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First evidence of retained sexual capacity and survival in the pyrethroid resistant *Sitobion avenae* (F.) (Hemiptera: Aphididae) SA3 super-clone following exposure to a pyrethroid at current field-rate

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Abstract

The grain aphid* Sitobion avenae *is a prolific pest of cereal crops worldwide, controlled effectively with pyrethroid insecticides. However, the classic knock down resistance (*kdr*) mutation, L1014F on the* S. avenae *sodium channel gene, has been identified as the cause of the recently observed heterozygous (*kdr-SR*) resistance in the SA3 grain aphid super-clone. Results indicate that the kdr-SR SA3 clone can survive pyrethroid exposure above twice the normal field rate, continuing to reproduce thereafter. Additionally, the SA3 clone was found to be capable of producing sexual oviparous morphs, able to lay eggs following pyrethroid exposure. This demonstrates that possession of the L1014F mutation does not preclude the capacity to produce sexual morphs. This makes the adoption of an effective resistance management strategy imperative, within a wider integrated pest management (IPM) approach to control grain aphid.

Keywords

Grain aphid • pyrethroid • reproduction • resistance • SA3 clone

Introduction

Ireland's tillage sector focuses largely on the production of cereals primarily for use in animal feed, brewing and malting. Approximately 8% and around 300,000 ha of Ireland's agricultural land is dedicated to cereal production, producing between 2.0 and 2.5 million tonnes of cereal annually, approximately 1% of the total EU production (DAFM, 2015). With Ireland regarded as having one of the highest yields in the world (Oerke, 2006), the productivity of the Irish tillage sector is maintained through intensive management and high agricultural inputs. Pests are altogether responsible for cereal crop losses ranging between 26% and 50% (Oerke and Dehne, 2004) with insecticides used as the main crop protection measure against aphids in Ireland (DAFM, 2012). Specifically, pyrethroid insecticides have been widely used to control cereal aphids, favoured for their rapid knock down effect, low cost and low environmental risk (Elliott *et al.,* 1978). Aphids, as the foremost cereal pest, have been the main target of pyrethroid applications (Dewar *et al.,* 2016). Cereal aphids affect the crop indirectly by vectoring barley yellow dwarf virus (BYDV) and directly through feeding damage in addition to the production of honeydew, which leads to the build-up of sooty moulds that impact photosynthetic activity (Dedryver *et al.,* 2010). Research during the 1990s in Ireland identified the grain aphid *Sitobion avenae* (Fabricius) as Ireland's main cereal pest and significant research led to the development of chemical control strategies, mainly in the form of appropriately timed pyrethroid insecticide sprays, still currently used today, to manage this pest and its transmission of BYDV (Kennedy and Connery, 2005, Kennedy *et al.,* 2010).

Indications of pyrethroid control failure were first detected in Ireland in 2013. The failure of pyrethroid insecticides to control grain aphids is linked to the possession of a knock down resistance (*kdr*) mutation, L1014F on the sodium channel gene in heterozygous-resistant (*kdr-SR*) grain aphids (Foster *et al.,* 2014). Irish samples of *S. avenae* collected from fields with suspected pyrethroid control-failures in 2013 were confirmed to carry this same mutation (M. Gaffney, unpublished results).

Insecticide resistance alleles are also associated with other phenotypic characteristics and compensatory mutations. In natural ecosystems with multiple complex interacting factors, the possession of resistance genes are often associated with fitness costs (Scott, 2017). Such 'reduction in fitness' within a wider ecological framework can be manifested in terms of altered life table parameters resulting in reduced population growth capacity, as illustrated in the case of diamondback moth *Plutella xylostella* (Steinbach *et al.,* 2017) with longer larval development and pupal periods recorded in resistant strains. The consequences of resistance gene possession can be relatively subtle, leading to altered behavioural capacities. For

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example, a reduction in the ability of insecticide-resistant peach-potato aphids (*Myzus persicae*) to respond effectively to aphid alarm pheromone, and so escape from an attack by natural enemies (Foster *et al.,* 2007).

As a preliminary step towards better understanding, the extent and likely consequences of *kdr* in grain aphid populations, the current study was undertaken to quantify the relative capacity of the *S. avenae* to survive pyrethroid exposure, and reproduce.

