

PROJECT FINAL REPORT

Supplementary file

Grant Agreement number: PIEF-GA-2008-220876

Project acronym: **SENSE**

Project title: **Social evolution: novel insights from the neglected
sphecid wasps**

Funding Scheme: **Madame Curie IEF**

Date of latest version of Annex I against which the assessment will be made: **24.4.2008**

Final report: **final report**

Period covered: **from 23 June 2008 to 22 October 2010**

Name, title and organisation of the scientific representative of the project's coordinator¹:

**PD Dr. Robert J. Paxton, School of Biological Sciences,
Queen's University Belfast, BT9 7BL, UK**

Tel: **+44-28-90972127**

Fax: **+44-28-90975877**

E-mail: **r.paxton@qub.ac.uk**

Project website² address: **not applicable**

¹ Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement .

² The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

Declaration by the project coordinator and Madame Curie Fellow

I, as coordinator of this project and in line with the obligations as stated in Article II.2.3 of the Grant Agreement declare that:

- The attached periodic report represents an accurate description of the work carried out in this project for this reporting period;
- The project (tick as appropriate)³:
 - has fully achieved its objectives and technical goals for the period;
 - X** has achieved most of its objectives and technical goals for the period with relatively minor deviations.
 - has failed to achieve critical objectives and/or is not at all on schedule.
- The public website, if applicable
 - is up to date
 - is not up to date
- To my best knowledge, the financial statements which are being submitted as part of this report are in line with the actual work carried out and are consistent with the report on the resources used for the project and if applicable with the certificate on financial statement.
- All beneficiaries, in particular non-profit public bodies, secondary and higher education establishments, research organisations and SMEs, have declared to have verified their legal status. Any changes have been reported under the section Project Management in accordance with the Grant Agreement.

Name of the Coordinator: Dr Robert J. Paxton.....

Name of the Madame Curie Fellow: Dr Manuela Giovanetti.....

Date: ...20.../ ...9.../ ...2011...

For most of the projects, the signature of this declaration could be done directly via the IT reporting tool through an adapted IT mechanism.

³ If either of these boxes below is ticked, the report should reflect these and any remedial actions taken.

1. FINAL PUBLISHABLE SUMMARY REPORT

SENSE: Social evolution: novel insights from the neglected sphecid wasps

SENSE was a two year (24 month) research project addressing the evolution of social behaviour in the Hymenoptera, integrating field experiments with molecular genetic laboratory studies of a Mediterranean ground-nesting wasp whose females sometimes nest alone and sometimes share with other females. The main objective of the research was to open up empirical study of a major evolutionary transition, that from solitary to social lifestyles, in the understudied 'sphecid' wasps, which the project has achieved in part through the successful development of novel genetic markers. The project simultaneously aimed to enhance the scientific and generic skills of a promising European researcher, Dr Manuela Giovanetti, an Italian national who undertook the research project in the United Kingdom.

One of the most amazing phenomena in the animal kingdom is the evolution of true sociality (eusociality), as exemplified by the ants, some bees, some vespid wasps and the termites. The phenomenon is of interest to all of us because of the parallels that social insects share with human social behaviour and human society. Among these well-known social insect societies, nest-mates cooperate for the successful growth and reproduction of the colony, with the workers often foregoing all reproduction and sometimes even dying to defend the colony. In the vast majority of these insect societies, there is just one or just a few mother queens that lay most or all of the eggs and all the worker individuals of a single colony therefore show a close genetic relationship (high relatedness) to one-another. The queen is also the dominant individual in the colony. Theories have been developed to justify the success of such mother-daughter eusocial systems. Central among these theories is 'inclusive fitness' or 'kin selection' theory, which explains why workers act altruistically and cooperate for the good of the colony: they do so because they help their close relatives and thereby enhance the transmission to future generations of their own genes, if not directly then indirectly through the enhanced reproductive success of relatives.

The Sphecidae is a large family of wasps that is found across the world. Studies to date of the group have often been descriptive and anecdotal, with some species of sphecid wasps labelled as social or potentially eusocial. This is surprising as they are only distantly related to the ants, bees, vespid wasps and termites. Sphecid wasps would therefore represent an independent origin of eusociality in the insects. Yet the group has rarely been investigated. Since sphecid wasp societies comprise only small groups of females, they provide an ideal model to test hypotheses related to the evolution of social behaviour, especially the role therein of kin selection (inclusive fitness) in favouring the transition from solitary nesting to eusocial nesting. *Cerceris rubida* (Jurine) is a very small but social member of the Sphecidae. Females are active during the hot Mediterranean summer, dig tunnels in the soil and hunt small beetles to feed to their larvae. It was the focus of this project.

