Inheritance of Resistance to Temephos in a Laboratory Selected Strain of the Potential Mosquito Vector of West Nile Virus (*Culex pipiens*) from Tunisia

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Abstract: The inheritance pattern of resistance was investigated in laboratory-induced temephos resistant and susceptible strains of *Culex pipiens*. The field population collected in Northwestern Tunisia was subjected to temephos selection and after 6 generations in the laboratory identified as Bou.tem6. Different genetic crosses were carried out between resistant and sensitive strains. The F1 offspring of the reciprocal crosses between the Bou.tem6 and S-Lab strains showed high levels of temephos resistance indicating that the resistant gene is completely dominant. The dose-mortality lines of reciprocal crosses to a sensitive colony showed the same projection and confirmed the autosomal inheritance of the resistance. Dose/mortality data for backcross showed that resistance to temephos is multifactorial. Our results were discussed in relation with previous studies.

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Keywords: *Culex pipiens*, temephos, inheritance of resistance, dominant, multigenic, autosomal.

1. Introduction:

Mosquito-borne diseases (malaria, hemorrhagic fevers, encephalitis, Filariasis) are the most important cause of mortality and morbidity, particularly in tropical areas (Horsfall, 1955; Service, 2003; Mullen, 2009). The prevention of these diseases is essentially based on the control of insect vector populations. Anti-vector control can be physical (sanitation), biological (use of pathogens or predators), genetic (introduction of sterile males) and/or chemical (synthetic or biological insecticides such as bacterial toxins). Although control with synthetic insecticides is not preferred, in many cases it remains the only possible approach (Davidson, 1964; Mukhopadhyay et al., 1993; Ben Cheikh et al., 1998; Bisset et al., 1999; Martinez-Torres et al., 1999; Corbel et al., 2007; Tantely et al., 2010; Toma et al., 2011; Jones et al., 2012; Pocquet et al., 2013; Tabbabi et al., 2016; 2017).

The resistance to organophosphate insecticides can be explained by mechanisms that prevent these insecticides from reaching their target, or reduced sensitivity of the target (AChE) (Raymond et al., 2001). Mosquitoes whose resistance is due to increased production of esterases have a genome characterized by amplification of the *Est-3* and *Est-2* genes. Genetic innovation consists of a gene amplification (Guillemaud et al., 1996; Guillemaud et al., 1997). The second mechanism corresponds to a modification of acetylcholinesterase encoded by the *Ace-1* gene in *Culex pipiens* (Scott, 1990; Feyereisen, 1995; Taylor and Feyereisen, 1996, Weill et al., 2002), whose mutation reduces its affinity for two major families of insecticides used in public health (organophosphates and Carbamates) and leads to resistance.

Understanding the mode of inheritance helps in resistance detection, monitoring, modeling, and risk assessment (Sayed et al., 2000). Hence, the present study aims to understand how the factors involved in resistance to temephos are transmitted by crossing the resistant strain established with the sensitive strain S-Lab. The hybrids (F1, F2) and the descendants of the backcross are tested by temephos and the resistances are compared to the parent strains.

2. Materials and Methods:

2.1. Mosquito Strains

One strain of mosquitoes was used in this study. The colony was derived from a collection of *Culex pipiens* eggs and larvae from Boussalem, Northwestern Tunisia, which was received in 2004. This colony was subjected to temephos selection and After 6 generations in the laboratory identified as Bou.tem6. A long-established laboratory reference strain, S-Lab was used for comparisons.

2.2. Insecticides

Two technical grade insecticides were used for selection and bioassay: the organophosphates temephos (9l‰; American Cyanamid, Princeton, NJ) and the

carbamate propoxur (997o; Mobay). Two synergists were used to help detect detoxification enzymes involved in resistance: S,S,S tributyl phosphorothioate (DEF), an esterase inhibitor, and piperonyl butoxide (PB), an inhibitor of mixed function oxidases.

2.3. Selection and Bioassay Procedures

In this study, we undertook a laboratory selection of the larvae of this population by exposing them to doses of temephos sparing only 50 to 75% of their numbers, with the aim of obtaining a strain as much as possible homozygous for the resistance character. This approach is useful for studying the mode of inheritance of resistance. Bioassay tests utilized standard methods (Georghiou et al., 1966; 1987). Data were subjected to probit analysis (Finney, 1971) using a BASIC program (Raymond, 1985). Resistance ratios were calculated at the median lethal concentration (LC50) and LC95 by comparing the estimated lethal concentration values of the Boussalem strains with those of the susceptible S-Lab strain. Synergism tests were similar to the bioassay tests except that 0.5 ml of the desired concentration of synergist was added to each cup, followed by the concentration of insecticide.