Materials and Methods

Aphid collection, rearing and *kdr* testing

Single *S. avenae* apterae collected from individual winter barley fields from Counties Carlow, Cork, Louth and Wexford in Ireland, were identified using Blackman's key to the Aphidinae (Macrosiphini) (Blackman, 2010), and subsequently, maintained on spring barley plants *Hordeum vulgare* (var. Propino) in individual cages (21 x 21 x 21 cm) at 20 ± 1ºC with a 16:8 h photoperiod (light:dark), to establish 16-clonal lineages of asexually-reproducing aphids at long day-length conditions. The parent aphid from each lineage was removed and suspended in 50 µl of a 300 mM extraction buffer prepared as 0.3 M sucrose, 0.3 M NaCL, 60 mM Tris HCL pH 8, aligned to the Louis (1997) protocol for genomic DNA extraction, ahead of testing for the *kdr* mutation L1014F using a Taqman Polymerase Chain Reaction (PCR) assay (Foster *et al.,* 2014). Probe and primer sequences were provided by Rothamsted Research UK, where the analysis was carried out on an ABI 7900 HT RT-PCR system.

After rearing the individual clonal lineages in long day-length conditions for approximately 20 weeks, cages were transferred to an incubator at 16 ± 1ºC with a 12:12 h photoperiod for a further 6-week period, before being returned to long-day length conditions in preparation for testing.

Pyrethroid exposure

Aphids from individual colonies were exposed to a standard pyrethroid insecticide (analytical standard, Lambda(λ)-cyhalothrin PESTANAL® 31058, procured from Sigma-Aldrich) in 34 cm^2 glass vials coated with 500 µl of a pre-prepared acetone solution (technical grade Acetone, procured from Fisher Scientific, 10162180). An average of fifteen adult and/or late-nymphal instar apterous aphids from each of the 16 clonal lineages were placed in pre-coated vials held vertically in a constant light incubator at 18 ± 1ºC for 5 hours, while following the protocol of Foster *et al.* (2014). Three vial treatments were prepared with two replicate vials for each combination of aphid clone x pyrethroid treatment:

- Treatment 1 (untreated control) - vials prepared with 500 µl of acetone alone
- Treatment 2 (pyrethroid above standard field rate) - vials prepared with λ-cyhalothrin dissolved in 500 µl of acetone at a concentration of 75 ng/cm^2
- Treatment 3 (pyrethroid above twice the standard field rate) - vials prepared with λ-cyhalothrin dissolved in 500 µl of acetone at a concentration of 150 ng/cm^2

Following exposure, a single surviving aphid was selected from each vial treatment of respective heterozygote (*kdr*-SR) lineages and homozygote (*kdr*-SS) lineages exposed to Treatment 1 only, as no individuals from *kdr*-SS lineages survived pyrethroid exposure unaffected beyond the initial 5 h test period. Selected aphids transferred to a barley shoot in an Austin tube (Austin *et al.,* 1991), were maintained in long day-length conditions and observed daily over a period of up to 18 days.

Reproduction after pyrethroid exposure and confirmation of sexual reproduction

During the post-exposure period, several aphids from both *kdr*-SS and *kdr*-SR genotypes were observed to have produced viviparous nymphs or eggs in the Austin tubes. These were consistently removed and stored in 90% ethanol.

All surviving aphids were later viewed under the microscope to determine whether scent glands (pseudosensoria), indicative of oviparae (Favret and Miller, 2012), were present on their meta-tibiae. The legs of specimens were removed and preserved in 90% ethanol for later photography.

Confirmation of the *kdr*-status and the clonal identity of sexual specimens

A further Taqman PCR assay was used to confirm the *kdr*-status of all sexual aphid morphs. Genotypes of sexual *S. avenae* specimens from the study were then examined at five microsatellite loci: Sm10, Sm12, Sm17, SaΣ4 and S16b using the published primer pair sequences (Simon *et al.,* 1999, Llewellyn *et al.,* 2003, Wilson *et al.,* 2004) and a published protocol (Malloch *et al.,* 2016). Sequencing was carried out on an ABI 3730 DNA analyser at the James Hutton Institute with the results interpreted using GeneMapper® Applied Biosystems (2005).

