

A STUDY OF GENETIC VARIATION IN POPULATIONS OF OLD WORLD SCREWORM FLY, *Chrysomya bezziana* (DIPTERA, CALLIPHORIDAE), FROM THE GULF REGION TO INDONESIA AND ITS IMPLICATIONS FOR CONTROL BY THE STERILE INSECT TECHNIQUE

(Studi Variasi Genetik pada Lalat *Chrysomya bezziana* (Old World Screwworm Fly) di Daerah Teluk dan Indonesia serta Implikasinya terhadap *Sterile Insect Technique*)

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ABSTRAK

Lalat *Chrysomya bezziana* (the Old World Screwworm Fly [OWSF], Diptera: Calliphoridae) adalah penyebab utama myiasis di daerah tropis. Lesi yang disebabkan oleh infestasi larva ini mengakibatkan ternak sangat menderita sehingga peternak mengalami kerugian ekonomis yang cukup besar. Karakteristik biologis OWSF identik dengan lalat *Cochliomyia hominivorax* (the New World Screwworm Fly). Sifat ini juga mirip dengan lalat daging, *Wohlfahrtia magnifica* (Diptera: Sarcophagidae) yang tersebar di wilayah mediteranian Basin, Benua Eropa dan Asia. Penyebaran lalat *W. magnifica* and OWSF yang semakin luas menjadi pemicu untuk mempelajari genetiknya. Analisis sekuen DNA mitokondria telah membuktikan adanya dua garis keturunan di dalam populasi *W. magnifica* di Eropa. Data ini memberikan dukungan yang kuat terhadap studi genetik OWSF yang menyimpulkan adanya dua ras di dalam populasi OWSF, yaitu ras sub-Saharan Africa dan ras wilayah teluk termasuk Asia. Penelitian terbaru saat ini difokuskan pada ras wilayah teluk dan Asia dengan melihat sekuen-sekuen yang lebih panjang pada salah satu gen di mitokondria dan inti sel. Sementara beberapa haplotipe yang sama telah tersebar di daerah teluk sampai Malaysia dan Hongkong, pulau-pulau di Indonesia menunjukkan haplotipe yang berbeda bahkan ada beberapa yang unik dan hanya ditemukan di pulau tertentu. Kondisi ini memberi kesan bahwa wilayah-wilayah tersebut tidak memungkinkan dijadikan tujuan untuk re-invasi jarak jauh selama percobaan aplikasi *Sterile Insect Technique* (SIT). Satu atau lebih banyak lagi pulau di Indonesia sangat memungkinkan untuk dijadikan kandidat pada saat percobaan SIT di lapang, terutama pada daerah-daerah yang nantinya menjadi penghalang terhadap penyebaran lalat ini ke Australia.

Kata Kunci: Lalat *Chrysomya bezziana*, Myiasis, DNA

ABSTRACT

The Old World Screwworm Fly (OWSF), *Chrysomya bezziana* (Diptera: Calliphoridae) is the major cause of traumatic myiasis in the tropical regions of the Old World. The lesions caused by its larval infestations on livestock caused great pain and suffering and result in considerable economic losses to farmers. The biology of OWSF is remarkably similar to that of the New World screwworm fly, *Cochliomyia hominivorax* (Diptera: Calliphoridae). It is also similar to a fleshfly, *Wohlfahrtia magnifica* (Diptera: Sarcophagidae), found in the Mediterranean Basin, continental Europe and Asia. There has been recent expansion of the distributions of *W. magnifica* and OWSF, stimulating comparative studies of their genetic status. Analyses of mitochondrial DNA sequences demonstrated two lineages of *W. magnifica* in Europe and gave strong support for recognizing two races of OWSF, one from sub-Saharan Africa and the other from the Gulf region and Asia. Recent studies have concentrated on the latter race, looking at longer mitochondrial gene sequences and nuclear gene sequences. While some haplotypes are shared in countries from the Gulf region to Malaysia and Hong Kong, the islands of Indonesia mostly show different haplotypes, some unique to individual islands. This suggests that these regions would not be subject to much long-distance re-invasion during a Sterile Insect Technique (SIT) trial and that one or more of the Indonesian islands would be a good candidate for an

SIT trial, especially where they could then become a buffer against potential movement of the species to Australia.

