and kept in colchicine-hypotonic solution for 20 min, fixed in acetic-ethanol, and permanent preparations were made according to the Imai squash method[6]. The staining has been performed by Feulgen reaction.

*Results.* The spermatogonial and oogonial metaphases of all reproductives from Sardinian and Apulian populations show a diploid complement of 42 chromosomes formed of 10 pairs of biarmed and 11 pairs of acrocentric chromosomes. No unequal pair to be regarded as heteromorphic chromosomes was found (figure, a). In about 2000 late diplotene and first meiotic metaphases of 6 males of Sardinian strain, the pairing configurations of chromosomes of all the meiotic figures show 17 bivalents and 4 bivalents linked in chain (figure, b). The chain should be a consequence of heterozygous reciprocal translocations between 4 pairs of chromosomes, 3 medium sized meta- or submetacentrics and 1, at the end of the chain, probably acro- or telocentric. The simplest interpretation of the observed meiotic association of 4 is obtained when the following sequence is assumed: $4 - 2^4 - 2 - 1^2 - 1\text{-}3^1 - 3 - 4^3$. In 6 out of 2000 first meiotic metaphases a ring was observed; the frequency is of the same order as that of a crossing-over in a short chromosomal segment. The ring is thus most likely the consequence of a chiasma at the short arms of the chromosome 4. About 1500 first meiotic metaphases of 6 males of Apulian strain were analyzed: 3 males show a set of 21 bivalents (figure, c); the other 3 show 19 bivalents plus an association of 2 bivalents aligned in chains (figure, d). The suggested sequence would be: $2^1 - 1 - 1^2 - 2$.

*Discussion.* The diploid complement of Italian populations of *R. lucifugus* is 2n = 42 for both males and females. The same complement was found in French populations, also for *R. santonensis* (Feytaud)[7]. No heteromorphic pair was found in either sex; however, mitotic chromosomes are tiny and small differences might have escaped observation. The analysis of male meiotic complements shows interchange complexes, different between and within populations. The Sardinian population displays a translocation heterozygosity involving 4 pairs of chromosomes. Among the analyzed Apulian males, 3 showed 21 bivalents and no translocation, 3 showed 19 bivalents plus an interchange complex of 2 pairs of chromosomes.

Translocation heterozygosities are common in plants[8] but rare in animals[9]. In animals they are believed to be associated with inbreeding as in roaches[10], or parthenogenesis as in some aphids species[11], or a balanced polymorphism as in certain marine snails[12]. Recently permanent segmental interchange complexes were discovered in males of *Incisitermes schwarzi* (Banks), *Kalotermes approximatus* Snyder and *Neotermes castaneus* (Burmeister), Kalotermitidae of Florida[13]; the authors suggest that the interchange complexes are related to sex chromosomes (neo Y). In *R. lucifugus* sex chromosomes in males were not discovered so far and the reciprocal translocations are probably autosomic. The differences of interchange complexes among Italian populations suggest a possible implication of this chromosomic mechanism in the geographic evolution also in relation to the budding mechanism of founding colonies[5], which increases the inbreeding. In this condition, reciprocal translocations may assure a balanced system with greater adaptive value.

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## Chlorpyrifos (Dursban®) resistance in *Culex pipiens pipiens* L. from Southern France: Inheritance and linkage

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*Summary.* In *Culex pipiens* mosquitos from Southern France, resistance to the organophosphorus insecticide chlorpyrifos is due to the dominant allele ($Chl^R$) of an autosomal gene. The *Chl* gene is localized between the *a-Gpd* and *Est-2* loci at 26.8 and 5.8 units of crossing-over respectively.

Pasteur and Sinègre[1] have described a very significant correlation between the frequency of an aliesterase isozyme, $Est\text{-}2^{0.64}$, and the degree of chlorpyrifos resistance in the natural populations of *Culex pipiens pipiens* from Southern France[2,3]. The present investigation was undertaken in order to determine whether $Est\text{-}2^{0.64}$ allele is itself responsible for the resistance, or whether these 2 factors are associated because of some linkage relationships.

*Material and methods.* 3 autogenous strains of *Culex pipiens* were used to perform the various crosses: SG, S7 and S5. The genetics of chlorpyrifos resistance was accomplished by mass crossing some 50 males with 50 virgin females of the desired genotypes, while the linkage relationships between resistance and the *Est-2* and *a-Gpd* loci were analyzed on the offspring of individual crosses. Chlorpyrifos resistance was tested on 3rd instar larvae[1] and *Est-2* and *a-Gpd* genotypes were analyzed by starch electrophoresis[4,5].