Results

Kdr genotypes

The initial Taqman PCR assays detected the L1014F *kdr* mutation in seven of the 16 (44%) field-collected clonal lineages, confirming their possession of heterozygous pyrethroid knockdown resistance (*kdr*-SR). The other nine tested lineages (56%) were identified as fully pyrethroid-susceptible *kdr*-SS genotypes.

Survival and reproduction following the pyrethroid bioassay

Grain aphid survival was high in the *kdr*-SR group immediately following the λ-cyhalothrin bioassay with 204 out of 212 (96%) surviving the untreated control, 116 out of 200 (58%) surviving the 75 ng/cm^2 concentration, and 47 out of 204 (23%) surviving the 150 ng/cm^2 concentration. A summary of aphid survival and subsequent reproduction by observed individuals is provided in **Table 1**. In total, 29-adult apterous aphids were individually observed in tubes following the pyrethroid bioassay. Twenty of these individuals were representative of all the seven confirmed *kdr*-SR clones and nine aphids were from the confirmed *kdr*-SS lineages that had survived the untreated (acetone only) treatment. Eleven of the observed aphids (38%) were viviparae, eight (28%) were oviparae and observed to produce eggs with five of these being *kdr-SR* individuals, whilst 10 (34%) were not observed to reproduce during the experiment. Two of the observed aphids shed their exoskeleton to become alates during the observational period. Only one of these alate aphids was observed subsequently to reproduce, producing live nymphs.

Pseudosensoria were observed on the meta-tibiae of egg-laying aphids, with the exception of one damaged aphid, confirming their status as probable sexual oviparous forms. The *kdr*-status of all egg-laying females was confirmed as matching that of their original field-lineage. Microsatellite genotyping indicated that all three individuals derived from pyrethroid-susceptible, *kdr*-SS, lineages were the SA27 clone. All *kdr*-SR oviparae were determined to be the SA3 clone. The *kdr*-heterozygous SA3 clone was collected from widely separated locations within the main cereal-growing region of Ireland, in Counties Carlow, Cork and Wexford (**Table 2**).

Discussion

These data provide the first evidence that the widely occurring *kdr*-heterozygote SA3 clone can survive pyrethroid contact, and is able to continue reproducing parthenogenetically under laboratory conditions at a comparable rate to the unexposed individuals of the susceptible (*kdr*-SS) SA27 clone, potentially explaining observations of increasing insecticide failure in the

Table 1. Survival of observed aphids (in days) and their reproduction over a period of 14-days following exposure to the λ -cyhalothrin bioassay treatments

Kdr-genotype x pyrethroid treatment	(*n*)	Survival (days)			Nymphs produced			Eggs produced		
		mean	median	mode	(*n*)*	total	rate**	(*n*)*	total	rate**
kdr-SS x Acetone Control (0 ng/cm^2)	*9*	9	13	14	*4*	57	1.02	*3*	9	0.21
kdr-SR x Acetone Control (0 ng/cm^2)	*7*	14	14	14	*4*	34	0.61	*2*	4	0.14
kdr-SR x λ-cyhalothrin (75 ng/cm^2)	*6*	6	12	14	*1*	16	1.14	*2*	3	0.11
kdr-SR x λ-cyhalothrin (150 ng/cm^2)	*7*	8	11	14	*2*	10	0.36	*1^*	0	0.00

*observed aphids that produced either asexual nymphs or eggs, respectively.
**rate of reproduction calculated as the number of progeny per individual, per 14-days.
^reproduction occurred outside of the 14-day period.

Table 2. Field origin, genetic identities and treatment history of all *S. avenae* individuals with observed capacity to produce sexual forms and lay eggs following the pyrethroid bioassay

Field Location	Kdr Genotype	Oviparous capacity (following bioassay treatment)	Confirmed clonal genotype
Co. Carlow	*kdr*-SS	Yes (untreated control)	SA 27
Co. Cork	*kdr*-SR	Yes (untreated control)	SA3
Co. Cork	*kdr*-SR	Yes ([b]150 ng/cm^2)	SA3
Co. Carlow	*kdr*-SR	Yes (untreated control)	SA3
Co. Carlow	*kdr*-SR	Yes ([a]75 ng/cm^2)	SA3
Co. Carlow	*kdr*-SS	Yes (untreated control)	SA 27
Co. Louth	*kdr*-SS	Yes (untreated control)	SA 27
Co. Wexford	*kdr*-SR	Yes ([a]75 ng/cm^2)	SA3

