We are well into 2014 and I am convinced that for many of you a productive period lays ahead or is already in full development. The last weeks, several new publications were circulated among the TEAM members. These are proof of the fact that fruit fly research remains very active in the different regions covered by TEAM and beyond, but also of the various fields of research that are tackled by some of us. We have seen papers ranging from pheromone communication and chemosensory genes to host use patterns, trapping and attractant testing to name just a few topics. We hope you enjoy these periodic circulations of newly published research findings just as the in depth presentation of a particular research field in this newsletter. This time we bring you an invited paper by Daniel Hahn and Giancarlo Lopez-Martinez of, respectively, the University of Florida and the New Mexico State University, entitled: ‘Antioxidants and the oxygen effect: an example of how simple environmental treatments can alter insect physiology, reduce stress, and improve sterile insect performance’. Success in SIT (Sterile Insect Technique) all relates back to the fitness and competitiveness of your reared insects. It they perform well, compared to the wild flies, the impact of your SIT program will be considerably higher. Methodologies to improve the ‘quality’ of your reared product are, therefore, an important aspect of further SIT development. Daniel and Giancarlo discuss the effect that radiation has on the male performance, and in particular the impact of the free radicals produced by the irradiation. But more importantly they will also explain on how they conducted experiments to illustrate reducing that damage. I will not disclose the finer details of this research but leave it to the readers to discover this themselves by reading their paper…

Talking about new research findings, the place to be this year will be the 9th International Symposium of Fruit Flies of Economic Importance, which will take place in Bangkok (Thailand) from 12 to 16 May, less than a month from now. This four-yearly international gathering of fruit fly researchers is the ideal opportunity to get acquainted with the latest findings in a wide range of topics. With more than 380 people already registered, 30 oral presentations, more than 130 posters, several satellite and pre- and post-conference gatherings, it promises to be once again an excellent symposium, not to mention the fact that it will be great to meet old friends as well as ‘new kids on the block’. You will find some more detailed information on the program further in this newsletter. In any case I would like to take this opportunity to already thank both the Steering and the Organizing Committees for the energy and time invested in the preparation of this Symposium. For those who are still hesitant to register, don’t miss out on this opportunity!

In addition to this gathering, there will also be the 8th International Congress of Dipterology that will take place in Potsdam (near Berlin, Germany) this year, where a separate Tephritoidea session will be organized. And we are already slowly planning for the next TEAM meeting in 2016, to be held in Stellenbosch, South Africa. More news and information for this latter event will be provided in the forthcoming issues of the TEAM newsletter.

This issue further highlights some other new developments. For Africa, next to the ongoing Indian Ocean Regional TC project (co-ordinated by Rui Cardoso of IAEA), there are two new networking projects launched. One is an ERAfrica project co-ordinated by Aruna Manrakhan of Citrus Research International (South Africa); a second one is a Belgian Science Policy funded project co-ordinated by myself. These two new initiatives, the partners involved and their objectives, will be shortly presented. Then there is also the announcement of a newly developed electronic multi-entry identification key to African fruit flies, a tool that will hopefully make identification less cumbersome for the non-taxonomic specialist.

Finally we present Marco Falchetto, a young doctoral researcher from the University of Pavia, who just finished his PhD dissertation on the molecular analysis of chemosensory perception in the Mediterranean fruit fly. Marco also provides us with a summary of his research findings.

As always, we would kindly like to ask you to communicate to us any news or activities that you would like to see included in the next TEAM newsletter.

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ANTIOXIDANTS AND THE OXYGEN EFFECT: AN EXAMPLE OF HOW SIMPLE ENVIRONMENTAL TREATMENTS CAN ALTER INSECT PHYSIOLOGY, REDUCE STRESS, AND IMPROVE STERILE INSECT PERFORMANCE

The sterile insect technique (SIT) is a common tool used in area-wide integrated pest management programs for Tephritid flies, as well as other pest insects from tsetse flies to moths. In field release programs sterile males must be able to disperse, live long enough to find females, and successfully mate with those females. The performance of sterile males is absolutely critical for the success of SIT programs; especially SIT programs for Tephritid flies that have highly competitive lek mating systems, like the Mediterranean fruit fly Ceratitis capitata (medfly). For these lekking fly species the sexual competitiveness of mass-reared sterile males cannot be substantially less than that of wild males. Yet, sterile male performance, particularly sexual competitiveness against wild males and mating compatibility with wild females, can be diminished at many steps during the SIT process including: initial colonization, mass rearing, sterilization, shipping and handling to release facilities, treatment at release facilities, and eventually the process of field releases that may include chilling and transport before distribution by hand or by aircraft. Getting dropped out of an airplane must be stressful! Stresses at each of these levels have individually been shown to reduce male performance (Calkins and Parker 2005), but the compounding of several of these stresses together is of even greater concern for active field release programs. In an attempt to compensate for poor male performance some programs release enormous numbers of sterile males, but increasing over-flooding ratios can severely reduce the practicality and increase costs of SIT programs. Thus, stringent quality control practices are important for all SIT programs and additional research that improves sterile insect quality by reducing stresses incurred at any point in the multilayered SIT system are needed.