Key Words: *Chrysomya bezziana* Fly, Myiasis, DNA

INTRODUCTION

Infestations of living vertebrates, including humans, by fly larvae are called myiasis (HALL and WALL, 1995). The term 'screwworm' is commonly applied to two species of blowfly, the New World screwworm fly (NWSF), *Cochliomyia hominivorax* (Diptera: Calliphoridae) and the Old World Screwworm Fly (OWSF), *Chrysomya bezziana* (Diptera: Calliphoridae) (HALL, 2004). Both of these are obligate parasites as larvae, causing traumatic myiasis. They are unable to develop on carrion. A third fly species, a fleshfly named Wohlfahrt's wound myiasis fly, *Wohlfahrtia magnifica* (Diptera: Sarcophagidae), is a screwworm fly in every aspect except name (HALL and FARKAS, 2000). The three species represent a remarkable example of convergent evolution and share a number of physical, physiological and behavioural characteristics that fit them for a parasitic life style and differentiate them from their non-parasitic relatives. In addition, all three species have recently been involved in range extensions or in introductions to completely new areas, which have given rise to a need for control or eradication programmes (LINDQUIST *et al.*, 1992; SIDDIG *et al.*, 2005; SOTIRAKI *et al.*, 2005).

It is important to be aware of the genetic diversity of pest populations because of possible variation in relevant biological traits, such as insecticide resistance and mating compatibility. For example, the presence of one relatively invariant genetic race of OWSF throughout mainland Asia, if confirmed, will be of major practical significance for the sterilisation programme, because it would indicate that a mass-rearing facility could be started and replenished with just one local population of flies, and sterile males from this facility could then be released anywhere in Asia. Knowledge of the geographical make up of recently introduced species is also important

to determine how they arose so that the avenues of introduction can be shut down.

Previous research had demonstrated that OWSF occurs as two mitochondrial DNA races, one in sub-Saharan Africa and the other throughout Asia, from the Persian Gulf to Malaysia (HALL *et al.*, 2001). However, this conclusion was based on the analysis of only the last 279 base pairs (bp) of Cytochrome b (Cyt b), because of the difficulties of extracting amplifiable DNA from old specimens. In the current report, these findings were extended by a phylogeographic analysis of the last 717 bp of the 3' end of Cyt b, as well as 401 bp of the single-copy nuclear gene Elongation factor-1 alpha (EF-1 alpha). The aims of the study were: (i) to investigate the phylogeography of OWSF in Indonesia and the Gulf region and to use the results to infer the potential of OWSF to re-invade a region where there is a Sterile Insect Technique (SIT) programme; (ii) for comparison, to study the phylogeography of *W. magnifica* in outbreak situations of Morocco and Crete to determine their history and origins.

MATERIALS AND METHODS

General methods are given in HALL *et al.* (2001) and TESTA *et al.* (2002). Muscle blocks were dissected from 3 segments of individual larvae (cuticle removed), which had been stored in 80% ethanol. Alternatively, muscle was scraped from inside the thorax of individual adults, which had been dry-pinned or preserved in ethanol.

The DNazol method was used to extract total genomic DNA. DNA fragments were amplified by the polymerase chain reaction (PCR) and directly sequenced using the ABI BIG DYE Terminator Kit and 377 Automated Sequencer (TESTA *et al.*, 2002).

Sequences were aligned with Sequencher software (Gene Codes Corporation) and analysed phylogenetically with PAUP* 4.0 software (SWOFFORD, 2002).