[a]above standard field rate; [b]above twice standard field rate.

field against this pest. At present, alternatives to pyrethroids registered for use on cereal crops are limited in the British Isles. In practice, the options for the control of aphids and in particular, the control of barley yellow dwarf virus (BYDV) that they transmit (Kennedy and Connery, 2001) is limited to two compounds (neonicotinoids and pyrethroids). However, widespread concerns about the environmental impact of neonicotinoids (Godfray *et al.,* 2014) called into question their continued use and through the passing of EU legislation in 2018 (EU, 2018) has led to the withdrawal of three neonicotinoid actives for outdoor use.

The outcome of simulating temperature and light conditions commonly experienced in the field at the time of pyrethroid application, was the stimulation of oviparous development in both SA27 (susceptible) and SA3 (resistant) clones. It can be inferred from our observations that alate morphs and female oviparae were produced within the originally asexual colonies that led to the observation of retained sexual reproductive capacity in SA3 individuals surviving pyrethroid exposure. This unanticipated observation demonstrates that possession of the *kdr*-SR mutation (L1014F) does not preclude known sexual reproductive strategies in *S. avenae* (Simon *et al.,* 1999, Dedryver *et al.,* 2001). Sexual capacity retains the possibility of gene-flow through a range of (androcyclic, holocyclic and intermediate) breeding systems that primarily facilitate overwintering survival in harsher winter conditions, and also ensures the capacity to respond effectively to evolutionary pressures through natural selection (Dedryver *et al.,* 2001, Papura *et al.,* 2003, Loxdale and Lushai, 2007).

The potential for sexual crossing between *kdr*-heterozygote SA3 individuals in field populations greatly increases the likelihood of producing *kdr*-homozygotes among the offspring. The consequences of this in terms of resistance phenotype are not known at present since *kdr*-homozygotes have yet to be detected in the field (our unpublished results). However, it does seem likely that individuals carrying two mutated copies of the gene would show greater resistance than those with one mutated and one sensitive allele, as seen in other aphid species, such as *Myzus persicae* (Martinez-Torres *et al.,* 1999). The situation is complicated because *kdr* (and closely related super-*kdr* alleles) are recessive mutations in all species that have been studied in detail except for aphids, where heterozygotes are phenotypically resistant (Fontaine *et al.,* 2011, Foster *et al.,* 2014). For example, early studies on *kdr* in the housefly (*Musca domestica*) identified it as a recessive trait located on chromosome 3 (Farnham, 1977) and this was later confirmed by genetic crossing studies that linked resistance to the sodium channel gene in houseflies and German cockroaches (*Blattella germanica*) (Williamson *et al.,* 1993, Dong and Scott, 1994). Genetic studies in other insects, for example, *Plutella xylostella*, have also shown that this resistance trait is recessive (Schuler *et al.,* 1998). The reason that *kdr*-heterozygotes are found to be resistant in aphids is unclear, but may be connected to the unusual two-subunit structure of the sodium channel gene in aphids (Amey *et al.,* 2015).

Our observations on the survival, reproduction and retained sexual capacity in the SA3 clone, have major relevance to future strategies for controlling BYDV incidence, which is the driver of insecticide application to cereal crops. They suggest that a continued over-reliance on pyrethroid insecticides is likely to exacerbate existing resistance, possibly even provide further selection pressure for additional forms of pyrethroid resistance, such as super-*kdr* or metabolic-based mechanisms, and potentially generate homozygous *kdr* genotypes through sexual crossing between *kdr*-heterozygote males, if produced under autumn conditions, and oviparous females.

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References

Amey, J.S., O'Reilly, A.O., Burton, M.J., Puinean, A.M., Mellor, I.R., Duce, I.R., Field, L.M. Wallace, B.A., Williamson M.S. and Davies, T.G.E. 2015. An evolutionarily-unique heterodimeric voltage-gated cation channel found in aphids. *FEBS Letters* **589**: 598-607.

Applied Biosystems. 2005. GeneMapper® Software Version 4.0. Foster City, CA, USA.

Austin, A.B.M., Tatchell, G.M., Harrington R. and Bale, J.S. 1991. A method for rearing cereal aphids in a small space. *Entomologia Experimentalis Et Applicata* **61**: 91-93.

