Chapter 5

CHROMOSOMAL LOCALISATION OF FACTORS UNDERLYING ALCOHOL RESISTANCE IN *DROSOPHILA MELANOGASTER* SELECTED AT DIFFERENT LIFE STAGES

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Abstract.

ADU-FF is a *Drosophila melanogaster* strain homozygous for the Adh^F allele and selected for increased adult ethanol tolerance. LAR-SS is a strain homozygous for Adh^S and selected for increased juvenile ethanol tolerance. In both cases, crosses between selected and controle lines (respectively CON-FF and CON-SS) were performed, and the offsprings were tested for adult ethanol resistance (ADU-FF x CON-FF) or juvenile ethanol resistance (LAR-SS x CON-SS). In both cases, results show no X-chromosome effect. In the first case, results indicate a general codominant effect for the increase in adult ethanol resistance, while in the second case results indicate a dominant effect for the increase in juvenile resistance. Crosses with a balanced marker line were performed to build genotypes in order to analyse the effects of X, second and third chromosomes of the two selected lines on the increase in adult and juvenile ethanol tolerance. In all cases, the X-chromosome has no effect, while the second chromosome always has a significant effect on adult and on juvenile tolerance to ethanol. The third chromosome from LAR-SS has no effect on ethanol tolerance, but the third chromosome from ADU-FF has a significant effect on juvenile tolerance and on male adult tolerance. The role of each chromosome in the increase in alcohol tolerance according to the life stage is discussed.

INTRODUCTION

Resistance to an environmental stress such as alcohol in *Drosophila melanogaster* is a complex trait which may involve several mechanisms. Because this species feeds and breeds on decaying fruits in which ethanol concentrations may exceed 12%, due to fermentation of sugars by micro-organisms (McKenzie and McKechnie, 1979), the level at which *D. melanogaster* is able to survive toxic concentrations of alcohol in its environment is particularly important for both juvenile and adult stages.

Alcohol dehydrogenase (ADH) plays an essential role in the metabolic pathway of ethanol degradation and in survival on ethanol supplemented medium (van Delden, 1982; Geer *et al*., 1990). At low concentrations, ethanol can be exploited as food. Higher concentrations are toxic. The two common alleles of the *Adh* gene, Adh^S and Adh^F, differ in their *in vitro* ADH activity. Adh^{FF} flies exhibit an ADH activity three to four times higher than Adh^{SS} flies while heterozygous flies have an intermediate ADH activity (van Delden, 1982; Chambers, 1988, 1991; Heinstra 1993). Adh^{FF} individuals, homozygous for the fast allele show generally a higher adult resistance to ethanol and a higher egg-to-adult survival on ethanol medium compared to Adh^{SF} and Adh^{SS} individuals (van Delden, 1982; Geer *et al*., 1990; Chambers, 1991; Heinstra, 1993; van Delden and Kamping, 1997). However, the relation between in vitro ADH activity and alcohol tolerance is not fully resolved (Gibson *et al*., 1979; Ziolo and Parsons, 1982; Oakeshott *et al*., 1983, 1984; Mercot *et al*., 1994). Alcohol tolerance appears to be a complex trait in which many factors other than ADH activity may be involved, including developmental time, body weight or lipid content (Geer *et al*., 1986; Hoffmann and Parsons, 1989a, b; Barros *et al*., 1991; Miller *et al*., 1993; Jenkins *et al*., 1997; see also Chapters 2 and 3 of this thesis).

Furthermore, in nature the selection pressure due to alcohol probably acts at different levels according to the life stage. The flying adults are very mobile and are able to move to feeding sites with a suitable alcohol concentration, while larvae are bound to a single feeding site. Boulétreau and David (1981) concluded that the selective pressure of ethanol in *D. melanogaster* occurs mainly during the juvenile stages.

We have performed selection experiments for increased alcohol tolerance in relation to the life stage. The lines selected at the adult stage (ADU) presented an increase in adult alcohol tolerance but not in egg-to-adult survival on ethanol medium, while the lines selected at the juvenile stages (LAR) showed an increase in egg-to-adult survival but not in adult tolerance, except for LAR-SS males (see Chapters 2 and 3 of this thesis). Adult and larval body weight, developmental time and adult and larval protein content did not show a clear effect related to the observed increase in tolerance (Chapter 2). *Adh* expression, ADH activity and the phenomenon of induction of the *Adh* gene play certainly a role in the increase in alcohol tolerance for both juvenile and adult stages (see chapter 4), but is not the only factor involved. Other loci are certainly involved, modifying for instance activities of other enzymes like glycerophosphate dehydrogenase (GPDH), octanol dehydrogenase (ODH), aldehyde oxydase (AOX), phosphogluconate mutase (PGM), or esterase (EST), potentially linked to alcohol tolerance (Pecsenye *et al*., 1997; Bokor and Pecsenye, 1997; Ochando and Ayala, 1999). Other traits like membrane composition (Geer *et al*., 1986; Miller *et al.*, 1993), or behavioral traits like oviposition behavior (Kamping and van Delden, 1990; van Delden and Kamping, 1990; Cadieu *et al*., 1997; Siegal and Hartl, 1999) may also play a role in the increase in alcohol tolerance according to the life stage. This may lead to a complex genetic situation involving major genes and polygenes, either or not located at the second chromosome.

