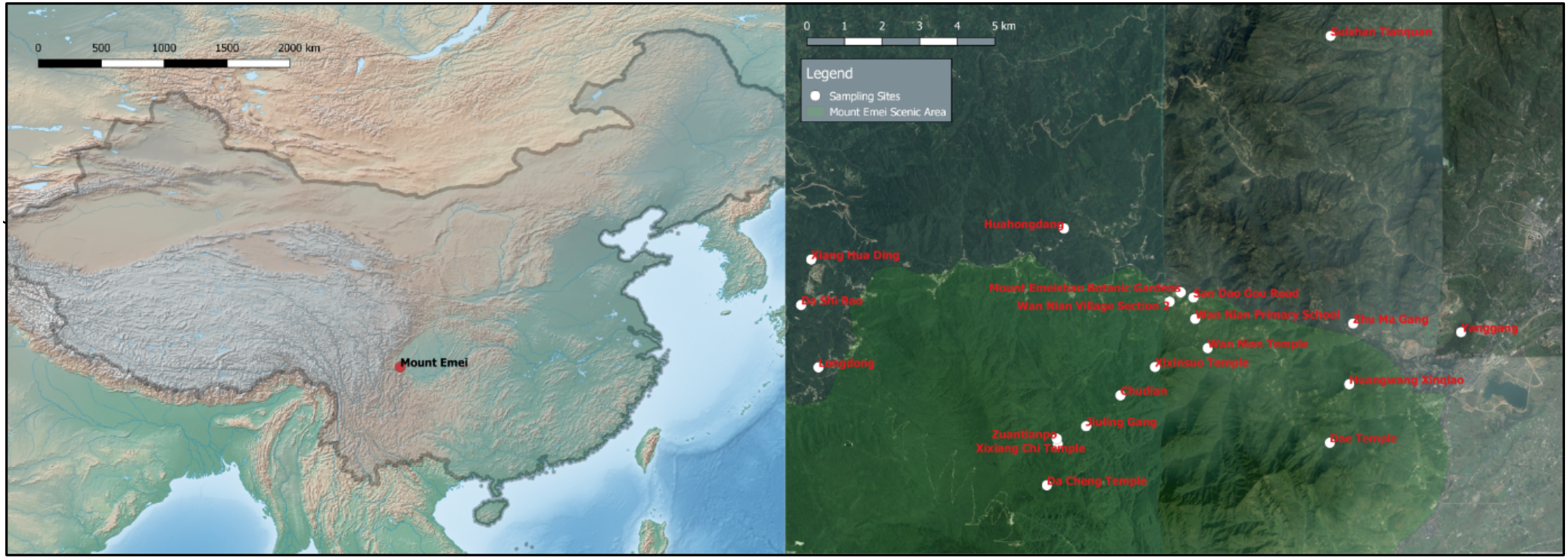

Figure 2. The location of: Mount Emei within China (left); and sampling sites at Mount Emei Scenic Area (right)

Table 2. Summary of gall morphotypes collected during expedition.

Type of gall induce	Number of gall morphotypes
Gall wasp	68
Midge	15
Mite	3
Psyllid	2
Scale insect	2
Lepidoptera/Coleoptera	10
Total	100

Table 3. Summary of oak gall morphotypes and host trees.

Tree Genus	Number of tree species encountered	Number of trees searched	Number of oak gall morphotypes collected
Castanea	2	58	2 [2]
Castanopsis	3	64	8 [0]
Cyclobalanopsis	4	23	16 [0]
Lithocarpus	3	25	9 [0]
Quercus	4	77	33 [10]
Total	16	247	68 [12]

Dissection revealed that many of the galls were fresh, and gall inducer larva were obtained from 27 of the 68 oak gall morphotypes. Although these cannot be used to formally describe new species, DNA sequences obtained from the larva can be added to datasets of known species to determine proximate identity, and to match generations of the same species. The presence of immature gall wasp larva at this time suggests that these species will overwinter within the galls, and adult gall wasps will not emerge until Spring 2018.