Blackman, R. 2010. 'Handbooks for the Identification of British insects: Aphids-Aphidinae (Macrosiphini)'. Royal Entomological Society, UK, 420 pages.

DAFM. 2012. 'Pesticide usage in Ireland'. Arable Crops Survey Report. Department of Agriculture Food and Marine (DAFM). Available online: http://www.pcs.agriculture.gov.ie/media/pesticides/content/sud/pesticidestatistics/Pesticde%20Usage%20-%202012%20Arable%20Survey%20Report.pdf [Accessed 14 July 2018], 92 pages.

DAFM. 2015. 'Food Wise 2025'. Available online: https://www.agriculture.gov.ie/media/migration/foodindustrydevelopmenttrademarkets/agri-foodandtheeconomy/foodwise2025/report/FoodWise2025.pdf [Accessed 14 July 2018], 102 pages.

Dedryver, C.-A., Hullé, M., Le Gallic, J.-F., Caillaud M.C. and Simon, J.-C. 2001. Coexistence in space and time of sexual and asexual populations of the cereal aphid *Sitobion avenae*. *Oecologia* **128**: 379-388.

Dedryver, C.-A., Le Ralec, A. and Fabre, F. 2010. The conflicting relationships between aphids and men: a review of aphid damage and control strategies. *Comptes Rendus Biologies* **333**: 539-553.

Dewar, A.M., Ferguson, A., Pell, J.K., Nicholls C. and Watts, J. 2016. 'A review of pest management in cereals and oilseed rape in the UK' Research Review No. 86. AHDB Cereals & Oilseeds, Kenilworth, UK. Available online: https://cereals.ahdb.org.uk/publications/2016/july/08/a-review-of-pest-management-in-cereals-and-oilseed-rape-in-the-uk.aspx [Accessed 14 July 2018], 249 pages.

Dong, K. and Scott, J.G. 1994. Linkage of *kdr*-type resistance and the para-homologous sodium-channel gene in german cockroaches (*Blattella germanica*). *Insect Biochemistry and Molecular Biology* **24**: 647-654.

Elliott, M., Janes, N.F. and Potter, C. 1978. The future of pyrethroids in insect control. *Annual Review of Entomology* **23**: 443-469.

EU. 2018. Legislation of the European Union L132. *Official Journal of the European Union* **61**: 31-40.

Farnham, A.W. 1977. Genetics of resistance of houseflies (*Musca domestica* L.) to pyrethroids. I. Knock-down resistance. *Pesticide Science* **8**: 631-636.

Favret, C. and Miller, G.L. 2012. 'AphID'. Identification Technology Program, CPHST, PPQ, APHIS, USDA; Fort Collins, CO. Available online: http://AphID.AphidNet.org/ [Accessed 15 May 2017].

Fontaine, S., Caddoux, L., Brazier, C., Bertho, C., Bertolla, P., Micoud A. and Roy, L. 2011. Uncommon associations in target resistance among French populations of *Myzus persicae* from oilseed rape crops. *Pest Management Science* **67**: 881-885.

Foster, S.P., Paul, V.L., Slater, R., Warren, A., Denholm, I., Field, L.M. and Williamson, M.S. 2014. A mutation (L1014F) in the voltage-gated sodium channel of the grain aphid, *Sitobion avenae*, is associated with resistance to pyrethroid insecticides. *Pest Management Science* **70**: 1249-1253.

Foster, S.P., Tomiczek, M., Thompson, R., Denholm, I., Poppy, G., Kraaijeveld, A.R. and Powell, W. 2007. Behavioural side-effects of insecticide resistance in aphids increase their vulnerability to parasitoid attack. *Animal Behaviour* **74**: 621-632.

Godfray, H.C.J., Blacquiere, T., Field, L.M., Hails, R.S., Petrokofsky, G., Potts, S.G., Raine, N.E., Vanbergen A.J. and McLean, A.R. 2014. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. *Proceedings of the Royal Society B* **281:** 20140558.

Kennedy, T. and Connery, J. 2001. Barley yellow dwarf virus in winter barley in Ireland: yield loss and timing of autumn aphicides in controlling the MAV-strain. *Irish Journal of Agricultural and Food Research*, **40**: 55-70.