2.4. Esterase Assay

Total esterase activity in individual, frozen adult mosquitoes (3 days postemergence) from Bou.nat and Bou.tem6 strains was determined according to the method of Pasteur et al. (1981, 1988).

2.5. Inheritance of Temephos Resistance

To determine the mode of inheritance of the resistance genes, we crossed the homozygous strain for resistance to temephos previously described and the sensitive strain S-Lab as well as crosses F1xF1 and cross-tests F1xS-Lab. We isolated the nymphs individually in plastic tubes filled 1/3 with water and covered with a loose cotton tip to be able to identify the sex of the mosquito at hatching and ensure that females used are blank. At the emergence, the adults are sorted by sex. All the males of one strain are introduced into a cage with virgin females of the other strain and vice versa. This allowed us to make crosses in both directions.

3. Results:

The linearity of concentration-mortality curves was accepted (P<0.05) only for Bou.tem6. T, ♀Boutem6×♂SL.T, ♂Boutem6×♀SLF1.T and ♀F1Boutem6×♂SL.T (Table 1, Figure1).

Table 1: *Inheritance pattern of temephos resistance in Culex pipiens.*

Name of population	LD_{50} (a)	LD_{95} (a)	Slope (b)	H (Df)	RR_{50} (c)	RR_{95} (c)
S-Lab.T	0.0012 (0.0011-0.0014)	0.0062 (0.0047-0.0094)	2.34± (0.22)	1 (3)	-	-
Bou.tem6. T	0.1488 (0.0887-0.2586)	0.6513 (0.1578-3.4516)	2.56± (0.47)	3.04 (2)	119.64 (82.08-174.39)	103.95 (36.02-299.93)
♀Boutem6×♂SL.T	0.0220 (0.0051-0.0947)	0.4014 (0.0084-22.0954)	1.30± (0.37)	7.76 (2)	17.71 (10.60-29.58)	64.07 (16.19-253.55)
♂Boutem6×♀SL.T	0.0248 (0.0209-0.0295)	0.1625 (0.1205-0.2397)	2.01± (0.15)	1 (2)	19.95 (16.47-24.18)	25.93 (16.53-40.68)
♀Boutem6×♂SLF1.T	0.0034 (0.0028-0.0043)	0.0551 (0.0364-0.0951)	1.37± (0.10)	1 (3)	2.80 (2.35-3.34)	8.79 (5.66-13.67)
♂Boutem6×♀SLF1.T	0.0103 (0.0045-0.0234)	0.4221 (0.0644-2.8807)	1.02± (0.16)	5.92 (5)	8.32 (5.84-11.86)	67.36 (28.71-158.03)
♀F1Boutem6×♂SL.T	0.0032 (0.0015-0.0068)	0.0341 (0.0080-0.1518)	1.61± (0.27)	5.33 (3)	2.64 (1.73-40.1)	5.44 (2.23-13.31)
♂F1Boutem6×♀SL.T	0.0048 (0.0037-0.0065)	0.0938 (0.0487-0.2528)	1.27± (0.13)	1 (2)	3.88 (3.20-4.71)	14.97 (8.60-26.05)

Bou: Boussalem; nat: natural population; tem: temephos; S-Lab: reference strain;
(a) In mg/liter, 95% CI in parentheses.
(b) Standard errors in parentheses.
H: Heterogeneity, (df): testing linearity of the probit mortality/log dose response.
(c) RR, resistance ratio (LC50 of the population considered / LC50 of S-Lab); 95% CI in parentheses.

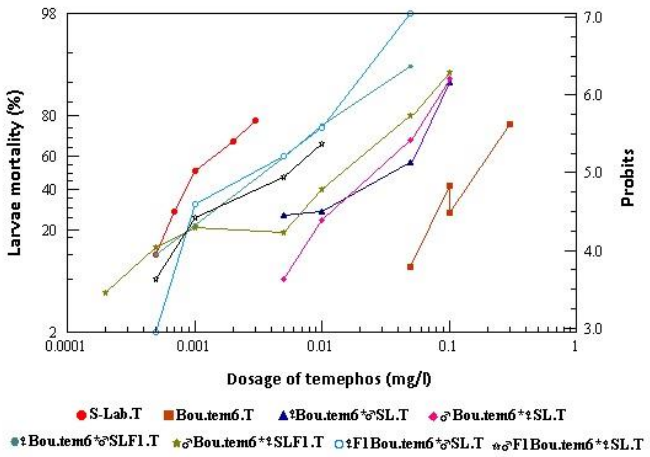

Figure 1. Curves of Inheritance pattern of temephos resistance in *Culex pipiens*.