In order to clarify the role of other loci than *Adh* (localized at 2-50.1), we performed a localization experiment to establish whether other chromosomes than chromosome 2, which contains the *Adh* locus, contribute to the increased tolerance in the juvenile and adult life stages. For this purpose, experiments were performed to assess chromosomal effects of X, second and third chromosome on both juvenile and adult resistance to alcohol. Furthermore, results are complemented by alcohol resistance measurements of F1 offspring of crosses between selected and control lines.

MATERIAL AND METHODS

Experimental stocks and selection procedures:

The two selected lines ADU-FF and LAR-SS (see Chapter 2) were chosen for the chromosomal exchange experiment because they showed the highest response to selection. ADU-FF exhibited the highest increase in adult alcohol resistance compared to the control line CON-FF, while its increase in juvenile alcohol tolerance was limited. LAR-SS presented the highest increase in juvenile tolerance compared to CON-SS, with no significant increase in adult tolerance after 20 generations of selection (Chapter 2), becoming significant for males after 40 generations (Chapter 3).

The procedures of selection are detailed in Chapter 2. Shortly, a line homozygous for the Adh^F allele, CON-FF, was kept on standard medium (18g agar, 54g sucrose, 32g dead yeast and 100mg ampiciline per 1000ml water) without ethanol, while a replicate of this line, ADU-FF, was selected for 40 generations for increased adult ethanol tolerance. For this purpose, one-week-old adults were kept on 18% ethanol medium during the time necessary for killing approximately a quarter of the flies. The survivors were then transferred to standard medium without ethanol to start a new generation. Another line, homozygous for the Adh^S allele, CON-SS, was kept on standard medium, while a replicate of this line, LAR-SS was selected for 40 generations for increased juvenile resistance to ethanol. In the later case, one-week-old females were allowed to lay eggs on standard medium, and the young eggs were transferred to ethanol medium (12%) in which larvae developed. Just after emergence, adults were transferred to standard medium.

Experiments:

- Cross between control lines and adapted lines:

For CON-FF and ADU-FF on the one hand, and for CON-SS and LAR-SS on the other hand, all possible crosses were effectuated. For each cross, 50 virgin one-week-old males were transferred to bottles containing the same number of virgin females on fresh standard medium. Males and females were kept together for two days, and then transferred to new bottles with fresh food to allow the females to lay eggs on standard

medium (a maximum of 300 eggs per bottle to avoid crowding effects). Crosses between CON-SS and LAR-SS were performed to test the juvenile alcohol tolerance. The eggs were then transferred to a medium supplemented with ethanol in order to test egg-to-adult survival. Crosses between CON-FF and ADU-FF were performed to test adult alcohol tolerance. For this experiment, the eggs were kept on standard medium, without ethanol (also 300 eggs per bottle). F1 males were collected after emergence from pupae, kept separately for one week on regular food before the adult survival test was started.

- Chromosomal exchange lines:

To study the effects of the X, second and third chromosomes on alcohol tolerance, a balanced line, with dominant markers, lethal when homozygous, was used to construct genotypes with different combinations of chromosomes from the control line or from the adapted line. The balanced line used carried two markers on the second chromosome (*Cy* and *Pm*), and two markers (*Sb* and *Tm3*) on the third chromosome. See Lindsley and Zimm (1992) for further details. These markers change a phenotypic character (wing shape, eye color or bristle number) in the adult fly when they are heterozygous, and allow the selection of the suitable genotype.

The crossing scheme is presented in Figure 1. The goal of these experiments was to construct genotypes carrying X, second and third chromosomes from the adapted or the control line (parental lines), and lines carrying all possible combinations of chromosomes from control and adapted lines. At the end of the procedure, 8 genotypes were constructed with chromosomes from CON-FF and ADU-FF, and 8 additional genotypes with chromosomes from CON-SS and LAR-SS.

Totally, 16 genotypes were tested for egg-to-adult and adult resistance to ethanol:

$\frac{Xc}{Xc\text{ or }Y}$; $\frac{2c}{2c}$; $\frac{3c}{3c}$ $\frac{Xa}{Xa\text{ or }Y}$; $\frac{2c}{2c}$; $\frac{3c}{3c}$ $\frac{Xa}{Xa\text{ or }Y}$; $\frac{2c}{2c}$; $\frac{3a}{3a}$ $\frac{Xa}{Xa\text{ or }Y}$; $\frac{2a}{2a}$; $\frac{3c}{3c}$

$\frac{Xa}{Xa\text{ or }Y}$; $\frac{2a}{2a}$; $\frac{3a}{3a}$ $\frac{Xc}{Xc\text{ or }Y}$; $\frac{2a}{2a}$; $\frac{3a}{3a}$ $\frac{Xc}{Xc\text{ or }Y}$; $\frac{2c}{2c}$; $\frac{3a}{3a}$ $\frac{Xc}{Xc\text{ or }Y}$; $\frac{2a}{2a}$; $\frac{3c}{3c}$

A “c” indicates a chromosome from the control line (CON-SS or CON-FF), and an “a” indicates a chromosome from the adapted line (LAR-SS or ADU-FF). For each cross, 50 virgin males and 50 virgin females with the suitable genotype were put together on standard medium. After two days, the flies were transferred to a new bottle with fresh medium to allow the females to lay eggs.