There were three specific objectives of the research of Dr Giovanetti:

- 1) to evaluate the degree to which nestmate females cooperate and share in worker activities such as foraging versus egoistic reproduction i.e. are they eusocial;
- 2) to determine whether nestmates are related i.e. could kin selection explain their sociality; and
- 3) to demonstrate experimentally the factors that modulate cooperation and reproductive division of labour, particularly relatedness, nestmate number (colony size), age, and size of individuals i.e. can we demonstrate experimentally that kin selection is important in favouring cooperation.

The fieldwork to address these objectives was undertaken at three Mediterranean sites and consisted of observations carried on in front of an aggregation of nests. She individually marked females and nests and recorded the behaviour of individuals during their period of activity across three summer seasons, 2008, 2009 and 2010. Data we collected included the number of provisioning trips wasps performed back and forth to their nest, if such provisioning trips were devoted to hunting beetles (and then coming back to the nest with a prey item), if wasps interacted with other con-specific females and how they interacted (aggression, tolerance). In each nest, we found up to 5 females, and nest-mates adopted a division of labour based on their reciprocal differences in size. These data suggest a division of labour amongst nestmates, a hallmark of eusociality. Yet many wasps even changed nest (and eventually task) during their lifespan, arguing against eusociality in the species (Giovanetti and Jacobi, manuscript submitted for publication).

Cerceris rubida clearly represents an excellent study system to investigate the central theory explaining social evolution, kin selection, and WD Hamilton's predictions of high relatedness among nest-mates in eusocial insects. To address this question, which was our second objective, we developed polymorphic microsatellite DNA markers for *C. rubida* (as used in human genetic 'fingerprinting' to detect criminals and to ascertain paternity/maternity) and genotyped individually marked wasp individuals from two Mediterranean populations, collected after observing their behaviour (the task they performed, their behaviour with other nest-mates or other females living close to their own nest). The laboratory work at Queen's University Belfast employed individual wasps that were collected in the field and preserved in alcohol. It consisted first in improvement of existing protocols to extract DNA from the samples collected in the field; the small size of the individual wasps caused initial problems. Once we had obtained DNA from wasps, we proceeded to the isolation of novel genetic (microsatellite DNA) markers and development of suitable polymerase chain reaction oligonucleotides, short stretches of synthetic DNA that are 100% homologous to *C. rubida* DNA and that can be used to amplify over one million-fold a specific stretch of *C. rubida* DNA, but no other organism's DNA. Finally, we tested our genetic markers (the PCR primers) on wasps, found those that were variable within the populations (i.e. which were polymorphic), and analysed collected wasp individuals with those polymorphic 'loci'.

Our genetic results show that *C. rubida* nestmates exhibit very low relatedness, with relatedness effectively no different from zero i.e. nestmates are unrelated; we prepare these results for publication (Giovanetti and Paxton, manuscript in preparation). This is a surprising result because it means that kin selection, the main theoretical paradigm for understanding social evolution, seems not to play a role in favouring social behaviour in this wasp.

These striking data resulting from the two-year Marie Curie Individual Grant to Dr. Giovanetti (SENSE; FP7-PEOPLE-2007-2-1-IEF; N° 220876) have been presented by Dr. Giovanetti to an international audience interested in social evolution in insects: the International Union for the Study of Social Insect (IUSSI), at their August 2010 conference, following on from her introduction of the project to the same audience two years before (IUSSI Conference 2008). This was the first study in which microsatellite DNA analysis has been undertaken with a social sphecid wasp, and her data greatly interested many outstanding researchers in this field. In fact, our striking results demonstrated a lack of relatedness among females sharing a nest and showing division of labour (Giovanetti and Paxton, manuscript in preparation). This is extremely intriguing from an evolutionary perspective; social behaviour may offer other selective benefits than those inclusive fitness benefits garnered through high genetic relatedness among nestmate females. Relatedness plays an important role in many social species, and Hamilton's theory of inclusive fitness had shed light on the possible reasons behind reproductive altruism. However, other factors are included in Hamilton's inclusive fitness theory (e.g. ecological, physiological and demographic factors), and their importance will probably emerge in future when addressing the evolution of unrelated helpers – as in *C. rubida*.

The major impacts of the results of this project are within the scientific community.