In most SIT programs insects are sterilized by ionizing radiation at a specific target dose delivered as gamma rays, x-rays, or an electron beam. At these target doses, ionizing radiation is very effective at generating the double-stranded DNA damage needed to produce random, dominant lethal mutations that render the offspring of irradiated insects unable to complete development. Irradiation creates double-strand DNA breaks through both direct transfer of energy breaking the covalent bonds between DNA nucleotides during irradiation and through the action of free radicals or other pro-oxidant molecules that may induce breaks during irradiation and for some time after. However, bombarding cells with sufficient irradiation to induce a high frequency of dominant lethal mutations also leads to substantial off target damage that substantially reduces sterile males performance (Bakari et al. 2005). One of the molecular consequences of exposing living organisms to ionizing radiation is the formation of free radicals, the most common being the superoxide anion that is formed when water in cells or body fluids is split by direct energy transfer (Olive 1998). Oxygen radicals and their reaction products, collectively termed reactive oxygen species (ROS), continue to damage cellular components (e.g., DNA, proteins, and membrane lipids) after the initial irradiation event. Oxidative damage associated with exposure to ionizing radiation is likely to be a major mediator of poor sterile insect performance in current SIT applications. The negative side effects of sterilization by irradiation on insect performance are so important that they are a major motivation for the development of genetic or transgenic approaches to inducing sterility (Schetelig and Handler 2012). Yet, other approaches to reducing off-target irradiation stress, including oxidative damage, exist and some are even simple, effective, and very affordable.

Sterile Insect Technique programs would benefit from manipulations that could limit off-target oxidative damage from irradiation while maintaining the double-stranded DNA damage necessary to induce sterility. A long history of irradiation research on vertebrate cell lines and in the context of human cancer research has repeatedly shown that substantial chromosomal damage occurs during the initial irradiation bout, but that oxidative damage to cellular components outside the nucleus, such as mitochondria or ribosomes in the cytosol, continues to damage cells for hours to days after irradiation (Wallace 1998, Kim et al. 2008). Thus, our group hypothesized that manipulations enhancing cellular defenses against oxidative stress could help to improve sterile insect performance by reducing some of the harmful off-target effects of irradiation, while maintaining sterility. Fortunately, one such manipulation, low-oxygen conditioning had been known to have beneficial effects on sterile insect performance for more than 40 years (Hooper and Katiyar 1971).

Numerous studies on medflies and other Tephritids have shown that males from late pupae in the pharate adult stage that are irradiated in either anoxic atmospheres (below 1 kPa O$_2$) or hypoxic atmospheres (below 10 kPa O$_2$) typically perform better in mating competitiveness tests, and often also other quality control parameters, than males from pupae irradiated in normal atmospheres (normoxia = 21 kPa O$_2$) while maintaining sterility at target doses (Hooper and Katiyar 1971, Sharp et al. 1975, Ohinita et al. 1976, Bakri et al. 2005, Nestel et al. 2007). These low-oxygen atmospheres can be induced by hermetically sealing insects in bags or other containers and either flushing these containers with gases like nitrogen or helium or simply allowing the insects to consume oxygen in the sealed container for some period of time prior to irradiation. Because low-oxygen conditioning is so effective at reducing the negative side effects of irradiation sterilization, the international fruit fly SIT quality control manual suggests that pupae be hermatically sealed and allowed to deplete environmental oxygen in packaging prior to irradiation and several major fruit fly production facilities, including the major medfly facility at El Pino, Guatemala, seal pupae in gas-tight bags allowing
them to develop a low-oxygen atmosphere prior to irradiation (FAO/IAEA/USDA 2003). However, not all tephritid fly SIT programs use low-oxygen conditioning prior to irradiation and SIT programs for other insects such as tsetse flies or moths also do not use low-oxygen conditioning despite some evidence for benefits (Mutika and Parker 2006, Robinson 1975). Two important barriers exist for more uniform adoption of low-oxygen conditioning prior to and during irradiation across SIT programs. First, insects tolerate low-oxygen environments much better than vertebrate animals and exposure to low-oxygen can be beneficial if the duration is short and insect metabolism is suppressed by keeping them cool. However, keeping insects hypoxic or anoxic for too long can cause steep declines in performance, especially if low-oxygen is coupled with high holding temperatures. Thus, low-oxygen conditioning treatments must be carefully parameterized with respect to temperature and holding time to prevent a beneficial treatment from becoming harmful. Second, although low-oxygen conditioning has been known to have organism-level benefits for more than 40 years, the physiological mechanisms underlying this beneficial response had not been elucidated. This gap in knowledge about the mechanisms underlying low-oxygen conditioning is also a barrier to further research and implementation in SIT programs.

Our recent work fills some of this gap in mechanistic knowledge about the low-oxygen effect. Several authors in the SIT literature have speculated that the low-oxygen effect is largely due to the removal of gaseous oxygen in the environment surrounding pupae so that fewer reactive oxygen species and other pro-oxidants are formed during the irradiation process. Radicals produced from gaseous oxygen probably do contribute to oxidative damage during irradiation sterilization of insects. However, much work on irradiating cells in the context of cancer biology has shown that the majority of reactive oxygen species and other pro-oxidants are derived from splitting water in the cytosol of cells or the surrounding medium rather than from gaseous oxygen (von Sonntag 1987, Wallace 1998, Kim et al. 2008). Thus, we expect that other mechanisms may contribute more to the protective effects of low-oxygen conditioning.