Tests for alcohol tolerance:

- Adult survival on ethanol medium:

Adult survival was measured in plastic vials (75 mm high, 25 mm diameter) containing 9 ml of standard medium supplemented with 30 per cent v/v ethanol. One-week-old virgin males or females were transferred to the test vials (ten flies per vial, 10 replicates per line) and the number of dead flies was regularly counted in each vial in order to calculate the lethal time 50 (LT50). In the crossing experiment between CON-FF and ADU-FF, only the males were tested for survival. For the chromosomal exchange genotypes, results for both sexes were obtained. In all cases, a control was effectuated on vials with standard medium without ethanol.

- Egg-to-adult survival on ethanol medium:

One-week-old mated females were transferred for 24 hours in bottles with standard medium and a drop of living yeast to stimulate egg-laying and avoid egg-retention. Then the females were transferred to vials with standard medium and allowed to lay eggs for four hours. The eggs were then transferred within eight hours after egg-laying to the test vials (5 replicates per line) containing food supplemented with 21 per cent v/v ethanol. The number of eggs was 50 per vial, and the ratio between the number of adults emerging and the initial number of eggs gave the egg-to-adult survival. In all cases, a control was effectuated on vials with regular food, without ethanol.

Statistical analysis:

A probit transformation was used to calculate the lethal time 50 (LT50). Egg-to-adult survival was analysed after an Arc-sin transformation. ANOVAS and Tukey tests for multiple comparison of means were performed by using Statistix 4.0 Analytical Software. The level of significance for the Tukey tests was five per cent.

RESULTS

On control medium, without alcohol, results of both juvenile and adult survival were not significantly different between the various lines for all experiments.

Crosses between control and adapted lines

- Adult survival on ethanol medium:

Figure 2A shows significant differences in male survival on ethanol medium between the different F1's after the various crosses between the control line CON-FF and the adapted line ADU-FF. F1 males with both parents from CON-FF had a lower survival than F1 males with both parents from ADU-FF. Males resulting from a cross between control and adapted lines presented an intermediate adult survival. The result was identical when the mother came from the adapted or from the control line. Apparently there was no X-chromosome effect in view of the identical outcome of the reciprocal crosses. The intermediate result observed for the males resulting from a cross between ADU-FF and CON-FF may suggest that a single gene with a codominant effect is involved in the increase in adult alcohol tolerance in the selected line ADU-FF, or that eventually several loci may be involved, some with a dominant effect and others with a recessive effect.

- Egg-to-adult survival on ethanol medium:

Figure 2B shows significant differences in egg-to-adult survival on ethanol medium between the offspring resulting from crosses between control line CON-SS and adapted lines LAR-SS. When both parents were from the control line, egg-to-adult survival was significantly lower than when at least one of the parents came from the adapted line. No difference in egg-to-adult survival was observed when both parents or when only one (either mother or father) came from the adapted line. For the two reciprocal crosses between control and adapted lines (CON-SS and LAR-SS; Figure 2B), the results of egg-to-adult survival were very close, which suggest a limited effect of the X-chromosome. Egg-to-adult survival in these two reciprocal crosses was similar to the survival when both parents were from LAR-SS, which indicates that a gene with a dominant effect is involved in the increase in juvenile alcohol tolerance in the selected line.

Chromosomal exchange genotypes

- Adult survival on ethanol medium

For each sex and *Adh* genotype, results of adult survival for the eight chromosomal exchange lines were compiled in one file and analysed by an ANOVA, using the X, the second and the third chromosomes as the main factors. Results are summarized in Table 1 for both sexes and both *Adh* genotypes. In all cases, the X chromosome had no effect on adult survival, while the second chromosome had always a significant effect in all cases. The third chromosome had a significant effect only in Adh^{FF} males. In the three other cases (i.e. Adh^{SS} males and females, and Adh^{FF} females), the effect of the third chromosome on adult survival was not significant. Interactions between the chromosomes did not show significant effect on adult survival on ethanol medium, except for one case: the interaction between second and third chromosomes in Adh^{SS} males (Table 1).

Mean values for each chromosome and Tukey tests for comparison of means between the chromosomes from the adapted line or from the control line are presented in Table 2. It confirms that there was no difference in adult survival when the X chromosome came from the adapted line or the control line. However, the mean value when chromosome 2 came from the adapted line was always significantly higher than when it came from the control line, for both *Adh* genotypes and both sexes. For the third chromosome, the increase in adult survival when the chromosome came from the adapted line was not significant in Adh^{SS} males and females and in Adh^{FF} females, but significant in Adh^{FF} males (Table 2).

Figure 3 details the results of adult survival on ethanol medium for all chromosomal exchange genotypes. The differences observed previously (see Chapter 2 and 3 of this thesis) in adult survival between the two *Adh* genotypes, were confirmed, with a distinct higher survival in Adh^{FF} lines compared to Adh^{SS} lines. The high sensibility to ethanol observed for Adh^{SS} females (see Figure 1 of Chapter 2, after 20 generations of selection, and Table 3 of Chapter 3 after 40 generations of selection) is also confirmed here (Figure 3).

In all four cases, the two lines carrying second and third chromosomes from the adapted lines (LAR-SS for Adh^{SS} lines, and ADU-FF for Adh^{FF} lines) showed higher survival than the two lines with second and third chromosome from the control lines (CON-SS and CON-FF respectively). It indicates that the chromosomal exchange experiment worked out well. The differences between the two genotypes differing only for the X chromosome were not significant, which confirm that the effect of the X chromosome on adult survival, was small or absent.