Monitoring of gall rearings is ongoing, but as of March 2nd approximately 3018 adult insects had emerged. These were mainly inquilines, and parasitoids from the families Braconidae and Torymidae. There were however adult oak gall wasps from two of the novel gall morphotypes – a ball shaped gall from the undersides of leaves of *Quercus faberi* and *Q. serrata*, and a 'birds head' shaped gall from the buds of *Lithocarpus cleistocarpus* and *L. megalophyllus* (see plate 2). This 'birds head' gall is only the third type to have ever been successfully reared from trees of the genus *Lithocarpus*, and initial study of the adult morphology suggests that it represents a novel gall wasp genus. Both species shall be formally described from the adult specimens.

Plate 4. Novel oak gall morphotypes that were successfully reared during expedition - a ball shaped gall found on *Quercus faberi* and *Q. serrata* (left); and a 'birds head' shaped gall found on *Lithocarpus cleistocarpus* and *L. megalophyllus* (right)

Galls of *Dryocosmus kuriphilus* were found on trees of *Castanea mollisima* at 9 sites, and approximately 200 galls were collected for rearing. The closely related species *D. zhuili* was also found on *C. henryii* at 5 sites. Adults of *D. kuriphilus* usually emerge during July-August, and so they are unlikely to appear in the gall rearings, but dissection revealed that many galls still contained parasitoid larva which are expected to emerge as adults during Spring 2018.

A sample of 120 specimens including larva and adult insects was selected for sequencing at Zhejiang University (ZU). Initial sequencing efforts will focus on the mitochondrial CO1 gene (i.e. DNA barcode), with further sequencing of nuclear genes for novel oak gall wasp specimens.

Further Work

This expedition was scheduled to coincide with the maturation of asexual generation oak galls. As most gall wasp species have two generations each year, a further expedition has been arranged for April 2018 to coincide with the maturation of sexual generation galls. By revisiting many of the same sites, it is expected that sexual generation gall collections will not only reveal further new gall types, but will allow for matching of gall generations via DNA barcoding of larva and adult specimens.

Gall rearings shall continue to be monitored by Dr Fang and his team at Emeishan Biological Resources Research Station. Many insects are likely to overwinter within the galls, and so monitoring shall continue for at least 12 months. Emerging specimens shall be preserved in ethanol, and transferred to Zhejiang University for non-destructive DNA extraction and sequencing. Adult oak gall wasp specimens shall be loaned to the University of Edinburgh, and formally described by the project team in collaboration with Dr George Melika - a world renowned expert in Cynipid taxonomy. Formal descriptions of novel species will be published in taxonomic journals. DNA sequence data for novel gall wasp species shall be combined with existing datasets to generate a comprehensive phylogeny of the Cynipini tribe, which shall be used to assess patterns of host switching, and the relationship between morphology and host plant diversity. The results shall be published in an appropriate evolutionary journal.

There has been very little previous taxonomic study of oak gall inquilines and parasitoids in Asia, and so DNA barcode sequence data shall be used to develop an intermediate species level taxonomy for the collected specimens. Interaction networks shall be constructed and used to examine to overlap between communities associated with *D. kuriphilus* on Chestnut, and galls on other hostplant genera.

Summary

The objective of this expedition was to sample galls from across the range of Fagaceae host plants present on Mount Emei. Fieldwork was conducted at varied sites between 522m to 2288m elevations, and 68 oak gall wasp morphotypes were sampled from trees of the Fagaceae genera *Quercus, Cyclobalanopsis, Castanopsis, Lithocarpus,* and *Castanea*. As most of these gall-types were previously unrecorded (56/68), this represents a substantial addition to knowledge of Asian gall wasps. Specimens of adult gall wasps have so far been obtained for 2 gall-types, and these shall be formally described in collaboration with taxonomic experts. One of these is particularly exciting, as it is only the 3rd gall wasp species to be successfully reared from *Lithocarpus*, and preliminary study of its adult morphology suggests that it represents a novel gall wasp genus. DNA sequence data from adult gall wasp specimens shall be combined with existing data to generate a comprehensive phylogeny of the Cynipini tribe. DNA barcode data from inquiline and parasitoid specimens will be generated and used to assess the overlap between communities from galls of *Dryocosmus kuriphilus,* and other galls.