In a wide range of animal taxa, including invertebrates like insects and bivalves to vertebrates like fish and humans, when exposed to low-oxygen conditions cells express a conserved response that includes down-regulating mitochondrial metabolism and energy production and up-regulating a suite of cellular protective mechanisms including molecular chaperone proteins, small molecules metabolites like trehalose, and antioxidants. This conserved hypoxia-reperfusion response protects cells from the rush of reactive oxygen species and other pro-oxidants that would be produced by mitochondrial metabolism when tissues are re-perfused with oxygen; this response has been studied very well in several contexts in human health and disease including low-oxygen conditions associated with strokes, heart attacks, and cancer (Hermes-Lima and Zenteno-Savin 2002). In fact, conditioning with short bouts of hypoxia has been shown to lessen the negative impacts of simulated strokes or heart attacks in laboratory animals, and this approach of giving small bouts of hypoxia is even gaining some traction clinically in preventing second heart attacks in human patients (Sanchis-Gomar et al. 2012). Beyond vertebrate biomedical work, studies on the fly Drosophila melanogaster had previously shown that exposure to low oxygen up-regulated cellular protective mechanisms, including the transcription of several key antioxidant enzymes (Gorr et al. 2006, Liu et al. 2006). Thus, we predicted that SIT relevant regimes of low-oxygen conditioning prior to and during irradiation that had been previously shown to improve the performance of sterile males did so by up-regulating antioxidants and reducing oxidative damage to cellular components from reactive oxygen species and pro-oxidants.

Figure 1. Elevated antioxidant capacity in response to anoxia during treatment and recovery (A) was correlated with both isoforms of Superoxide dismutase (Cu-Zn SOD in B and Mn SOD in C) and Glutathione Peroxidase (D).

We tested our hypothesis in the Caribbean fruit fly, Anastrepha suspensa (caribfly), a species with a long history of SIT work in the context of previously active field release programs and as a model for flies with current SIT programs like medflies and the Mexican fruit fly, Anastrepha ludens. Exposing late caribfly pupae 48 hours prior to adult emergence to one hour of anoxia, generated by replacing the normal atmosphere with nitrogen, caused a substantial increase in total antioxidant activity by Trolox (Fig. 1a and Lopez-Martinez and Hahn 2012), a measure primarily of small molecule antioxidants like glutathione. Further consistent with our hypothesis, we also showed that low-oxygen conditioning increased the activity of two critical antioxidant enzymes, mitochondrial superoxide dismutase (MnSOD, Fig. 1c) and cytosolic glutathione peroxidase (GPx, Fig. 1d), but not cytosolic superoxide dismutase (Cu-ZnSOD, Fig. 1b), even in the absence of irradiation stress. Consistent with previous studies, we also showed that low-oxygen conditioning improved mating competitiveness in small-cage choice tests, while maintaining sterility. At the target dose for SIT of 70 Gy females preferred low-oxygen conditioned sterilized males 3 to 1 over males sterilized under ambient oxygen (Fig. 2). Furthermore, females mated equally with low-oxygen conditioned males sterilized with 70 Gy and unsterilized males that received no irradiation (Fig. 2). At higher doses of irradiation from 200-400 Gy low-oxygen conditioning also improved...
adult emergence and flight ability (Lopez-Martinez and Hahn 2012). In agreement with our observation of higher antioxidant activity, low-oxygen conditioned pupae irradiated at 70 Gy sustained lower levels of oxidative damage to lipids and proteins than pupae irradiated at 70 Gy in ambient air (Fig 3).

Figure 2. Anoxia-irradiated males mated significantly more than those irradiated in an oxygen environment (A), but were equally successful at mating as control unirradiated males (B).

In biomedicine, antioxidant activity and oxidative stress are most often associated with studies of longevity and healthspan, the ability to maintain activity and function into old age (Ames 1993) and there is a substantial history of using tephritid flies as models for longevity research (Carey et al. 2006, Papadopoulos et al. 2011). In a second study, we showed that sterile adult males from caribfly pupae that received low-oxygen conditioning during irradiation at the target dose for SIT (70 Gy) were indeed longer lived than sterile males that were irradiated in air, and that this effect of lifespan extension was even more pronounced at higher irradiation doses up to 400 Gy (Lopez-Martinez and Hahn 2014). Our previous work on antioxidants, oxidative damage, and mating competitiveness had been done in young males near the peak of their sexual activity (10 days old in caribflies, Lopez-Martinez and Hahn 2012). Subsequently, we showed that low-oxygen conditioned irradiated male caribflies also had less oxidative damage in old age (30 days old) and retained their mating competitiveness longer than pupae that were sterilized in air. When sterile males were 30 days old, young 10 day old females preferred low-oxygen conditioned males 9 to 1 over males sterilized in air. Thus, low-oxygen conditioning has the potential to not only improve the performance of young sterile flies, but also to increase the healthspan of sterile flies. The economy and efficacy of SIT programs could be improved if sterile males could maintain their performance, particularly their mating competitiveness, longer in the field.

Figure 3. Anoxia-irradiated males mated significantly more than those irradiated in an oxygen environment (A), but were equally successful at mating as control unirradiated males (B).