RESULTS

Cytochrome b

Phylogenetic analysis was based on 717 bp of the 3' end of Cyt b, amplified by PCR as one fragment, or as two overlapping fragments if the DNA was degraded. Four OWSF lineages were discovered: lineage 5 in Iraq, Iran and Malaysia; lineage 6 in the Indonesian island of Sulawesi, and previously in Papua New Guinea (HALL *et al.*, 2001); lineage 7 in the Indonesian islands of Java and Sulawesi; and lineage 8 only in the Indonesian island of East Sumba. Further studies by one of us (A. Wardhana) extend this result in Indonesia revealing 13 haplotypes, with a clear separation (i.e. no shared haplotypes) between those populations in Sumatra and those on all islands to the east.

Pairwise genetic distances between these Cyt b lineages were 0.28 – 0.84% (or 2 – 6 out of 717 bp). Insect mitochondrial DNA diverges at the rate of 1.0 – 2.5% per million years and, therefore, the first lineage divergence of the OWS studied occurred 112,000 – 840,000 years ago.

Studies of outbreak populations in Morocco and Greece examined a similar sized fragment of Cyt b to that studied for OWSF. Specimens were found to belong to one of eleven unique mitochondrial DNA haplotypes. The Moroccan haplotypes belonged to the Cyt b lineage of Spain/France. There was a clear and statistically significant difference between the genetic composition of the central and northern faunas in Morocco. There were shared haplotypes, but each area had unique and rare haplotypes. Specimens from Crete belonged to one of two haplotypes of the central/eastern European lineage and differed by at least three nucleotide changes from the Moroccan haplotypes.

EF-1 alpha

Phylogenetic analysis was based on 401 bp, amplified by PCR as one fragment. This analysis was only undertaken with *C. bezziana*. Only 3 substitutions were discovered, all synonymous at third-base sites of deduced amino acid codons. There was no phylogeographic distribution of the 5 alleles

and 5 genotypes recognized from 25 individual OWS. One homozygous genotype predominated in all geographical populations.

DISCUSSION

Studies of *W. magnifica* in Europe and the Mediterranean Basin showed that there are two distinct lineages, one in the Iberian Peninsula and France, the other elsewhere in Europe, extending to Iran. All specimens from Crete were found to belong to the eastern lineage. Hence, it is clear that the outbreak populations of *W. magnifica* on Crete did not originate from Spain, an early hypothesis that was considered because of the importation of Spanish sheep. The analyses are consistent with the occurrence of *W. magnifica* on Crete being a recent event, supporting the reports of local farmers and veterinarians who claim not to have encountered it before 1999.

In contrast to the situation in Crete, studies in Morocco support the hypothesis that the recent increase in cases of myiasis due to *W. magnifica* results from a resurgence of historical endemic populations rather than an introduction. Analysis showed that Moroccan specimens belonged to one of six unique mt DNA haplotypes, all in the lineage of Spain/France. The genetic diversity was high in all areas examined. Had there been a recent introduction of *W. magnifica*, it would most likely have come from a single source, for example in a consignment of infested animals, with just one or two haplotypes. We found significant genetic differences in populations collected from the northern and central regions of Morocco, indicating that there are barriers to fly dispersal, the most obvious being the Rif mountains.

The reasons for the greater diversity of *W. magnifica* in Morocco compared to elsewhere in the Mediterranean Basin and in Europe deserve further study, because this could explain how the species has spread. It is possible that the degree of genetic diversity in any area could be linked to factors, such as animal trade, that can overcome natural barriers to pest dispersal. Thus, the commonality of haplotypes in areas as widely separated as Crete and Hungary could be a result of more extensive trade within Europe

compared to that within Morocco. *Wohlfahrtia magnifica* is certainly more widespread in Morocco than government veterinarians were until recently aware, but its genetic diversity is consistent with the species having been present in Morocco for a long time, where it maintains a low prevalence (< 1%). Conversely, on Crete there was a higher prevalence in livestock (up to 15%) and other evidence points to it being a genuinely recent introduction.