Kennedy, T. and Connery, J. 2005. Grain yield reductions in spring barley due to barley yellow dwarf virus and aphid feeding. *Irish Journal of Agricultural and Food Research*, **44**: 111-128.

Kennedy, T., McDonald, J., Connery J. and Purvis, G. 2010. A comparison of the occurrence of aphids and barley yellow dwarf virus in minimum-till and conventional-till autumn-sown cereals. *The Journal of Agricultural Science* **148**: 407-419.

Llewellyn, K., Loxdale, H., Harrington, R., Brookes, C., Clark S. and Sunnucks, P. 2003. Migration and genetic structure of the grain aphid (*Sitobion avenae*) in Britain related to climate and clonal fluctuation as revealed using microsatellites. *Molecular Ecology* **12**: 21-34.

Louis, C. 1997. Isolation of genomic DNA. In: 'The Molecular Biology of Insect Disease Vectors', Springer, Dordrecht, pages 159-163.

Loxdale, H.D. and Lushai, G. 2007. Population genetic issues: the unfolding story using molecular markers. In: 'Aphids as Crop Pests' (eds. H.F. Van Emden and R. Harrington), CAB International, Oxfordshire, UK, pages 31-67.

Malloch, G., Foster, S. and Williamson, M. 2016. 'Monitoring pyrethroid resistance (kdr) and genetic diversity in UK populations of the grain aphid, *Sitobion avenae* during 2015'. AHDB-Potatoes Agriculture & Horticulture Development Board, Stoneleigh Park, Kenilworth, Warwickshire, UK, CV8 2TL. Available online https://potatoes.ahdb.org.uk/sites/default/files/publication_upload/R480%20Final%20Report_2015%20season.pdf [Accessed 14 July 2018], 25 pages.

Martinez-Torres, D., Foster, S.P., Field, L.M., Devonshire A.L. and Williamson, M.S. 1999. A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera : Aphididae). *Insect Molecular Biology* **8**: 339-346.

Oerke, E.-C. 2006. Crop losses to pests. *The Journal of Agricultural Science* **144**: 31-43.

Oerke, E.-C. and Dehne, H.-W. 2004. Safeguarding production—losses in major crops and the role of crop protection. *Crop Protection* **23**: 275-285.

Papura, D., Simon, J., Halkett F. and Delmotte, F. 2003. Predominance of sexual reproduction in Romanian populations of the aphid *Sitobion avenae* inferred from phenotypic and genetic structure. *Heredity* **90**: 397-404.

Schuler, T.H., Martinez-Torres, D., Thompson, A.J., Denholm, I., Devonshire, A.L., Duce I.R. and Williamson, M.S. 1998. Toxicological,

electrophysiological, and molecular characterisation of knock-down resistance to pyrethroid insecticides in the diamondback moth, *Plutella xylostella* (L.). *Pesticide Biochemistry and Physiology* **59**: 169-182.

Scott, J.G. 2017. Evolution of resistance to pyrethroid insecticides in *Musca domestica*. *Pest Management Science* **73**: 716-722.

Simon, J.C., Baumann, S., Sunnucks, P., Hebert, P., Pierre, J.S., Le Gallic, J.F. and Dedryver, C.-A. 1999. Reproductive mode and population genetic structure of the cereal aphid *Sitobion avenae* studied using phenotypic and microsatellite markers. *Molecular Ecology* **8**: 531-545.

Steinbach, D., Moritz G. and Nauen, R. 2017. Fitness costs and life table parameters of highly insecticide-resistant strains of *Plutella xylostella* (L.) (Lepidoptera : Plutellidae) at different temperatures. *Pest Management Science* **73**: 1789-1797.

Williamson, M.S., Denholm , I., Bell C.A. and Devonshire, A.L. 1993. Knockdown resistance (kdr) to DDT and pyrethroid insecticides maps to a sodium channel gene locus in the housefly (*Musca domestica*). *Molecular and General Genetics* **240**: 17-22.

Wilson, A.C., Massonnet, B., Simon, J.C., Prunier-Leterme, N., Dolatti, L., Llewellyn, K.S., Figueroa, C.C., Ramirez, C.C., Blackman, R.L. and Estoup, A. 2004. Cross-species amplification of microsatellite loci in aphids: assessment and application. *Molecular Ecology Resources* **4**: 104-109.