Recent companion work in an invasive lepidopteran pest that is a target for control using SIT in the Southeastern United States, the cactus moth Cactoblastis cactorum, showed similar benefits of low-oxygen conditioning prior to and during irradiation (Lopez-Martinez et al. 2014). Male cactus moths are irradiated as adults at a target dose of 200 Gy to induce F1 sterility. Exposure to low-oxygen conditions also increased antioxidant activity in cactus moths and male cactus moths that received low-oxygen conditioning prior to and during irradiation had less oxidative damage to lipids and proteins, lived longer, flew farther, and were more sexually competitive in female choice tests than males irradiated in air (Lopez-Martinez et al. 2014). Treatments like low-oxygen conditioning that boost antioxidants and other cellular protective mechanisms may be even more important for SIT programs focused on lepidopteran pests because lepidopterans require higher doses of irradiation to induce F1 sterility than tephritids typically require to induce complete sterility, thus also probably inducing more off-target damage that could reduce sterile moth performance (Carpenter et al. 2005). Of note, the above studies linking cellular protective mechanisms with increased performance and longevity of irradiation sterilized males have been performed under relative stress-free laboratory conditions. Ultimately, we need strategies that improve the performance of sterile insects in the field. Ongoing
work in our laboratories is focused on both larger-scale tests of performance in field cages and in field releases.

From the perspective of physiological approaches to reduce the negative side-effects of irradiation sterilization on insects we would like to use approaches that maximize protection of the somatic components of cells while maximizing nuclear DNA damage, particularly in the germ-line tissues. In this work we have established a link between an environmental treatment that improves post irradiation sterile male performance, low-oxygen conditioning, and antioxidant activity in two cellular compartments outside the nucleus, the mitochondria and cytosol, as contributing physiological mechanisms. Yet, antioxidants are unlikely to be the whole story for the performance-enhancing effects of low-oxygen conditioning. Exposure to low-oxygen environments activates a suite of transcription factors, including the hypoxia-inducible factor (Gorr et al. 2006, Liu et al. 2006), that alter the transcription of numerous downstream genes that may play important roles in protecting somatic cellular components against irradiation-induced stress, including molecular chaperones and other small molecules like trehalose.

Beyond the off-target oxidative stress incurred during irradiation, there are many other points in SIT systems at which insects may lose performance. For example, other environmental manipulations like chilling during knockdown of emerged adults for distribution by aircraft or heating during the shipping process can also potentially induce oxidative stress that may degrade the performance of sterile insects (Calkins and Parker 2005, Shelly et al. 2013). Thus, treatments that increase antioxidants and other cellular protective mechanisms may reduce the performance loss from individual stressors and also the compounding of multiple stresses across a SIT system. Ongoing work in our laboratories is exploring whether manipulations of antioxidants or other stress protection mechanisms may apply to steps in the SIT process beyond irradiation. Approaches that boost antioxidants do indeed protect insects from performance losses at these other steps beyond irradiation, these approaches could also be applied to systems that may use genetic, transgenic, or paratransgenic methods of sterilization without irradiation in the future. Additional careful study from biochemical, cellular and whole organism perspectives is needed to identify other stress-resistance mechanisms that could be manipulated to improve the performance of sterilized insects during sterilization by irradiation and at multiple other stressful points in the SIT process, as well as novel ways to manipulate those candidate mechanisms.


References


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MOLeCuLAR AnALYSIS OF CHEMOSENSORY PERCePTION IN THE MEDITERRANEAN FRUIT FLY, CERATITIS CAPITATA

MARCO FALCHETTO
Dissertation for a Ph.D. in Cellular Biology,
Department of Biology and Biotechnology,
University of Pavia (Italy) 2013

The research activities of this Ph.D. focused on the implementation of the basic knowledge of the olfactory and reproductive biology of insect pest species of high agricultural and public health relevance, with the final aim of improving the current control methods, including those based on the Sterile Insect Technique.

In this study, the Mediterranean fruit fly, Ceratitis capitata (medfly), was used as model species, and the experimental approach, as well as the extensive molecular information made available in the recent years on this pest, was then transferred to the tsetse fly Glossina morsitans morsitans.

The medfly is an extremely important agricultural pest due to its polyphagy and its invasive potential (Liquido et al., 1991; Malaclrida et al., 2007). Chemosensory behaviour plays a key role in the detection of odors during host fruit localisation for oviposition and in the detection of pheromone cues during mate pursuit (Diaz-Fleischer et al., 2000; Eberhard 2000; Whittier et al., 1992). In insects, odour perception is regulated by a molecular pathway that involves multigene families including odorant-binding proteins (OBPs), chemosensory proteins (CSPs) and odorant receptors (ORs) (Leal 2013). Within the OBP family, pheromone binding proteins (PBPs) are proven to be involved in insect sexual communication, but the molecular basis underlying this process is still unknown for most Diptera insects and is currently the target of several research efforts.