The results of the cytochrome b analysis of OWSF indicate that there is a single species in Indonesia and the Gulf and that this shows strong phylogeographic structure, with DNA lineages and haplotypes being markers for regional populations. This is a very similar result to that for *W. magnifica* although factors involved in creation of the regional diversity might be the same or different for the two species, e.g., glacial events for *W. magnifica* (e.g. Schmitt, 2007), mainly island events for OWSF. Malaysia and the Gulf shared the same Cyt b lineage of OWSF and one haplotype. The lineages diverged <1 million years ago. The results suggest that long-distance natural re-invasion during a SIT programme is unlikely between the OWSF populations from the Gulf, Malaysia and Indonesia. The more likely threat is of human assisted reintroductions. The results with EF-1 alpha indicate that there is a single species of OWSF in Indonesia and the Gulf and that EF-1 alpha provides a useful marker for the species *C. bezziana*, compared with the widespread *C. albiceps* and *C. megacephala*.

WALLACE (1892) made the observation that, "In terms of biogeography, Java differs from Sumatra, Borneo and the Malay Peninsula far more than either of the latter three do from each other". Our results fit in with this interpretation of the regional biogeographical lines of separation, with Java samples of OWSF being distinct from those in Sumatra and all other regions to the west.

Our results showed that some Indonesian islands have distinctive mt-DNA lineages, indicating that there has been little natural or human assisted dispersal of OWSF in the region. This is an encouraging result, which suggests that the chances of success of island by island eradication programmes are high. It also suggests that one or more of the Indonesian islands would be well suited to a

SIT trial and success in the region would act as a buffer against spread of OWSF to Australia. For both *W. magnifica* and OWSF, molecular studies have great value in planning for control and eradication programmes as well as in biosecurity programmes.

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REFERENCES

- HALL, M.J.R. 2004. New World screwworm (*Cochliomyia hominivorax*) and old world screwworm (*Chrysomya bezziana*). In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees), Fifth Edition, Volume 1. World Organisation for Animal Health, Paris, France. Chapter 2.2.8., pp. 370 – 379.
- HALL, M.J.R. and R. WALL. 1995 Myiasis of humans and domestic animals. *Advances in Parasitology* 35: 257 - 334.
- HALL, M.J.R. and R. FARKAS. 2000. Traumatic myiasis of humans and animals. In: PAPP, L. and B. DARVAS (Eds.). *Contributions to a Manual of Palaearctic Diptera*, Science Herald, Budapest. Chapter 1.18, pages 751 – 768..
- HALL, M.J.R., W. EDGE, J. TESTA, Z.J.O ADAMS and P.D. READY. 2001. Old World screwworm fly, *Chrysomya bezziana*, occurs as two geographical races. *Med. Vet. Entomol.* 15: 393 – 402.

- LINDQUIST, D.A., M. ABUSOWA and M.J.R. HALL. 1992. The New World screwworm fly in Libya: A review of its introduction and eradication. *Med. Vet. Entomol.* 6: 2 - 8.
- SCHMITT, T. 2007. Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. *Frontiers in Zoology.* 4: 11.
- SIDDIG, A., S. AL JOWARY, M. AL IZZI, J. HOPKINS, M.J.R. HALL and J. SLINGENBERGH. 2005. The seasonality of Old World screwworm (*Chrysomya bezziana*) in the Mesopotamia valley in Iraq. *Med. Vet. Entomol.* 19: 140 - 150.
- SOTIRAKI, S., A. STEFANAKIS, M.J.R. HALL, R. FARKAS and J.F. GRAF. 2005. Wohlfahrtiosis in sheep and the role of dicyclanil in its prevention. *Vet. Parasitol.* 131: 107 - 117.
- SWOFFORD, D.L. 2002. PAUP: *Phylogenetic Analysis Using Parsimony*, version PAUP*4.0. Smithsonian Institution Press, Washington DC.
- TESTA, J.M., J. MONTOYA-LERMA, H. CADENA, M. OVIEDO and P.D. READY. 2002. Molecular identification of vectors of *Leishmania* in Colombia: Mitochondrial introgression in the *Lutzomyia townsendi* series. *Acta Tropica.* 84: 205 - 218.
- WALLACE, A.E. 1892. *Island Life*. MacMillan and Company, London, New York. xx + 563 pp.