For the Adh^{FF} females, the two genotypes with the second chromosome from the adapted line and the third from the control line (named “cac” and “aac” in Figure 3) showed an adult survival significantly higher than the two genotypes with the second chromosome from the control line and the third from the adapted line (“cca” and “aca” in Figure 3). In these last two cases, adult survival was close to the two genotypes with both second and third chromosome from the control line (“ccc” and “acc”), which indicates that for Adh^{FF} females the third chromosome had no effect on the increase in adult survival in the selected line. The major effect was due to the second chromosome in this case.

For the three other cases, Adh^{SS} males and females, and Adh^{FF} males, the two genotypes “cac” and “aac”, with the second chromosome from the adapted line, showed adult survival higher than the two genotypes “cca” and “aca” with the second chromosome from the control line. This suggests a higher effect of the second chromosome than the third, but as the differences in survival were not significant, it was impossible to conclude only with these figures. From the ANOVAs and comparisons of means presented in Table 1 and Table 2, it can be conclude that the positive effect on survival of the second chromosome from the adapted line occurs in all cases, while a positive effect of the third chromosome occurs only in Adh^{FF} males. A significant interaction between chromosomes 2 and 3 is observed in Adh^{SS} males.

- Egg-to-adult survival on ethanol medium

As for the adults, results of egg-to-adult survival for the eight Adh^{SS} lines and the eight Adh^{FF} lines were compiled in one file , and an ANOVA was performed for each *Adh* genotype using X, second and third chromosome as the main factors. In both *Adh* genotypes, the X chromosome has no significant effect on juvenile survival, while the effect of the second chromosome was significant (Table 3). The effect of the third chromosome was not significant in Adh^{SS} lines, but significant in Adh^{FF} lines. All interactions between these three chromosomes did not show a significant effect on egg-to-adult survival for both *Adh* genotypes.

The results of egg-to-adult survival on ethanol medium are detailed in Figure 4. In Adh^{SS} lines, only the 2 chromosomal exchange genotypes carrying both second and third chromosomes from LAR-SS, the adapted line, showed a higher survival than the 2 genotypes carrying second and third chromosomes from CON-SS. For the 6 other genotypes, the differences in egg-to-adult survival were not significant. The combination of second and third chromosome from the adapted line is necessary to obtain the increase in egg-to-adult survival. In Adh^{FF} lines, the two genotypes carrying both second and third chromosomes from the adapted line ADU-FF exhibited the higher juvenile survival, while the two genotypes with second and third chromosomes from the control line CON-FF showed the lower survival. Egg-to-adult survival was intermediate when only one of the second or the third chromosome was from ADU-FF, and the other from CON-FF. Both second as third chromosomes have an effect on the increase in juvenile tolerance to alcohol, but the highest effect is observed when both these two chromosomes are present.

Table 4 presents for each of the three chromosomes the mean value of egg-to-adult survival for the four chromosomal exchange genotypes carrying a chromosome from the control line, and the mean value for the four genotypes with a chromosome from the adapted line. Surprisingly, survival was similar in both *Adh* genotypes when previously Adh^{FF} lines were more resistant than Adh^{SS} lines (see Table 4 of Chapter 2 and Table 6 of Chapter 3). The difference is observed in the Adh^{SS} genotype carrying the three chromosomes from CON-SS, the "ccc" line which exhibit an egg-to-adult survival rate of 0.29 (Figure 4), compared to the result observed previously for CON-SS with a survival rate of 0.18 (Table 7 of Chapter 3). Egg-to-adult survival rate is similar for the

genotype carrying the chromosomes from LAR-SS ("aaa") and the original LAR-SS line, respectively 0.42 (Figure 4) and 0.39 (Table 7 of Chapter 3).

Results in Table 4 confirm the results presented in Table 3. The increase in juvenile survival observed for the genotypes with an X chromosome from the adapted line compared to the genotypes with an X chromosome from the control line was not significant in both *Adh* genotypes. For the second chromosome, on the contrary, the mean survival was significantly higher when the chromosome came from the adapted line for both *Adh* genotypes. Finally for the third chromosome, the differences were not significant in Adh^{SS}, but were significant in Adh^{FF} in favor of the genotypes with chromosome three from the adapted line ADU-FF.

DISCUSSION

The rapid responses to selection (preliminary results after 10 generations of selection showed already a significant increase in tolerance to ethanol for both life stages) indicate considerable genetic variation for alcohol resistance in the initial populations, the two control lines. The relative specificity of the response according to the life stage (the adult selected line increased specifically its adult tolerance and the larval selected line increased essentially its juvenile tolerance) indicates that different mechanisms may have been selected in each case. However note that the LAR-SS line, selected for larval tolerance, also exhibited increased adult tolerance, though only in males. This is the reason that LAR-SS was also tested for adult tolerance and complementary, ADU-FF was tested for larval tolerance. The role of ADH in alcohol tolerance is unambiguous in both adult and juvenile life stages. However, we have shown in the previous chapters that ADH activity was not sufficient to explain the increase in tolerance in the selected lines. Furthermore, different mechanisms may have been selected according to the procedure of selection -adult or juvenile selection- but also according to the *Adh* genotype -Adh^{SS} or Adh^{FF}.