Results of resistant and susceptible strains crosses of *Culex pipiens* are presented in Table 1 and Figure 1. The F1 offspring of the reciprocal crosses between the Bou.tem6 and S-Lab strains showed high levels of temephos resistance. The RR50 of ♀Bou.tem6♂×SL.T and ♂Bou.tem6♀×SL.T are 17.71 and 19.95 respectively. However, the RR50 of ♀F1Bou.tem6♂×SL.T and ♂F1Bou.tem6♀×SL.T are low (2.64 and 3.88 respectively). The dominance of the resistant character(s) is clearly confirmed.

Resistance was not significantly different between the reciprocal F1 families (Figure l). The dose-mortality lines ♀Bou.tem6♂×SL.T / ♂Bou.tem6♀×SL.T and ♀F1Bou.tem6♂×SL.T /♂F1Bou.tem6♀×SL.T showed the same projection and confirmed the autosomal inheritance of the resistance.

Analysis of the temephos dose-response line of the backcross offspring (♀Bou.tem6♂×SLF1.T and ♂Bou.tem6♀×SLF1.T) revealed a distribution with a distinct plateau over a range of concentrations. Our results showed the heterogeneity of tested phenotypes.

Our results showed that neither esterases (or GST) inhibited by DEF nor P450 cytochrome mediated monooxygenases inhibited by PB played a role in the observed resistance of Bou.tem6. *Culex pipiens* of Bou.tem6 showed resistance to Propoxur which indicates an evidence of acetylcholinesterase insensitive.

4. Discussion

Our study on the mode of inheritance of resistance genes showed that factors of resistance to organophosphates are autosomal. Note that according to Bourguet et al. (1996), there are two loci AChE in *Culex pipiens*, *Ace-1* is involved in resistance to organophosphates and is autosomal, and *Ace-2* whose role is unknown, but is very strongly linked to sex. In addition, Raymond et al. (1986) showed that resistance to propoxur is due to an insensitive AChE-1 (organophosphate target) that is encoded by an autosomal gene.

It seems from our results that there is a considerable intra-strain phenotypic heterogeneity (the curves of dose-mortality response are nonlinear (Figure 1). This confirms the hypothesis that resistance to temephos is rather multifactorial. This resistance was associated with acetylcholinesterase insensitive activity. Resistance controlled by a single gene develops and spreads much more rapidly when compared to polygenic resistance (Tabashnik, 1986; Roush and McKenzie, 1987).

On the other hand, enzymatic conjugation (esterases, GST, and oxidases) does not seem to be involved in the resistance of the studied strain. This coincides with previous studies by Pasteur et al. (1999) which suggested the existence of a new mechanism not known (which does not appear to be affected by the synergists) responsible for the high resistance recorded in Tunisia. Several studies found the same conclusion: all enzymes inhibited by DEF and PB synergists (esterases, oxidases) play a very weak role in this enormous resistance and the mechanism involved remains to be elucidated (Ben Cheikh, 2003; 2009; Tabbabi et al., 2016; 2017).

5. Conclusion

The comparison of the temephos resistance of larvae from the different crosses showed that this trait is dominant and autosomal. In addition, the addition of synergists to temephos showed the noninvolvement of the enzymatic conjugation in the resistance of the studied strain, which confirms the hypothesis mentioned above.

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Conflicts of Interest:

Authors declared no conflicts of interest.

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References :

1. Ben Cheikh, H., Ben Ali-Haouas, Z., Marquine, M. & Pasteur, N. (1998). Resistance to organophosphorus and pyrethroid insecticides in *Culex pipiens* (Diptera: Culicidae) from Tunisia. *J. Med. Entomol* 35(3), 251-260.