*Results.* 1. Heredity of chlorpyrifos resistance. The larvae of the SG and S7 strains (or their hybrids) are all killed by chlorpyrifos concentrations equal or higher than 0.0015 ppm, while those of the S5 strain are not affected by chlorpyrifos concentrations below 0.0030 ppm (table 1 and figure, A). The log-probit regression lines do not overlap and they are both very steep: the slope has a value of 11.9 for the susceptible S7 or SG strains and of 12.1 for the resistant S5 strain. The resistance ratio ($LD_{50}$ resistant/$LD_{50}$ susceptible) can be estimated to 0.0080/0.0008, i.e. 10fold. The regression lines as well as the $LD_{50}$ of both susceptible (S7 and SG) and resistant (S5) strains have remained unchanged for more than 2 years (30 generations), suggest-

Table 1. Heredity of chlorpyrifos resistance in *Culex pipiens* from Southern France. Percentages of mortality observed after 24 h expositions to different chlorpyrifos concentrations. (F, females, M, males)

| Crosses | % of mortality at chlorpyrifos concentrations (in ppm) of | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.0004 | 0.0006 | 0.0008 | 0.0012 | 0.0015 | 0.0030 | 0.0060 | 0.0120 | 0.0240 |
| 1. *SS*×*SS* | | | | | | | | | |
| F S7×M S7 | 0 | 0 | 58 | 96 | 100 | 100 | 100 | 100 | 100 |
| F SG×M SG | 2 | 4 | 37 | 100 | 100 | 100 | 100 | 100 | 100 |
| F S7×M SG | 1 | - | 56 | - | 100 | 100 | 100 | 100 | 100 |
| 2. *RR*×*RR* | | | | | | | | | |
| F S5×M S5 | 0 | - | 0 | - | 0 | 1 | 6 | 98 | 100 |
| 3. *SS*×*RR* | | | | | | | | | |
| F SG×M S5 | 0 | - | 0 | - | 0 | 0 | 26 | 100 | 100 |
| F S7×M S5 | - | - | - | - | 0 | 0 | 33 | 100 | 100 |
| F S5×M SG | - | - | - | - | 0 | 2 | 18 | 100 | 100 |
| 4. *SR*×*SR* | | | | | | | | | |
| F (FSG×MS5) × M (FSG×MS5) | 0 | - | 16 | - | 30 | 24 | 31 | 98 | 100 |
| F (FS5×MSG) × M (FS5×MSG) | 0 | - | 22 | - | 35 | 24 | 74 | 100 | 100 |
| 5. *SR*×*SS* | | | | | | | | | |
| F (FS7×MS5) × M SG | 1 | - | 33 | - | 56 | 47 | 77 | 100 | 100 |
| F SG × M (FS7×MS5) | 0 | - | 10 | - | 60 | 56 | 82 | 100 | 100 |
| F SG × M (FSG×MS5) | - | - | - | - | 52 | - | - | - | - |
| F SG × M (FS5×MSG) | - | - | - | - | 52 | - | - | - | - |
| 6. *SR*×*RR* | | | | | | | | | |
| F S5 × M (FS5×MSG) | - | - | - | - | 2 | 8 | 6 | 88 | - |

ing that as far as susceptibility to chlorpyrifos is concerned, the strains are homogenous. (We will use thereafter the term *SS* for the genotype of susceptible mosquitos, and *RR* for the genotype of resistant ones.)

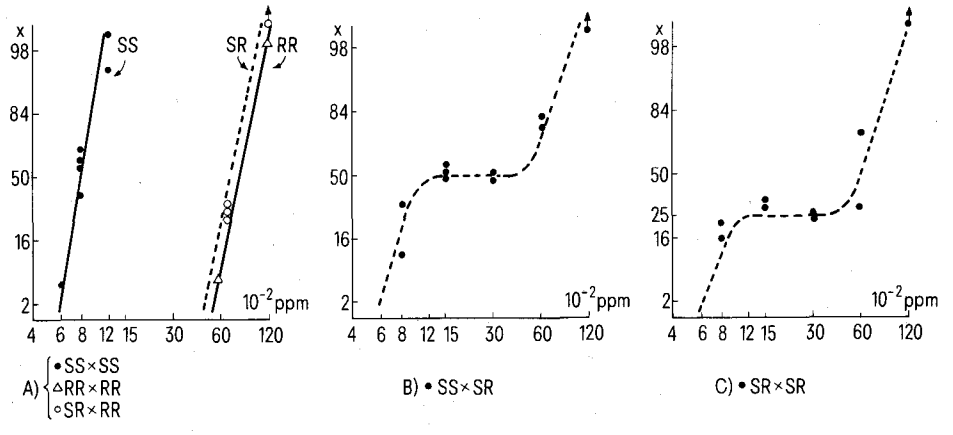
*SR* genotypes (i.e. the offspring of *SS*×*RR* crosses) demonstrate a mortality curve similar to that of the *RR* homozygotes (table 1, figure, A), although the mortality registered at 0.0060 ppm is somewhat higher (25 against 6%, $\chi^2 = 13.8$ for 1 df, $p < 0.001$). However, the mortality curves overlap over most of their range.