Dr. Manuela Giovanetti, recipient of the Marie Curie Individual Fellowship (FP7-PEOPLE-2007-2-1-IEF; ENV; Proposal N° 220876), currently at:
Università degli Studi di Napoli Federico II, Dept. ARBOPAVE, Via Università 100; 80055
PORTICI (NA), Italy.
Email address: manuela.giovanetti@gmail.com

Prof. Robert J. Paxton, Scientist in Charge of the Marie Curie Individual Fellowship (FP7-PEOPLE-2007-2-1-IEF; ENV; Proposal N° 220876), currently at:
Martin-Luther-Universität Halle-Wittenberg, Institute for Biology, Hoher Weg 8,
D-06099 Halle (Saale), Germany.
Email address: robert.paxton@zoologie.uni-halle.de

2. USE AND DISSEMINATION OF FOREGROUND

Section A (public) – DISSEMINATION MEASURES

POPULAR DISSEMINATION: At the beginning of the project, it was envisaged that fieldwork would have been carried out on Corsica. Due to unforeseen events (destruction of the field site by a 3rd party), the majority of the fieldwork was eventually carried out in a protected area of the Italian mainland near Mantova (Oasi Le Bine). Journalists of local newspapers were invited to interview Dr. Giovanetti. Two newspapers (La Gazzetta di Mantova and La Provincia) showed much interest. Journalists came at the protected area and Dr. Giovanetti illustrated to them the main goals of the project and replied to curiosities on Hymenopteran habits. Also a magazine (Corriere Vicentino) heard of her work and interviewed Dr. Giovanetti. She also provided a report in Italian for the Protected Area, to be posted in the future on their official website. Attached are copies of these three popular reports on the Madame Curie project (**Sense_Press_1_2008.pdf; Sense_Press_2_2008.pdf; Sense_Press_3_2008.pdf**).

SPECIALISED RESULTS: directly related to the research are two manuscripts (one on the methodology, another on the evolutionary significance of the results) in preparation and one has been submitted, for which we still await a reply from the editors and reviewers. Two additional manuscripts have been published by Dr Giovanetti that are indirectly related to the project.

MS in preparation: Giovanetti, M. & Paxton, R.J. Low relatedness among nestmates despite apparent reproductive division of labour in the social sphecid wasp *Cerceris rubida*. For *Behavioral Ecology and Sociobiology*.

MS in preparation: Giovanetti, M. & Paxton, R.J. Novel microsatellite loci for the social sphecid wasp *Cerceris rubida*. For *Molecular Ecology Resources*.

Submitted MS: Giovanetti, M. & Jacobi, B. Relation between body size and activity in a small social sphecid wasp, *Cerceris rubida*: thermal constraints or polyethism? Submitted to *Ethology, Ecology, Evolution*.

Attached as additional file: **Giovanetti_MS_2009.pdf**

MS in press: Aronne G, De Micco V, Giovanetti M, (2011) L'approccio interdisciplinare nello studio dell'impollinazione delle Angiosperme. *Informatore Botanico Italiano*, **43** (1): *in press*. Attached as additional file: **Giovanetti_MS_2011a.pdf**

Published MS: Giovanetti, M. & Aronne, G. (2011) Honey bee interest in flowers with anemophilous characteristics: first notes on handling time and routine on *Fraxinus ornus* and *Castanea sativa*. *Bulletin of Insectology* **64** (1): 77-82. ISSN 1721-8861.

Attached as additional file: **Giovanetti_MS_2011b.pdf**

Dr Giovanetti also attended 8 international conferences and 2 national conferences during the fellowship and presented 4 talks and 7 posters. In total, she actively participated (gave a talk or displayed a poster) at 8 of these 10 meetings.

Conference	Contribution & Authors
<i>International Union for the Study of Social Insects European quadrennial</i>	Talk 1: Polyethism in a sphecid wasp, <i>Cerceris rubida</i>: the importance of size. GIOVANETTI M., JACOBI B.