2. Ben Cheikh, R. (2003). Evolution et mode de transmission de la résistance aux insecticides chimiques chez des souches *Culex pipiens* soumises a une pression de sélection. Mémoire de DEA ; Université de Sousse, Tunisie.
3. Ben Cheikh, R., Berticat, C., Berthomieu, A., Pasteur. N., Ben Cheikh, H. and Weill, M. (2009). Genes conferring resistance to organophosphorus insecticides in *Culex pipiens* (Diptera: Culicidae) from Tunisia. *J Med Entomo*. 46(3), 523-30.
4. Bisset, J.A., Rodriguez, M.M., Diaz, C. & Alain Soca, L. (1999). Characterization of resistance to organophosphate insecticides, carbamates, and pyrethroids in *Culex quinquefasciaus* from the State of Miranda, Venzuela. *Rev Cubana Med Trop* 51(2), 89–94.
5. Bourguet, D., Raymond, M., Fournier, D., Malcolm, A.C., Toutant, J.P. and Arpagaus, M. (1996). Existance of two acetylcholinesterase in the mosquito *Culex pipiens* (Diptera: Culicidae). *J. Neurochem* 67(5), 2115-2123.
6. Corbel, V., N'Guessan, R., Brengues, C., Chandre, F., Djogbenou, L., Martin, T., Akogbéto, M., Hougard, J.M. & Rowland, M. (2007). Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, *West Africa. Acta Trop* 101, 207-216.
7. Davidson, G. (1964). DDT resistance and dieldrin resistance in *Cx. Pipiens fatigans*. *Ann Trop Med Parasitol* 58, 180–188.
8. Feyereisen, R. (1995). Molecular biology of insecticide resistance. *Toxicology Letters* 82-83, 83–90.
9. Finney, D.J. (1971). Probit Analysis. Cambridge University Press, Cambridge, UK.
10. Georghiou, G.P., Metcalf, R.L. & Gidden, E.E. (1996). Carbamate resistance in mosquitoes: selection of Culex pipiens fatigans Weidman (: Cx. quinquefasciatus Say) for resistance to Baygon. *Bull. WHO* 5, 691-708.
11. Georghiou, G.P., Wirth, M., Tran, H., Saume, E. & Knudsen, A.B. (1987). Potential for organophosphate resistance in Aedes aegypti (Diptera: Culicidae) in the Caribbean and neighboring Countries, *J. Med. Entomol* 24, 29O-294.
12. Guillemaud, T., Makate, N., Raymond, M., Hirst, B. and Callaghan, A. (1997). Esterase gene amplification in *Culex pipiens*. *Insect. Mol. Biol* 6(4), 319-327.
13. Guillemaud, T., Rooker, S., Pasteur, N. and Raymond, M. (1996). Testing the unique amplification event and the worldwide migration hypothesis of insecticide resistance genes with sequence data. *Heredity* 77, 535-543.
14. Horsfall, W.R. (1955). Mosquitoes: Their Bionomics and Relation to Disease. Hafner publishing, New York, pp.723.
15. Jones. C.M., Machin, C., Mohammed, K., Majambere, S., Ali, A.S., Khatib, B.O. & Kelly-Hope, L.A. (2012). Insecticide resistance in *Culex quinquefasciatus* from Zanzibar: implications for vector control programmes. *Parasit Vectors* 5, 78.
16. Martinez, Torres. D., Chevillon, C., Brun, Barale. A., Berge, J.B., Pasteur, N. & Pauron, D. (1999). Voltage dépendent Na+ channels in pyrethroid Résistant Cx. Pipiens L mosquitos. *Pestic Sci* 55, 1012–1020.
17. Mukhopadhyay, A.K., Sinha, S.N., Yadav, R.L. & Narasimham, M.V. (1993). Susceptibility status of Culex quinquefasciatus in Patna to insecticides. *Indian J Publ Health* 37(2), 57–60.
18. Mullen, G.R. & Durden, L. (2009). Medical and Veterinary Entomology, Mosquitoes (Culicidae) Woodbridge A. Foster and Edward D Walter. Vol. 2. Elsevier, Burlington, pp. 207–260.
19. Pasteur, N., Iseki, A. & Georghiou, G.P. (1981). Genetic and biochemical studies of the highly active esterases A′and B associated with organophosphate resistance in mosquitoes of the *Culex pipiens* complex. *Biochemical Genetics* 19, 909–919.
20. Pasteur, N., Marquine, M., Ben Cheikh, H., Bernard, C. and Bourguet, D. (1999). A new mechanism conferring unprecedented high resistance to chlorpyrifos in *Culex pipiens* (Diptera: Culicidae). *J. Med. Entomol*. 36(6), 794-802.
21. Pasteur, N., Pasteur, G., Bonhomme, F. & Britton-Davidian, J. (1988). Practical Isozyme Genetics. Ellis Horwood, Chichester, UK.
22. Pocquet, N., Milesi, P., Makoundou, P., Unal, S., Zumbo, B., Atyame, C., Darriet, F., Dehecq, J., Thiria, J., Bheecarry, A., Iyaloo, D., Weill, M., Chandre, F. & Labbé, P. (2013). Multiple Insecticide Resistances in the Disease Vector *Culex quinquefasciatus* from Western Indian Ocean. *PLoS One*. 8(10), e77855.
23. Raymond, M., Berticat, C., Weill, M., Pasteur, N. and Chevillon, C. (2001). Insecticide resistance in the mosquito *Culex pipiens*: what we have learned about adaptation? *Genetica*. 112-113.
24. Raymond, M., Foumier, D., Bergé, J.B., Cuany, A., Bride, J.M. & Pasteur, N. (1985). Single-mosquito test to determine genotypes with an acetylcholinesterase insensitive to inhibition to propoxur insecticide. *J. Am. Mosq. Control Assoc* l, 425-427.
25. Raymond, M., Fournier, D., Bride, J.M., Cuany, A., Bergé, J.B., Magnin, M. and Pasteur, N. (1986). Identification of resistance mechanisms in *Culex pipiens* (Diptera: Culicidae) from southern France: insensitive acetylcholinesterase and detoxifying oxidases. *J. Econ. Entomol.* 79, 1452.
26. Roush, R.T. & McKenzie, J.A. (1987). Ecological genetics of insecticide and acaricide resistance. *Annual Review of Entomology* 32, 361–380.