The offspring of *SR*×*RR* crosses behave in presence of chlorpyrifos as do *RR* homozygotes, while those of *SR*×*SS* and *SR*×*SR* crosses demonstrate a plateau around 50 and 25% mortality respectively (table 1 and figure, B and C). These plateaus correspond to chlorpyrifos concentrations which induce the death of all *SS* genotypes while all *SR* or *RR* genotypes remain alive. These mortalities are not significantly different from those expected for 1 locus segregating for a recessive and a dominant alleles. In conclusion, in *Culex pipiens* from Southern France, chlorpyrifos resistance is induced by a major gene, *Chl*, with 2 alleles $Chl^R$ and $Chl^S$ ($Chl^R$ being dominant).

2. Chromosome localization of the *Chl* locus. *a-Gpd*$^{1.30}$*Est-2*$^{1.00}$ $Chl^S$/*a-Gpd*$^{1.00}$ *Est-2*$^{0.64}$ $Chl^R$ mosquitos were obtained by crossing the SG and S5 strains[6]. They were backcrossed with *a-Gpd*$^{1.00/1.00}$ *Est-2*$^{Nul/Nul}$ $Chl^{S/S}$ mosquitos from the S7 strain. Such a backcross allows us to recognize the genotypes of any descendant: *a-Gpd* and *Est-2* genotypes are determined by electrophoresis, and $Chl^{SR}$ genotypes

Table 2. Linkage analysis of the *Chl* locus with *a-Gpd* and *Est-2* autosomal loci, from crosses between *a-Gpd*$^{1.30}$ *Est-2*$^{1.00}$ $Chl^S$/*a-Gpd*$^{1.00}$ *Est-2*$^{0.64}$ $Chl^R$ and *a-Gpd*$^{1.00/1.00}$ *Est-2*$^{Nul/Nul}$ $Chl^{S/S}$ genotypes

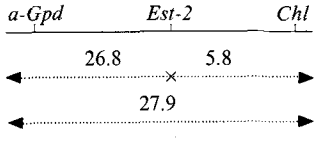
| Chromosome recovered from the heterozygous parent | After an exposition to chlorpyrifos of 0.0015 ppm | of 0 ppm |
|---|---|---|
| *a-Gpd*$^{1.30}$ *Est-2*$^{1.00}$ $Chl^S$ | - | 30 |
| *a-Gpd*$^{1.30}$ *Est-2*$^{1.00}$ $Chl^R$ | 3 | |
| *a-Gpd*$^{1.00}$ *Est-2*$^{0.64}$ $Chl^S$ | - | 41 |
| *a-Gpd*$^{1.00}$ *Est-2*$^{0.64}$ $Chl^R$ | 134 | |
| *a-Gpd*$^{1.30}$ *Est-2*$^{0.64}$ $Chl^S$ | - | 20 |
| *a-Gpd*$^{1.30}$ *Est-2*$^{0.64}$ $Chl^R$ | 45 | |
| *a-Gpd*$^{1.00}$ *Est-2*$^{1.00}$ $Chl^S$ | - | 6 |
| *a-Gpd*$^{1.00}$ *Est-2*$^{1.00}$ $Chl^R$ | 8 | |
| Total | 190 | 97 |




Chlorpyrifos resistance in *Culex pipiens*. Mortality curves: *A* of *SS*, *RR* and *SR* genotypes; *B* of *SR*×*SS* offspring; and *C* of *SR*×*SR* offspring (Chlorpyrifos concentrations are indicated in abscissa, and percentages of mortality in ordinates).

because all $Chl^{SS}$ must be killed by a 24-h exposition to 0.0015 ppm chlorpyrifos.