The analysis of medfly EST libraries and the genome sequence resulted in the identification of a number of OBP genes (Gomulski et al., 2008, 2012; Scolari et al., 2012; Siciliano et al., 2014a). Further molecular characterisation of five identified putative OBP genes (CcapOBP69a, CcapOBP19d-1, CcapOBP83a-1, CcapOBP83a-2 and CcapOBP28a) suggested a possible implication of these genes in odorant perception and represented a first step in the elucidation of the molecular pathway regulating olfactory behaviours in the medfly (Siciliano et al., 2014a). Recently, one of these proteins (CcapOBP83a-2) was expressed and used to examine the relative binding ability of the pheromone components emitted by sexually mature medfly individuals during the “calling” period. CcapOBP83a-2 displayed high specificity for (E,E)-α-farnesene, one of the five major compounds in the medfly male pheromone emission (Siciliano et al., 2014b).

In this regard, one research line of this Ph.D. project focused on:

1) The phylogenetic analyses of the identified OBP predicted amino acid sequences in relation to Drosophila melanogaster but also the available putative OBPs from three other tephritid species, i.e. the Oriental fruit fly Bactrocera dorsalis s.s., the Northern walnut husk fly Rhagoletis suavis, and the apple maggot Rhagoletis pomonella. Particular attention was devoted to the subset of OBP genes that may represent PBP-related protein (PBPRP) candidates;

2) The assessment of the relative transcript abundances of five PBPRP candidates in the main olfactory organs of each sex;

3) The analysis of the effects of maturation/mating/time of day on transcript abundances in the antennae of each sex;

4) The expression of one of these PBPRPs (CcapOBP69a) in a heterologous system and the subsequent binding assays to assess the affinities for components of the male pheromone;

5) The determination of CcapOBP69a three-dimensional structure using X-ray crystallography and the exploration of folding similarities and
sequence differences in relation to the previously studied CcapOBP83a-2 and other known OBPs’ X-ray structures.

The seventeen identified medfly OBP transcripts were classified in Classic (13), Minus-C (3) and Plus-C (1) subfamilies and their tissue distributions assessed by RT-PCR. Then we studied their phylogenetic affinities with the *D. melanogaster* pheromone binding protein related proteins (PBPRPs) and the other currently available putative OBPs from tephritid species. Interestingly, the seven medfly putative PBPRPs we found (CcapOBP69a, CcapOBP83a-1, CcapOBP83a-2, CcapOBP19d-1, CcapOBP28a, CcapOBP84a-1 and CcapOBP84a-2) are distributed in five well distinct clades together with sequences from the two *Rhagoletis* species. Each of these five clusters includes sequences sharing high similarity to the *Drosophila* DmelOBP69a/PBPRP1, DmelOBP83a/PBPRP3, DmelOBP19d/PBPRP2, DmelOBP28a/PBPRP5, and DmelOBP84a/PBPRP4 (Fig. 1).

![Figure 1. Phylogenetic relationships of tephritid OBP proteins. The unrooted maximum-likelihood (log likelihood =29096.49) tree was inferred using the Whelan and Goldman (2001) model and a discrete Gamma distribution and some invariable sites. Bootstrap values greater than 50% (1000 replications) are shown. Coloured circles indicate the different OBP subfamilies.](image)

Real Time quantitative PCR was then used to assess the relative transcript abundances of five of these putative medfly PBPRP genes in the antennae, maxillary palps and tarsi of virgin sexually mature males and females (Fig. 2). In both sexes, transcription is highest in the antennae for CcapOBP69a, CcapOBP19d-1, CcapOBP83a-1 and CcapOBP83a-2. CcapOBP83a-2 appears to be almost exclusively transcribed in the antennae, but the other three are also transcribed, at lower levels, in the palps (CcapOBP69a, CcapOBP19d-1, CcapOBP83a-1) and in the tarsi (CcapOBP19d-1), with relatively higher transcript abundance in the females. CcapOBP28a is instead present in the antennae, but is more abundant in the palps and tarsi, suggesting that this gene may have a biological role in all three tissues. Antennae are known to be the main olfactory tissues in the medfly (Bigiani et al., 1989). We thus determined the impact of maturation and mating on the transcript abundances of the five putative PBPRP genes in this tissue. A trend of increasing transcript abundance is evident as a consequence of female maturation for CcapOBP69a, CcapOBP19d-1, CcapOBP83a-1 and CcapOBP83a-2 (Fig. 3). Conversely, in males the only gene that displays a change in transcript abundance during maturation is CcapOBP83a-2 (unpaired t-test, P<0.05). None of the five genes appear to be modulated by mating in the females, whereas in the males there is a general trend of decreased transcriptional activity although this is significant only for CcapOBP69a. Finally, CcapOBP28a displayed a slight, but insignificant, reduction in transcript abundance related to maturation in both sexes and was not affected by mating.

![Figure 2. Transcript abundances of five OBP genes in the antennae, palps and tarsi of mature virgin males and females. Asterisks indicate significant differences in transcript abundances (*P<0.05, **P<0.01, ***P<0.001, unpaired 2-tailed t-tests with Sidák’s correction for multiple comparisons).](image)

In our insectary conditions, medfly display a daily bimodal pattern of sexual activity, with one peak at about 08:00–11:00 hrs and a second minor peak at about 13:00–16:00 hrs. To evaluate whether the transcriptional activities of the five putative PBPRP genes were similar during the two peaks, Real-Time quantitative PCR assays were performed on RNA from
antennae collected from sexually mature virgin individuals of both sexes at 09:00 and 14:00. We found a general trend of decreased transcript abundance in the afternoon compared to the morning in both sexes, with the exception of CcapOBP83a-1 in females. Interestingly, the reduction in transcript levels in the afternoon was statistically significant only for CcapOBP69a in male individuals (unpaired t-test, P=0.027).