27. Sayyed, A.H., Haward, R., Herrero, S., Ferre, J. & Wright, D.J. (2000). Genetic and biochemical approach for characterization of resistance to *Bacillus thuringiensis* toxin Cry1Ac in a field population of the diamondback moth, *Plutella xylostella*," *Applied and Environmental Microbiology* 66(4), 1509–1516.
28. Scott, J.G. (1990). Investigating mechanisms of insecticide resistance: methods, strategies and pitfalls, pp. 39-57. *In* R.T. Roush and B.E. Tabashnik [eds.], Pesticide resistance in arthropods. Chapman & Hall, New York.
29. Service, M.W. (2003). Medical Entomology for Students. Vol. 3. United Kingdom: Cambridge. University Press, Cambridge.
30. Tabashnik, B.E. (1986). Computer stimulation as a tool for pesticide resistance management," in *Pesticide Resistance: Strategies and Tactics for Management*, pp. 195–203, National Academy Press, Washington, DC, USA.
31. Tabbabi, A., Daaboub, J., Laamari, A. & Ben Cheikh, H. (2016). New Esterases Amplification Involved in Organophosphate Resistance in *Culex Pipiens* Mosquitoes from Tunisia. *The Journal of Middle East and North Africa Sciences* 2(12), 1-2.
32. Tabbabi, A., Laamari, A., Daaboub, J., Ben Jha, I. & Ben Cheikh, H. (2017). Cross-Resistance to Pyrethroid and Organophosphorus Insecticides Induced by Selection with Temephos in the Potential Mosquito Vector of West Nile Virus (*Culex Pipiens)* from Tunisia. *The Journal of Middle East and North Africa Sciences* 3(3), 25-29.
33. Tantely, M.L., Tortosa, P., Alout, H., Berticat, C., Berthomieu, A., Rutee, A. & Weill, M. (2010). Insecticide resistance in Cx. pipiens quinquefasciatus and Aedes albopictus mosquitoes from La ReunIon Island. *Insect Biochem Mol Biol* 40, 317–324.
34. Taylor, M. & Feyereisen, R. (1996). Molecular biology and evolution of resistance to toxicants, *"Molecular Biology and Evolution* 13(6), 719–734.
35. Toma, L., Menegon, M., Romi, R., De Matthaeis, E., Montanari, M. & Severeni, C. (2011). Status of insecticide resistance in *Culex Pipiens* field populations from northeastern areas of Italy before the with drawl of OP compounds. *Pest Manag Sci* 67(1), 100–106.

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