- Increase in alcohol tolerance in ADU-FF

The results of male survival after the two reciprocal crosses between the control line CON-FF and the adult selected line ADU-FF indicate that the X chromosome is not involved in the increase in adult tolerance in ADU-FF. This is confirmed by the results obtained for adult survival in the Adh^{FF} chromosomal exchange genotypes. For both sexes, the differences observed in adult survival between the two lines carrying the same second and third chromosomes but a different X are not significant. The intermediate result in adult survival observed for the F1 males from a cross between ADU-FF and CON-FF suggests two possibilities: a single gene with a codominant effect is involved in the increase in adult alcohol tolerance in the selected line ADU-FF, or several loci are involved, some with a dominant effect and others with a recessive effect.

For females, adult survival in the Adh^{FF} chromosomal exchange lines shows a clear effect of the second chromosome, while the positive effect of the third chromosome from ADU-FF is not significant. For males, both second and third chromosomes have a

significant effect on adult survival, though the effect of the second chromosome is larger. These results show the importance of the second chromosome in the increase in adult ethanol tolerance in ADU-FF. It is important to underline that the *Adh* locus is located on the left arm of the second chromosome at map position 50.1, which corresponds to chromosome band 35B3 (Woodruff and Ashburner, 1979). The *Adh* locus itself or its regulatory sequences, and particularly the distal promoter, as it is acting during the adult life stage, may be related to the increase in adult alcohol tolerance. It has been previously observed that ADH activity in this selected line is significantly higher than in CON-FF, the control Adh^{FF} line, for both sexes (see Table 4 of Chapter 3). However, the limited increase in adult ADH activity compared to the large increase in adult alcohol resistance in ADU-FF suggests that also other factors than *Adh* are involved. Furthermore, after 20 generations of selection, ADU-FF showed a significant increase in adult body weight and in developmental time (Chapter 2). Other factors, perhaps linked to the critical weight for pupation, may have been selected, because it seems possible that the larger flies are also the more resistant to an environmental stress (Parsons, 1973; Hoffmann and Parsons, 1989a, b, 1991; Jenkins *et al*., 1997; Hallas *et al*., 2002).

In Adh^{FF} chromosomal exchange genotypes, the third chromosome has an effect on adult survival, significant in males but not in females. Combined with the results of the crosses between CON-FF and ADU-FF, suggesting that several loci are involved, it can be assumed that various factors, located on both second and third chromosomes, have been selected in ADU-FF to increase more or less specifically adult alcohol tolerance.

The Adh^{FF} chromosomal exchange genotypes were also tested for egg-to-adult survival on ethanol medium. Previously (Chapter 3), we observed an increase in juvenile survival for ADU-FF compared to CON-FF, but this increase was not significant (Table 7 of Chapter 3). In the present experiment, the flies carrying both chromosomes 2 and 3 from the adapted line ADU-FF show higher juvenile resistance to alcohol. The factors selected in ADU-FF to increase adult tolerance are not totally specific to the life stage. The mechanism(s) involved in adult tolerance probably also contribute to juvenile tolerance. Both second and third chromosomes are involved also in egg-to-adult survival. As for adult resistance, the *Adh* locus certainly plays a role, but other factors may have an effect on both juvenile and adult stages. For instance, a longer

developmental time may be linked to a lower metabolic rate as an adaptation to environmental stress as seen in lines selected for increased dessication resistance (Hoffmann and Parsons, 1989a, b, 1991; Barros *et al*., 1991; Jenkins *et al*., 1997) and in lines selected for increased starvation resistance (Chippindale *et al*., 1996). It seems that the loci selected for increased adult tolerance, but involved to a lesser extent in juvenile tolerance, are located on both second and third chromosomes.

- Increase in alcohol tolerance in LAR-SS

LAR-SS was chosen for this experiment because it exhibited considerable increase in egg-to-adult survival on ethanol medium with an increase in male adult survival after 40 generations of selection (Tables 3 and 7 of Chapter 3). This Adh^{SS} line selected for increased juvenile tolerance showed a significant increase in egg-to-adult survival compared to the control line CON-SS, but did not exhibit higher larval ADH activity, higher larval body weight or a change in protein content (Table 6 of Chapter 2). Even developmental time, which may indicate a response in metabolic rate, was similar to the control line (Table 5 of Chapter 2) after 20 generations of selection. At this time, the increase in juvenile tolerance was already significant, but not the increase in adult tolerance. After 40 generations of selection, the increase in adult survival became significant in males but not in females (Table 3 of chapter 3). The mechanism involved in the selection for increased egg-to-adult survival on ethanol medium was then not totally specific to the life stage and also involved adult tolerance.

The results in egg-to-adult survival of the F1 offspring of the two reciprocal crosses between LAR-SS and CON-SS indicate that the X chromosome is not involved in the increase of juvenile tolerance in the selected line. Furthermore, the similar survival when only one of the parents or when both parents came from LAR-SS suggests that one or more genes with dominant effects have been selected.

The results of the Adh^{SS} chromosomal exhange genotypes in egg-to-adult survival on ethanol medium confirm that the X chromosome in not involved. The two genotypes with both second and third chromosomes from the adapted line LAR-SS exhibit a higher juvenile survival, while for the six other genotypes the differences in survival are not significant. It seems that both second and third adapted chromosomes are necessary to increase juvenile tolerance. The statistical analysis shows that the effect of the second

chromosome is significant while the effect of the third is not. However, the mean values for these two chromosomes, presented in Table 4, are quite close (i.e. 0.34 for the second chromosome from the adapted line, and 0.33 for the third chromosome). This points to epistatic interactions between loci located on both second and third chromosomes. According to the crossing experiment between CON-SS and LAR-SS, dominant effects are involved.

