Antonis Rokas

I.C.A.P.B.

Report for James Rennie Bequest

I recently applied and succeeded for an award which would cover my travel expenses for the First International Wolbachia Conference which took place in Kolymbari, Crete, Greece, June 7-12, 2000. James Rennie Bequest awarded me with £250 for travel expenses. This amount covered almost the full cost of the trip which consisted of my flight to Athens (£213) and the boat and bus tickets to Crete (£50 in total).

The purpose of my participation in the meeting was the presentation of a talk and a poster (an A4 reprint of the poster is attached). In both cases I clearly acknowledged the support of James Rennie Bequest. I received important feedback concerning both presentations of my work. Since both of the projects I presented are still unfinished, this feedback will be of great help in the write-up stage of both.

An important fact that emerged is the tremendous progress in the field of *Wolbachia* research. Almost all work presented in the conference is as yet unpublished and getting to know what people are working on at the moment and their immediate research plans will be, of course, very helpful.

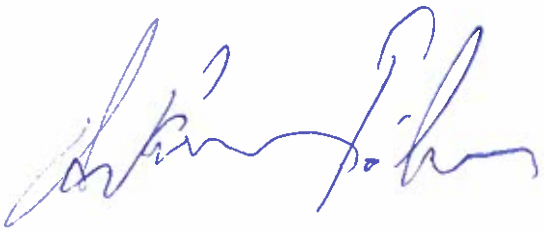
I also had the opportunity to discuss unofficially about post-doc opportunities in the UK and the United States. Various established researchers expressed a serious interest in my ideas (which is always good to know). Additionally, the informal style of the conference and the relatively small number of participants gave me the opportunity to discuss evolutionary aspects of the bacterial parasite *Wolbachia* with leaders in the field.

We (Dr. James M. Cook of Imperial College, London and myself) also succeeded in getting permission to write the meeting report for the review journal *Trends in Genetics* and we have already submitted the manuscript.

I would like to express my gratitude for your support in my participation in this conference. It was a great scientific and social experience!

With Kind Regards,

Antonis Rokas



The phylogenetic distribution of *Wolbachia* in the monophyletic clade of gallwasps (Hymenoptera: Cynipidae)

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What is a gall wasp?

Gallwasps (Hymenoptera: Cynipidae) induce galls on herbs (tribe "Aylacini"), roses (tribes Diplolepidini and Eschatocerini) and trees, particularly oaks (tribes Cynipini and Pediaspidini). Female wasps lay their eggs into plant tissue and when the larvae hatch they induce gall formation. Some gallwasps (tribe Synergini) have lost the ability to induce galls themselves and instead develop in (i.e. parasitise) the galls of the other gall inducing species. The clade of Cynipidae is ancestrally bisexual, but deviations to cyclical parthenogenesis (oak gallwasps) and obligate parthenogenesis (rose and oak gallwasps) are known. Cyclical parthenogens strictly alternate between one bisexual and a parthenogenetic generation every year and each generation produces a different type of gall. Parthenogenetic species have just one generation per year.

Introduction

Few studies have looked at prevalence of *Wolbachia* in monophyletic groups. The gallwasp family (Hymenoptera: Cynipidae) possess several characteristics that make them an ideal group for the study of *Wolbachia*. They are divided into six tribes (subfamilies) (Figure 1), four of which are bisexual and the other two are cyclical parthenogens. Additionally, a number of species have deviated to obligate parthenogenesis in both reproductive mode groups. Little work on *Wolbachia* has been done in gallwasps, except for the rose gallwasps, where it has been found that *Wolbachia* induces parthenogenesis (Plantard *et al.* 1998 *Proc. Roy. Soc. B* 265: 1075-1080). Here we investigate the following questions:

⇨ Is there any difference in prevalence or phenotype of *Wolbachia* infection between the two reproductive groups (bisexuals versus cyclical parthenogens)?

⇨ Is obligate parthenogenesis in oak gallwasps induced by *Wolbachia*?

⇨ Can we infer anything concerning the transmission patterns (horizontal/vertical) of *Wolbachia* among gallwasps?

Materials & Methods

⇨ 64 species of gallwasps were PCR-screened for *Wolbachia* infection using *ftsZ* gene primers. Wherever possible, individuals from many populations were screened.The data reported here are from our lab and from the literature (Plantard *et al.* 1999 *Insect Mol. Biol.* 8:185-191).

⇨ Representatives of each infected species were sequenced for the *Wolbachia* gene *wsp* and for the gallwasp host mitochondrial gene *cytochrome b*.

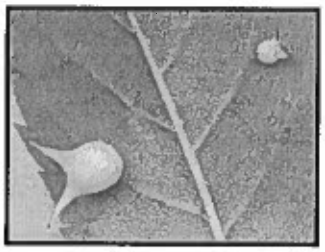
Results & Discussion

⇨ There is a statistically significant difference in the prevalence of *Wolbachia* infection in the groups with different genetic systems (Figure 1c). Cyclical parthenogens exhibit the rate of *Wolbachia* infection (19.6%) commonly found in various ecological assemblages. The bisexually reproducing group shows much higher levels of infection (53.6%) (Figure 1b).

⇨ Obligate parthenogenesis observed in oak gallwasps (tribe Cynipini) is not correlated with *Wolbachia* infection. Recent evidence suggests that several of these putatively parthenogenetic gallwasps are probably cyclical parthenogens with an as yet unidentified sexual generation (Atkinson *et al.* unpublished).

⇨ Study of transmission patterns of *Wolbachia* in gallwasps reveals extensive evidence for frequent horizontal transfer events within the Cynipidae. Outside the genus *Diplolepis* (discussed in Plantard *et al.* 1999), there are three other cases. For example, in one case four species (three Cynipini species and one Synergini) share a group A *Wolbachia* strain (also present in tsetse flies). Given that Synergini are parasites of Cynipini, this might be the result of a horizontal transfer from a parasitoid to its hosts! There is no evidence for vertical transmission and/or co-speciation of *Wolbachia* with its hosts as the phylogenetic relationships of the host are not congruent with that of the parasite. This is in agreement with results from rose gallwasps (Plantard *et al.* 1999) and members of the wasp genus *Trichogramma* (Schilthuizen & Stouthamer 1997 *Proc. Roy. Soc. B* 264: 361-66).

Here are some examples of spectacular gall formations:

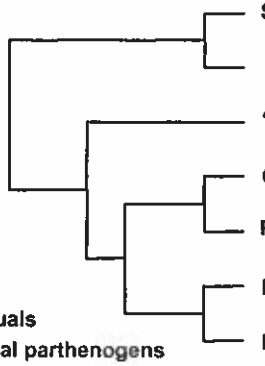


Leaf galls from *Diplolepis nervosa* (Tribe Diplolepidini).

Galls from *Neuroterus quercusbaccarum* (Tribe Cynipini).

Figure 1.

(a) Phylogenetic relationships within Cynipidae



(b) Proportion of species infected with *Wolbachia*

Synergini	7/12
"Aylacini"	4/9
"Aylacini"	
Cynipini	10/51
Pediaspidini	-
Eschatocerini	-
Diplolepidini	11/20

(c) The proportion of infected species with differing reproductive modes

Bisexuals: 22/41

Cyclical parthenogens: 10/51 (χ^2=10.17, d.f.=1, P<0.01)

Acknowledgements

We acknowledge the important contribution of Olivier Plantard 's published work on *Wolbachia* in cynipid gallwasps. Funding was provided by NERC, BBSRC, Royal Society and James Rennie Bequest. We thank Gordon Brown and an army of Hungarian foresters for their help with gall collecting.