The results given in table 2 show that *a-Gpd, Est-2* and *Chl* are localized on the same chromosome. *a-Gpd* and *Chl* are separated by 27.9 units of crossing-over, *Est-2* and *Chl* by 5.8 units, and *a-Gpd* and *Est-2* by 26.8 units. (*a-Gpd* and *Est-2* were found to be distant of 37.1 recombination units in a previous experiment[5] using SG and S7 strains only. This value is not significantly different from ours, $\chi^2 = 3.69$ for 1 df, $p > 0.05$.) The disposition of the 3 genes is therefore:




*Discussion.* In *Culex pipiens pipiens* from Southern France, chlorpyrifos resistance is determined by a single gene named *Chl* with 2 alleles, $Chl^S$ which is recessive, and $Chl^R$ dominant. The *Chl* locus belongs to the same linkage group as do the *a-Gpd* and *Est-2* loci and is at some 5 units of crossing-over from the later. *a-Gpd* and *Est-2* loci are known to be on an autosome[4,5]. De Stordeur[4] believes that *Est-2* does not belong to linkage group II because he observed an independant assortment between this locus and the *fc* (yellow larvae) locus generally attributed to the 2nd chromosome[7]. If this information is confirmed, it would mean that the locus inducing chlorpyrifos resistance is on a different chromosome than that bearing the *ma* (malathion resistance) locus in Japanese *Culex pipiens pallens*[8] and the *fe* (fenthion resistance) locus in Burman *Culex pipiens fatigans*[9].

It can be concluded that the *Est-2* locus, when it codes the $Est\text{-}2^{0.64}$ allele is not responsible for chlorpyrifos resistance, and therefore, the highly significant correlation observed between $Est\text{-}2^{0.64}$ frequency and chlorpyrifos resistance in natural populations of Southern France is most probably the result of a tight linkage between 2 independant loci.

The analysis of organophosphate resistant *Culex* of different geographic origins[10] has pointed out that all resistant strains demonstrate an extremely high level of esterase activity. This is not the case of the $Est\text{-}2^{0.64}$. In France, the allozyme demonstrating the highest activity is coded by the *Est-3* locus which is localized at some 3 units of crossing-over from *Est-2*. Experiments are actually underway to localize this last locus with respect to *Chl*.

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## Lysine estimation with the modified Udy-dye binding method in hexaploid wheat

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*Summary.* Lysine content in bread wheat (*Triticum aestivum* L.m. Thell) was determined by a modified Udy-dye binding method and was compared with that obtained from the amino acid analyzer. The values obtained from the 2 methods were correlated and the co-efficient was found to be +0.91 at 0.450 mg/ml dye concentration. The modified method is quick, less expensive and quite helpful for screening lysine at earlier generation in wheat breeding for improved grain quality content.

It has been reported[2-4] that lysine is the most limiting essential amino acid and is present in wheat much below the required amount needed for nutritional balance. About 80% of people in Pakistan and nearly 1 billion people in the world depend on wheat as their main staple food. The green revolution has been effective to a certain extent in meeting the caloric requirements of the underdeveloped countries, but so far no significant achievement have been made in improving the nutritive value of the bread wheat (*Triticum aestivum* L.m. Thell). This is probably due to the fact that the plant breeders in general are unable to screen larger plant population for basic amino acids. Although various methods for screening lysine have been reported[4-8], these methods are slow and time-consuming. Screening lysine with amino acid analyzer is not only slow but also unsuitable for mass screening. Laboratories with meagre financial support must devise less expensive and quick screening methods for lysine determination. A modified Udy-dye binding method for screening barley genotypes was tried with changed dye concentrations for estimating lysine content in hexaploid wheat. The present work deals with lysine screening in bread wheat and the lysine values thus obtained were compared with those determined from the amino acid analyzer.

*Material and method.* Protein estimation with Udy-method[10,11] is essentially based on the principle that the protein binds quantitatively with certain dyes and each protein has precise dye-binding capacity (DBC). Acid orange-12 (AO-12) when reacting with proteins, binds strongly with basic groups of amino acid, viz lysine, arginine, histidine, and forms insoluble protein complexes. The filtration of these complexes and calibration of unbound equilibrium dye concentration (EDC) through Udy-color analyzer enables us to estimate the protein content.

Slight modification[9] in the Udy-method[11] was made and low dye concentrations of reagent dye, as suggested[7], were used. Accordingly Udy-color analyzer was set at 20% transmission (T) with reference dye instead at 42% (T) to provide a wider range for calibration. At this setting 30% T was observed with 0.450 mg/ml or diluted dye concentration. Wheat flour of 9 wheat cultivars, with known analyzer lysine values, were reacted with the following reagent dye concentrations: 0.325, 0.350, 0.375, 0.400, 0.425, 0.450, 0.475, 0.500, 0.525 and 0.575 mg/ml. 100 mg of wheat flour