The Adh^{SS} chromosomal exchange lines were also tested for adult survival on ethanol medium. LAR-SS, selected for increased juvenile alcohol tolerance, presents also an increase in adult tolerance compared to CON-SS (Table 3 of Chapter 3). The two chromosomal exchange genotypes with both second and third chromosomes from LAR-SS showed a higher adult survival than the two lines with second and third chromosomes from the control line. At least one factor selected to increase juvenile resistance is not totally specific to this life stage. The X chromosome and the third chromosome do not present a significant effect on adult survival. Only the effect of the second chromosome is significant. However, it is clear that the combination of both the second and third chromosomes from the adapted line gives the highest adult survival. This again points to epistatic relations between genes on both chromosomes. The main factor(s) involved in both juvenile and adult ethanol tolerance is (are) probably located on the second chromosome which contains the *Adh* gene. It suggests that *Adh* itself or its regulatory sequences may be involved in the increase in ethanol tolerance in LAR-SS for both life stages, and a closer inspection of this region of second chromosome should be made in the selected lines compared to the control line.

For both selection procedures, it seems that several loci, involved in alcohol tolerance and located on both second and third chromosomes, have been selected. Some of these loci are more or less specific to a life stage, and may be linked to *Adh* and its regulatory sequences, or to other enzymes (Pecsenye *et al*., 1997; Bokor and Pecsenye, 1997; Ochando and Ayala, 1999), or to other traits like membrane composition (Geer *et al*., 1986; Miller *et al*., 1993), developmental time (Hoffmann and Parsons, 1989a, b, 1991; Barros *et al.*, 1991; Chippindale *et al*., 1996; Jenkins *et al*., 1997) or behavioral traits like oviposition behavior (Kamping and van Delden, 1990; van Delden and Kamping, 1990; Cadieu *et al*., 1997; Siegal and Hartl, 1999).

Table 1 : Summary of ANOVAS for adult survival on 30% ethanol medium for all chromosomal exchange lines. Chromosome X, 2 and 3 are the main factors.

		Adh^{SS}		Adh^{FF}	
		Males	**Females**	**Males**	**Females**
Chr. X	**(A)**	ns	ns	ns	ns
Chr. 2	**(B)**	***	**	***	***
Chr. 3	**(C)**	ns	ns	*	ns
A x B		ns	ns	ns	ns
A x C		ns	ns	ns	ns
B x C		**	ns	ns	ns
A x B x C		ns	ns	ns	ns

ns: not significant; * P<0.05; ** P<0.01; *** P<0.001

Table 2 : Mean lethal time 50 (LT50) values in hours for adult survival and Tukey tests for comparison of means for each chromosome of the chromosomal exchange genotypes. For Adh^{SS}, the control line was CON-SS and the adapted line LAR-SS. For Adh^{FF}, the control line was CON-FF and the adapted line ADU-FF.

		Adh^{SS}		Adh^{FF}	
		Males	**Females**	**Males**	**Females**
Chr. X	**control**	20.05[a]	5.18[a]	52.59[a]	69.64[a]
	adapted	19.84[a]	5.24[a]	47.51[a]	68.20[a]
Chr. 2	**control**	16.02[a]	3.73[a]	39.90[a]	38.69[a]
	adapted	23.86[b]	6.70[b]	60.20[b]	99.16[b]
Chr. 3	**control**	18.56[a]	4.55[a]	44.07[a]	62.21[a]
	adapted	21.33[a]	5.87[a]	56.03[b]	75.64[a]

For each chromosome, sex and *Adh* genotype, means with a different superscript letter between control and adapted are significantly different at the 5% level.

Table 3 : Summary of ANOVAS for egg-to-adult survival on 21% ethanol medium for all chromosomal exchange lines. Chromosome X, 2 and 3 are the main factors.

		Adh^{SS}	Adh^{FF}
Chr. X	**(A)**	ns	ns
Chr. 2	**(B)**	*	***
Chr. 3	**(C)**	ns	**
A x B		ns	ns
A x C		ns	ns
B x C		ns	ns
A x B x C		ns	ns

ns: not significant; * P<0.05; ** P<0.01; *** P<0.001

Table 4 : Mean values for egg-to-adult survival and Tukey tests for comparison of means for each chromosome of the chromosomal exchange genotypes. For Adh^{SS}, the control line was CON-SS and the adapted line LAR-SS. For Adh^{FF}, the control line was CON-FF and the adapted line ADU-FF. SE is the standard error.

		Adh^{SS}		Adh^{FF}	
		Mean survival	**SE**	**Mean survival**	**SE**
Chr. X	**control**	0.307[a]	0.014	0.297[a]	0.021
	adapted	0.322[a]	0.020	0.328[a]	0.029
Chr. 2	**control**	0.288[a]	0.013	0.250[a]	0.020
	adapted	0.341[b]	0.019	0.375[b]	0.022
Chr. 3	**control**	0.295[a]	0.016	0.267[a]	0.018
	adapted	0.334[a]	0.017	0.358[b]	0.028

For each chromosome and *Adh* genotype, means with a different superscript letter between control and adapted lines are significantly different at the 5% level.