Acknowledgements

This expedition was generously supported by grants from the Davis Expedition Fund (£6500), the Percy Sladen Memorial Fund (£1500), the Genetics Society (£1500), and the Royal Entomological Society (£400). International flights were covered by Zhejiang University (ZU), and all in-country food, travel, and accommodation were provided by Emeishan Biological Resources Research Station. The visiting team were hosted by Professor Sheng-Guo Fang from Zhejiang University (ZU), and fieldwork was conducted from Emeishan Biological Resources Research Station with Dr Zhi Qiang Fang. Assistance in the field was provided by Xiao Jie Li, Bang Kui Qi, & Da Jun Qi. DNA sequencing of specimens collected during the expedition will be conducted by Dr Zhu at Zhejiang University (ZU).

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Appendix 1 – Expedition Team

Back row - Frazer Sinclair, Da Jun Qi, Kasia Mikolajczak, Zhi Qiang Fang, Chang-Ti Tang; front row – Graham Stone, Jack Hearn.

Dr Jack Hearn – Jack is a Postdoc at the University of Edinburgh. His key research interest is in the interaction between ecological pressures and gene-level adaptation, for which the gall system with its discrete community has been a useful model. He has extensive experience of collecting gall wasps in Asia, Europe, and North America.

Dr Frazer Sinclair - Frazer completed a PhD at the University of Edinburgh in 2012, studying local adaption of European sessile oak and its effects on interactions with herbivorous gall wasps. Since graduating he has worked on several conservation projects, and has extensive international fieldwork experience.

Dr Chang-Ti Tang – Chang-Ti completed his PhD at the University of Taichung in 2015, studying the systematics and host associations of oak gall wasps in Taiwan. He is currently Postdoc researcher at the University of Edinburgh, exploring the phylogeography and host associations in the gall wasp genus *Dryocosmus*.

Miss Kasia Mikolajczak –Kasia completed her BSc degree at the University of Edinburgh in 2011, and for her research project she used DNA data to uncover cryptic species of oak gall parasitoids. She is currently completing a PhD at the University of Lancaster, studying psychological connection with nature in the Brazilian Amazon.

Professor Graham Stone – Graham is Professor of Ecology at the University of Edinburgh. For more than two decades he has used the oak gall system to research various themes in ecology and evolution.

Dr Zhi Qiang Fang – Dr Fang is a senior researcher at the Emeishan Botanical Gardens. He graduated from the Chengdu Institute of Biology, and his research focuses on understanding the community structure of forests in Sichuan.

Appendix 2 – Expenditure

An itemised record of all expenditure, with receipts, is available on request from Jack Hearn (jack.hearn@ed.ac.uk).

Table A2.1. Summary of expedition expenses by expense type.

Expense Type	Expenditure (£)
Equipment (inc. field kit, lab consumables, GPS, camera fixtures etc.)	4585.31
Visas	802.97
Medical expenses (inc. vaccinations, medical kits, insurance)	2227.01
UK travel & subsistence	596.17
International flights	3200.00
China travel and subsistence	3164.17
Total	14575.63

Davis Expedition Fund award dispersal

As of March 2018, £5698.48 of Davis Expedition Fund award has been spent on the Emeishan Gall Wasp Project (Table A2.2). The success of the expedition has led to an ongoing collaboration with our Chinese colleagues, who have agreed to fund two further trips to Emeishan. Because of this the remaining £801.52 is allocated for the following: £302 will be used for visas to China in April 2018 for expedition members Frazer Sinclair and Jack Hearn, and £499.52 will be used to cover subsistence in China for this visit. Receipts for these visas, proof of travel/flight confirmations, and subsistence spending will be available on request from Jack Hearn.

Source	Equipment	Visas	Medical expenses	UK travel & subsistence	International flights	China travel and subsistence	Total (£)
Genetics Society Heredity Fieldwork Grant (awarded to F Sinclair)		188.70	354.45	108.00			651.15
Royal Entomological Society Outreach Grant	400.00						400.00
Davis Expedition Fund	2685.31	614.27	1872.56	488.17		38.17	5698.48
Percy Sladen Memorial Fund	1500.00						1500.00
Zhejiang University					3200.00		3200.00
Emeishan Biological Resources Research Station						3126.00	3126.00
Total (£)	4585.31	802.97	2227.01	596.17	3200.00	3164.17	14575.63

Table A2.2. Summary of expedition expenses by funding source.