Based on the above described results, we chose CcapOBP69a for protein purification and relative binding studies aimed at the identification of its binding specificity to one or more of the medfly emitted chemicals involved in inter and intra-sex communication. This because CcapOBP69a displayed particularly important features that support its potential role in chemical perception. First, it is enriched primarily in the antennae, known to play a crucial role in intra- and inter-sex communication during courtship behavior (Nakagawa et al., 1973), and in the maxillary palps. Second, we know that medfly females reach sexual maturation two to three days after emergence, becoming receptive to the male sexual signaling for copulation, whereas males become sexually mature shortly after eclosion (Kaspi et al., 2002; Fletcher 1989). Thus the significant increase in CcapOBP69a transcript abundance in four day-old compared to one day-old females may be consequent to the synthesis of the molecular components required for mate recognition. Conversely, one day-old males are already able to mate and, as expected, we do not observe further increases in transcript abundance between one and four-day-old males. This is in accordance to the finding that sexual maturation itself induces profound transcriptional changes in adult medfly females and modest variations in males (Gomulski et al., 2012). Third, after mating, CcapOBP69a transcript abundance remained unaltered in females, possibly because they may employ this OBP to detect volatile host plant emissions for the localization of ripening/ripe fruits suitable for oviposition (Papadopoulos et al., 2006; Levinson et al., 2003). Instead, the significant reduction in transcript abundance in mated males may be related to the necessity to reallocate resources to restore depleted reserves prior to further courtship activities (Papadopoulos et al., 2010; Shelly et al., 2002). Finally, the decreased, although significant only in males, transcript abundance observed during the afternoon peak of male calling (pheromone release), compared to the morning peak, may reflect the reduction in afternoon calling activity observed in our laboratory.

Binding assays are now in progress, as well as the determination of CcapOBP69a three-dimensional structure using X-ray crystallography (Falchetto et al., in preparation). Since OBPs/PBPRPs are involved in the regulation of species-sex-specific behaviours related to host/mate location, the knowledge acquired for the medfly and other highly invasive tephritids will help develop novel species-specific attractants repellents for pest control programmes. The Sterile Insect Technique will particularly benefit from the development of new effective lures and traps. A deeper understanding of the three dimensional structures of olfactory proteins will enable the synthesis of molecules able to bind with higher affinity to the protein/s binding pockets, ultimately providing agents able to disrupt mating or oviposition behaviour.

Figure 3. Transcript abundances of five OBP genes in the antennae of 1 day immature (1 dV), 4 day mature virgin (4 dV) and 4 day-old mated (4 dM) males and females. Asterisks indicate significant differences in transcript abundances (*P<0.05, **P<0.01, unpaired 2-tailed t-tests).

References


Marco Falchetto initiated his PhD studies in October 2010 under the supervision of Professor Giuliano Gasperi, at the Laboratory of Insect Evolutionary Molecular Biology, Department of Biology and Biotechnology, University of Pavia (Italy). On December 2013, he successfully defended his PhD Thesis entitled “Novel molecular tools for the control of insect pest species of agricultural and public health importance”.

During his PhD, he worked on a project that aimed at acquiring further knowledge of the molecular mechanisms involved in odour and pheromone perception both in the medfly Ceratitis capitata, and in the tsetse fly Glossina morsitans morsitans. The long term goal of these studies is the identification of natural ligands of key chemosensory genes for the development of potential useful targets/tools to implement the current control strategies against these pests.

Dr. Falchetto’s research was partially supported by funding from the Italian Ministry of Education, University and Research (PRIN 20077RCHRW, awarded to Dr. Ludvik M. Gomulski). During the course of his PhD studies, Dr. Falchetto collaborated with the Department of Biological Chemistry, Rothamsted Research, Harpenden (United Kingdom), and worked as Postgraduate fellow at the Yale School of Public Health, Department of Epidemiology of Microbial Diseases (New Haven, CT, USA). He attended several training courses on bioinformatics, including ‘RNA-Seq data analysis using Bioinformatic tools’ (Vectorbase Workshop, University of Notre Dame, Indiana, USA, June 2013) and ‘Training school on NGS and arthropod symbiosis: from fundamental studies to pest and disease management’ (COST DSTG10010, Uppsala University, Sweden, April 2012).

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The 9th ISFFEI is approaching. As this newsletter is being prepared there are 388 Registered Participants with 9 Keynote speakers, 30 oral presentations and 137 posters. Several other activities will be held in tandem with the Symposium:

**Keynote speakers**

- **Anond Snidvongs** Applications of Geo-Informatics in Area-Wide Integrated Pest Management (Opening Speaker)
- **Donald McInnis** Can Polyphagous Tephritid Pest Populations Remain Undetectable over Years under Favorable Climatic and Host Conditions? (Session 1)
- **Phillip Taylor** The Tephritid Tardis: How Queensland Fruit Flies Escape in Time. (Session 2)
- **Massimiliano Virgilio** Led Zeppelin and the DNA Barcoding of Fruit Flies: “Stairway to heaven” or “Babe, I’m gonna leave you”? – A Pragmatic Approach Towards Workable Solutions. (Session 3)
- **Marc F. Schetelig** Past, Present and Future of Strain Development (Session 4)
- **Shanmugam Vijaysegaran** Bait Manufactured from Beer Yeast Waste and Its Use for Fruit Fly Management (Session 5)
- **Aruna Manrakhan** Use of Male Annihilation Technique for control of pest species in the Bactrocera group on Mainland Africa (Session 6)
- **John Sivinski** Technical Competition and the Fate of Augmentative Biological Control (Session 7)
- **Daniel Hahn** Management of Dormancy: A Review and Discussion of Importance for Biological Control Programs Including SIT (Session 8)
- **Stephanie Bloem** Pest Risk Analysis for Economically Important Tephritidae: The Crossroads between Science, Plant Protection and Safe Trade (Session 9)

**Activities in tandem with the 9th ISFFEI**

1. Workshop on the Characterization of Symbions of Fruit Flies of Economic Importance via Bioinformatic Approaches

**Satellite workshops:**

1. Second Research Coordination Meeting on Use of Symbiotic Bacteria to reduce Mass-Rearing Costs and Increase mating success in Selected Fruit pests in Support of SIT Application
   - Place: Montien Riverside
   - Dates: 4-5 May 2014
   - Place: Montien Riverside
   - Dates: 13-15 May 2014
3. APPPC Regional Training workshop on Bactrocera fruit fly surveillance, taxonomy and identification and area-wide management.
   - Place: Montien Riverside
   - Dates: 9-11 May 2014

**Kick-off meetings**

- ERAfrica Networking Programs for Africa (co-ordinator A. Manrakhan, Citrus Research Institute)
- FRUITFLYNET Networking Program of the Belgian Science Policy (co-ordinator M. De Meyer, Royal Museum for Central Africa)

**Information**

Information about the symposium can be found in [http://www.fruitflythailand.com](http://www.fruitflythailand.com)

For questions you may contact

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AN ELECTRONIC MULTI-ENTRY WEBKEY FOR AFROTROPICAL FRUGIVOROUS FRUIT FLIES

In Africa, the cultivation of (sub-) tropical fruits and certain vegetables like tomatoes and cucurbits is seriously hampered by both indigenous and introduced fruit fly pests. Most of these belong to a few genera such as *Bactrocera*, *Ceratitis* and *Dacus*. In order to develop any research or control program, an accurate identification of the pest species is a prerequisite. The morphological identification of African tephritids (including representatives of those economically important genera) relies on the use of classical single-entry (dichotomous) keys. However, the technical and rather specific terminology used in single-entry keys is a serious obstacle for non-specialists who are not acquainted with tephritid morphology and taxonomy. Additionally, species identification through single-access keys inevitably fails whenever the user is not able to select any of the dichotomous character states (e.g. due to inadequate taxonomical expertise, lack of clarity of the key, damaged specimen, etc.).

The Royal Museum for Central Africa (RMCA), in collaboration with the Belgian Directorate-General for Development Cooperation (through framework agreement with RMCA) and with the International Atomic Energy Agency (IAEA - Vienna, project,16859) developed a multi-entry identification key for African fruit-infesting tephritids. Eight character matrices that were previously used to develop single-entry identification keys for African *Bactrocera* and *Dacus*, as well as for some smaller and closely related genera like *Capparimyia*, *Carpophthoromyia*, *Ceratitis*, *Neoceratitis*, *Perilampsis* and *Trirhithrum*, were imported into Lucid 3.5, a software package specifically designed for development of multi-entry keys, and optimised to allow multi-entry species identification.

The result is a key that includes a total of 394 fruit fly species and comprises an initial key for genus identification as well as individual keys to all African representatives of eight genera (Bactrocera, Capparimyia, Carpophthoromyia, Ceratitis, Dacus, Neoceratitis, Perilampsis, Trirhithrum). These are based upon separate character sets for each genus (range 11-95 characters, 22-280 character states). Images and drawings are provided for (1) characters (showing where the character is found and how it looks like), (2) character states (showing the degree of morphological variability of character states) and (3) species (providing information about species morphological features). A formal description is provided for each species based upon the published literature. Information regarding the taxonomic status, host plant range, geographic distribution and collection specimens for species is available through hyperlinks to Encyclopedia of Life and to the Belgian Biodiversity Platform (as Belgian portal to GBIF). Links to the Barcoding of Life Database website (BOLD) allow verifying the availability and geographical sampling of DNA barcodes for each species.

The key is either accessible through the internet (http://keys.lucidcentral.org/keys/v3/fruitflies/) or it can be freely installed on PC/Mac (installers available upon request to the authors).

For further information, you can contact Massimiliano Virgilio
massimiliano.virgilio@africamuseum.be
In the previous newsletter (Nr 13, 2013) Mervyn Mansell of the University of Pretoria (South Africa) presented a network of fruit fly surveys in southern Africa (Malawi, Swaziland, Zimbabwe with possible extension to Botswana and Mozambique) funded by Trademark, Southern Africa (an implementation agency for the British Department for International Development). At the beginning of 2014, two new networking programs with partners in Europe and Africa were approved for funding. Although each of them is independent, there are some linkages and similarities between them both with regard to the objectives as well as the regional scope. Also, they could be of interest to other individuals or institutions in those regions, who would like to be informed on the developments of the projects. We, therefore, present briefly the programs and the contact points.