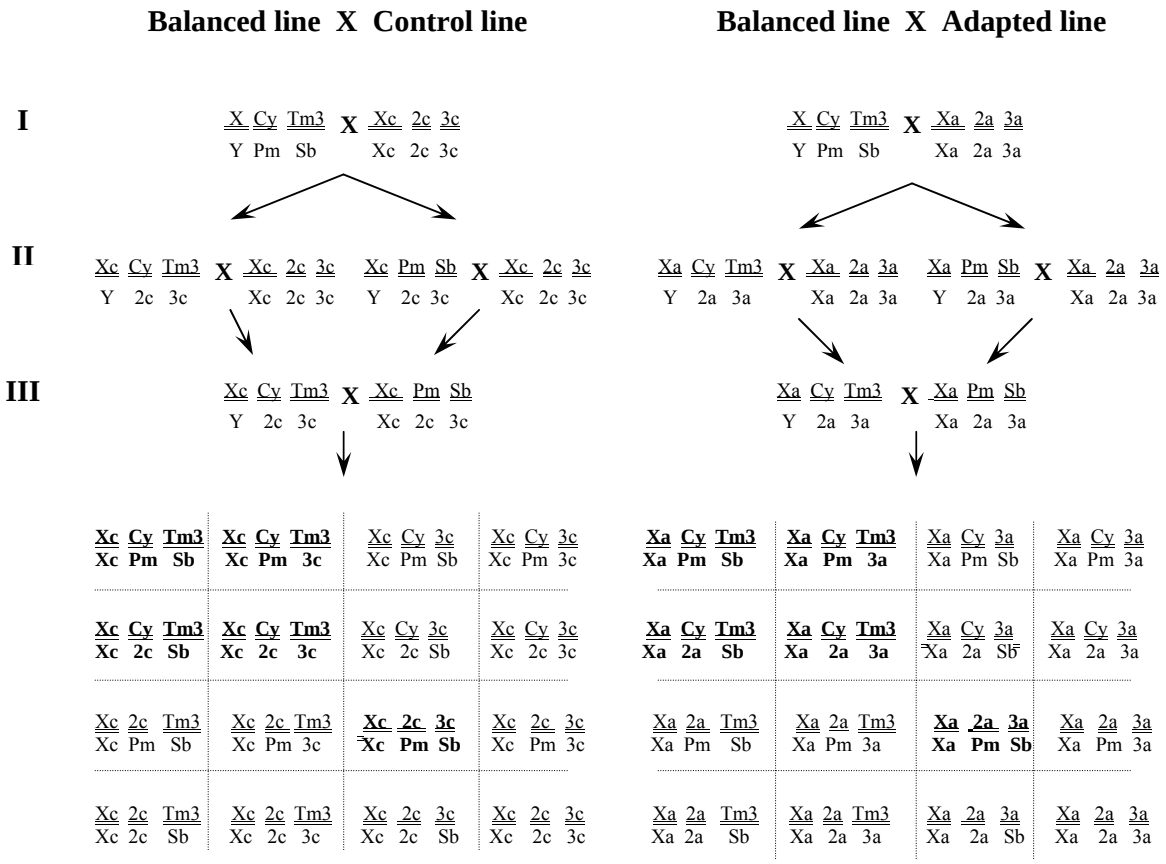

Figure 1A: The first three crosses (I, II and III) of the chromosomal exhange experiment. The goal was to construct two times 16 genotypes with all possible combinations of X, second and third chromosomes from the balanced line and the control line (indicated by a "c") or the balanced line and the adapted line (indicated by an "a"). From these two times 16 genotypes, 10 genotypes, shown in bold, were selected and used to continue the chromosomal exchange experiment.