conference La-Roche-en-Ardenne, Belgium 30/August-03/September 2008	
3 rd European Bee Research Conference Belfast, UK 7-11 September 2008	<p>Poster 1. <i>Fraxinus ornus</i> L. as a helpful provider of pollen for springtime activity of honeybee foragers. GIOVANETTI M., ARONNE G. at symposium: Behaviour and physiology or Wild plant pollination or Bee products</p> <p>Poster 2. Sexual harassment by <i>Andrena agilissima</i> males of foraging females. GIOVANETTI M., SANTORO D. at symposium: Non-Apis bees</p>
Recent Advances in Conservation Genetics 2009 Smithsonian Tropical Research Institute, Panamá 12-31 January 2009	<p>Poster 3 and Talk 2. SENSE. Social Evolution: novel insights from the neglected sphecid wasps. GIOVANETTI M.</p> <p>Attached as additional file: Giovanetti_poster_CONGEN_2009.pdf</p>
E-Biosphere London, UK 1-3 June 2009	no contribution
European Society for Evolutionary Biology biennial conference Turin, Italy 24-29 August 2009	<p>Poster 4. Adoption of an aggregated nesting habit: relative importance of biotic and abiotic factors for a solitary wasp. GIOVANETTI M.</p> <p>Attached as additional file: Giovanetti_poster_ESEB_2009.pdf</p>
Monitoraggio della fenologia vegetativa e riproduttiva e sue applicazioni. Turin, Italy 28 June 2010	<p>Poster 5. Importanza della fenologia nell'interazione impollinatore-fiore: <i>Andrena agilissima</i> (Scopoli) e <i>Raphanus raphanistrum</i> L. GIOVANETTI M., PAPADIA C.</p>
EuroScience Open Forum Turin, Italy 2-7 July 2010	<p>Organiser and invited speaker (Talk 3) of the symposium: “Dual Career: how many computations to transform it into an advantage?”</p>
V European Conference on Behavioural Biology Ferrara, Italy 16-18 July 2010	no contribution
XVI Congress of the International Union for the Study of Social Insects Copenhagen, Denmark 8-14 August 2010	<p>Poster 6. Social evolution and relatedness in a sphecid wasps. GIOVANETTI M., PAXTON R.J.</p> <p>Attached as additional file: Giovanetti_poster_IUSSI_2010.pdf</p>
105° Congresso della Società Botanica Italiana Milan, Italy 25-28 August 2010	<p>Talk 4. Diving as a bee: flower morphology and function in the perspective of insects. GIOVANETTI M., DE MICCO V. ARONNE G.</p> <p>Poster 7. Attività dell'ape da miele su una specie androdioica</p>

	anemofila, <i>Fraxinus ornus</i> L. (Oleaceae). GIOVANETTI M., ARONNE G.
--	--

Additional files uploaded:

Manuscripts

Giovanetti_MS_2009.pdf

Giovanetti_MS_2011a.pdf

Giovanetti_MS_2011b.pdf

Posters

Giovanetti_poster_CONGEN_2009.pdf

Giovanetti_poster_ESEB_2009.pdf

Giovanetti_poster_IUSSI_2010.pdf

This complete file also uploaded as: **SENSE_Supplement_Final_Report.pdf**

Section B (confidential or public)

Patents do not arise from this project. Rather, the novel genetic (microsatellite DNA) markers developed in this project will be made publically available, as is demanded of such research by the scientific community and international peer-reviewed science journals.

Research Training Assessment

The principal training element of the fellowship involved molecular genetic methods of analysis, in which Dr. Giovanetti had little background. She proved to be quick and alert in the laboratory. She quickly mastered the necessary skills and techniques for molecular genetic analysis: DNA extraction, PCR, cloning, DNA hybridization, DNA sequencing and sequence analysis, PCR primer design, microsatellite amplification, use of autosequencer and use of software for DNA sequence and DNA fragment analysis). She also quickly understood the general principles and theoretical background to those methods. I am therefore very content with the training element of her fellowship. Her successful job interview and current academic job at the University of Naples as a researcher are testament to her quality and skills, both former and acquired within the MC fellowship.

Researchers Assessment

Dr. Manuela Giovanetti integrated well into my research group, she became an active member, contributing usefully to in-group seminars and interacting well with other PhDs and postdocs. She actively supervised one visiting PhD student and she vigorously engaged in public dissemination of her project to the public and the scientific community through participation in numerous conferences. She was acutely aware of the benefits of training to enhance her science profile and generic skills and, in addition to the training in genetic methods that her project necessarily entailed, she undertook several courses organised by Queen's University Belfast for staff training and development. Her science training in genetic methods progressed well.

Research Training Outcomes

PIEF-GA-2008-220876 (SENSE) Supplementary file to Final Report

Dr Giovanetti performed extremely well and I think her research training was extremely successful. One point of critique is the low number of papers that have arisen from the project, which is in large part due to the difficulties encountered in the field (the need to locate additional field sites during the 1st year of the project, the small number of wasps per field site), and the need to undertake field work at a long distance (in a different country) from where laboratory work was undertaken and where I was located.

Your opinion about Madame Curie Actions

Madame Curie Fellowships offer an excellent opportunity for up-and-coming academics to engage in high-profile research at an important transition time in their careers. I think the funding principle is excellent.

Suggestion for improvement: reduce bureaucracy so that academics can focus on research and training.