**Surveillance trap**

There is an ERAfrica project on fruit flies that just has been approved. ERAfrica itself is a European Union initiative aimed at promoting a unified European approach to collaborating with Africa in the field of science and technology research for innovation and sustainable development. This particular fruit fly project is funded by different national agencies and provides a network between European and African partners. It is co-ordinated by Aruna Manrakhan of Citrus Research International, CRI (South Africa). Its main objectives are improving detection and monitoring methods and identification of fruit fly pests in Africa and the Indian Ocean region. Partners in this are Reunion (CIRAD), Ivory Coast (Centre National de Recherche Agronomique-CNRA), South Africa (CRI) and Belgium, Tervuren (Royal Museum for Central Africa- RMCA). This three year collaborative project is due to start not later than 1 June 2014 with a first meeting being planned at the International Fruit Fly Symposium in Bangkok in May 2014. Trapping surveys with new and standard attractants will be conducted over two years in South Africa and Ivory Coast. The CIRAD team in Reunion will focus on development of fruit volatiles for monitoring of female Afrotropical fruit flies which have a poor response towards currently available food-based attractants.

The second networking project is funded by BELSPO, the Belgian Science Policy (of the Belgian Federal Government). BELSPO wants to initiate development of networks between Federal Research Institutions (like the RMCA) and international partners. The RMCA will be co-ordinating a two year program, entitled ‘Monitoring Network for fruit flies in South-eastern Africa’ together with the Stellenbosch University (South Africa), the Sokoine University of Agriculture (Tanzania) and the Eduardo Mondlane University (Mozambique). All these institutions have ongoing monitoring activities in their own countries. These activities are diverse in nature and can be part of IPM program, detection surveys, long term monitoring, or experimental setups. The RMCA is involved in this on a one-by-one basis. The main objective of this network is to bring the different parties together, try to harmonize the different activities so that results can be compared, and facilitate further interactions between them. This will be done through a number of meetings over a period of two years that will include field visits, as well as international meetings where results can be presented. A kick-off meeting is also planned at the International Symposium in Bangkok.

**Trap preparation**

The RMCA team in Belgium will use specimens and information obtained from the trapping studies to test, modify and improve currently existing identification tools (multi-entry keys and barcode based identification methods).
Trap servicing

Both networks also have a clear regional and objective overlap with the Indian Ocean Regional TC project of the International Atomic Energy Agency (RAF5062) on preventing the introduction of exotic fruit fly species and implementing the control of existing species with the sterile insect technique and other suppression methods. This project is co-ordinated by Rui Cardoso of IAEA and among its objectives it deals with detection methods. Partners in this are Reunion, Mauritius, Seychelles, Madagascar, Tanzania, Mozambique and IAEA. It is, therefore, suggested that partners of the different networks meet up and discuss collaboration between them. Again, the forthcoming Symposium in Bangkok will be the ideal meeting place for this.

For further information on the networks, you can contact:

Aruna Manrakhan (CRI)
ERAfrica network
aruna@cri.co.za

Marc De Meyer (RMCA)
BELSPO fruitfly network.
demeyer@africamuseum.be

TRAVEL AWARD – UNIVERSITY OF THESSALY : MITRA MOEZIPOUR

The selection process has been completed and Dr. Mitra Moezipour has been selected for the travel award (short term research grant) supported by the 2nd TEAM meeting.

Mitra will work on a project regarding molecular and behavioral aspects of medfly oviposition in two laboratories of the University of Thessaly (Laboratory of Entomology and Agricultural Zoology, and Laboratory of Molecular Entomology).

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FORTHCOMING MEETINGS

9th INTERNATIONAL SYMPOSIUM ON FRUIT FLIES OF ECONOMIC IMPORTANCE
12-16 May 2014
Bangkok, Thailand
http://www.fruitflythailand.com/page/announcement

THE INTERNATIONAL CONFERENCE ON 'INSECTS TO FEED THE WORLD'
14-17 May 2014
Wageningen, The Netherlands

AN INTERNATIONAL SHORT COURSE IN INSECT CHEMICAL ECOLOGY
1 - 15 June 2014
Penn State University, Pennsylvania, USA
http://ento.psu.edu/events/international-short-course-in-insect-chemical-ecology

30th ANNUAL INTERNATIONAL SOCIETY OF CHEMICAL ECOLOGY MEETING
8-12 July 2014
University of Illinois, Urbana-Champaign, Illinois, USA
http://www.life.illinois.edu/isce-csiv/
THE 10TH EUROPEAN CONGRESS OF ENTOMOLOGY (ECE 2014)
3-8 August 2014
University of York, York, UK
http://www.royensoc.co.uk/meetings/20140803_ece2014.htm

8TH INTERNATIONAL CONGRESS OF DIPTEROLOGY
10-15 August 2014
Potsdam, Germany
http://www.icd8.org

XXV INTERNATIONAL CONGRESS OF ENTOMOLOGY (ICE 2016)
25-30 September 2016
Orlando, Florida, USA
http://www.ice2016orlando.org/

THIS NEWSLETTER

This newsletter is intended for the publication of subjects of interest to the members of TEAM. All content is solicited from the membership and should be addressed to the members of the editorial board.

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