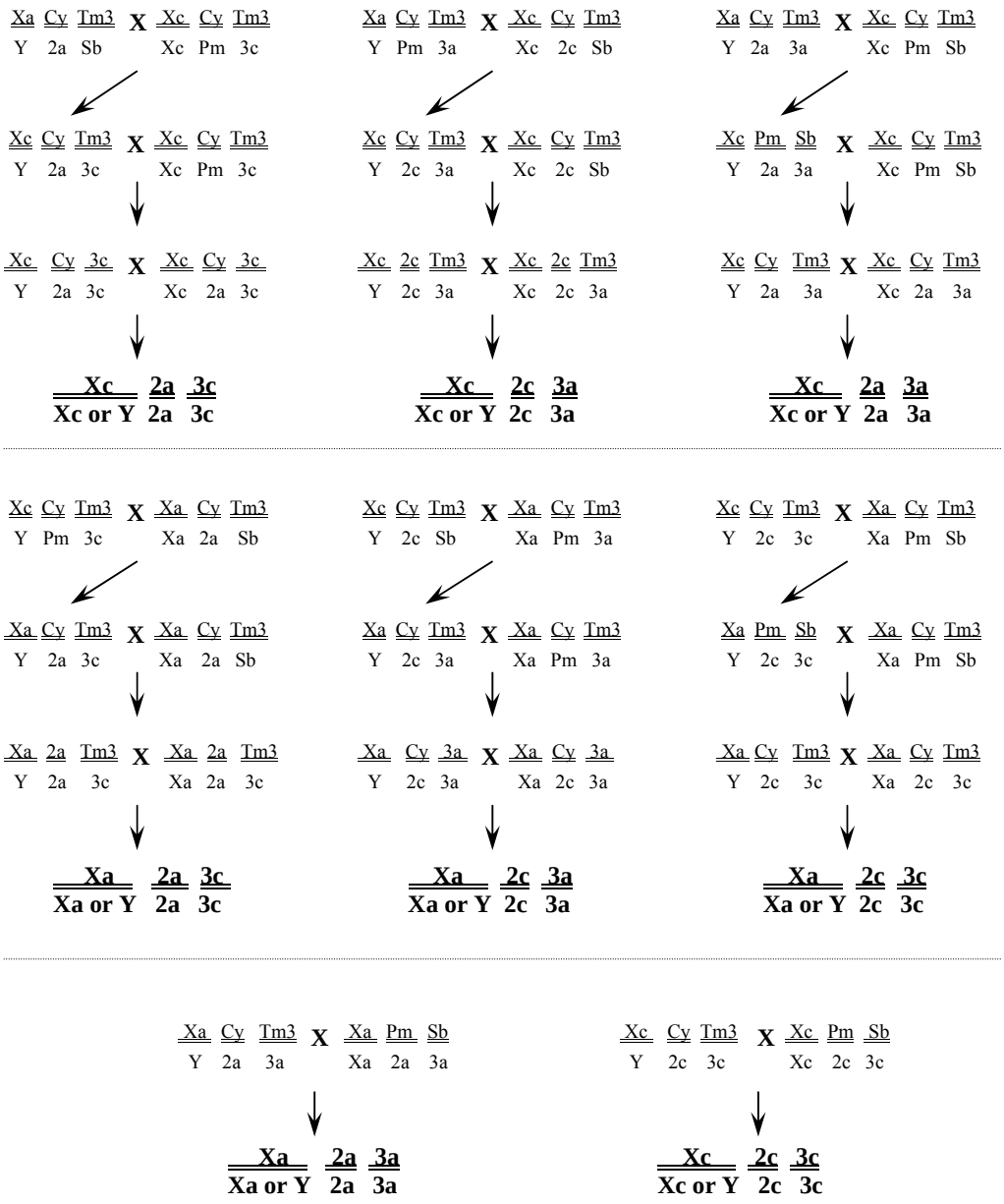

Figure 1B: The last crosses of the chromosomal exchange experiment. The goal was to construct the 8 genotypes with all possible combinations of X, second and third chromosomes from the control line (indicated by a "c") and the adapted line (indicated by an "a"), using the 10 genotypes selected during the first part of experiment.

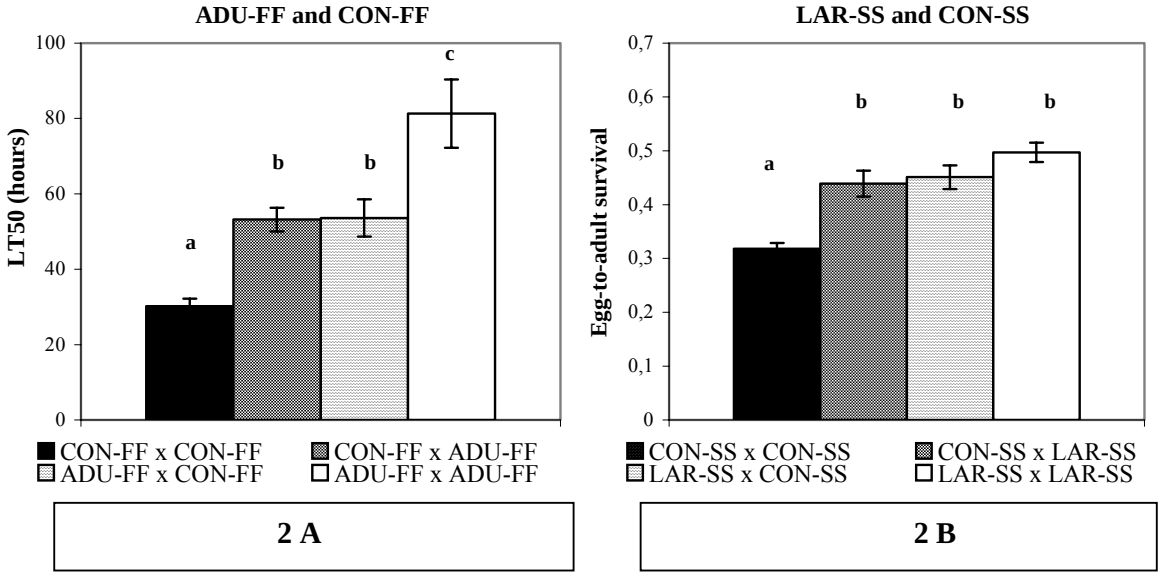

Figure 2: Adult male survival for F1 offspring for all possible crosses between ADU-FF and CON-FF lines (2A) and egg-to-adult survival for F1 offspring for all possible crosses between LAR-SS and CON-SS lines (2B). The father is indicated first, the mother second (e.g. CON-FF x ADU-FF means males from CON-FF and females from ADU-FF). Adult survival is the lethal time 50 (LT50) in hours at which 50% of the flies placed on 30% ethanol medium are dead. Egg-to-adult survival is the proportion of adults emerging from eggs placed on 21% ethanol medium. Vertical bars indicates the standard errors. Different letters above histograms indicate significant differences at the 5% level (Tukey tests for comparison of means).

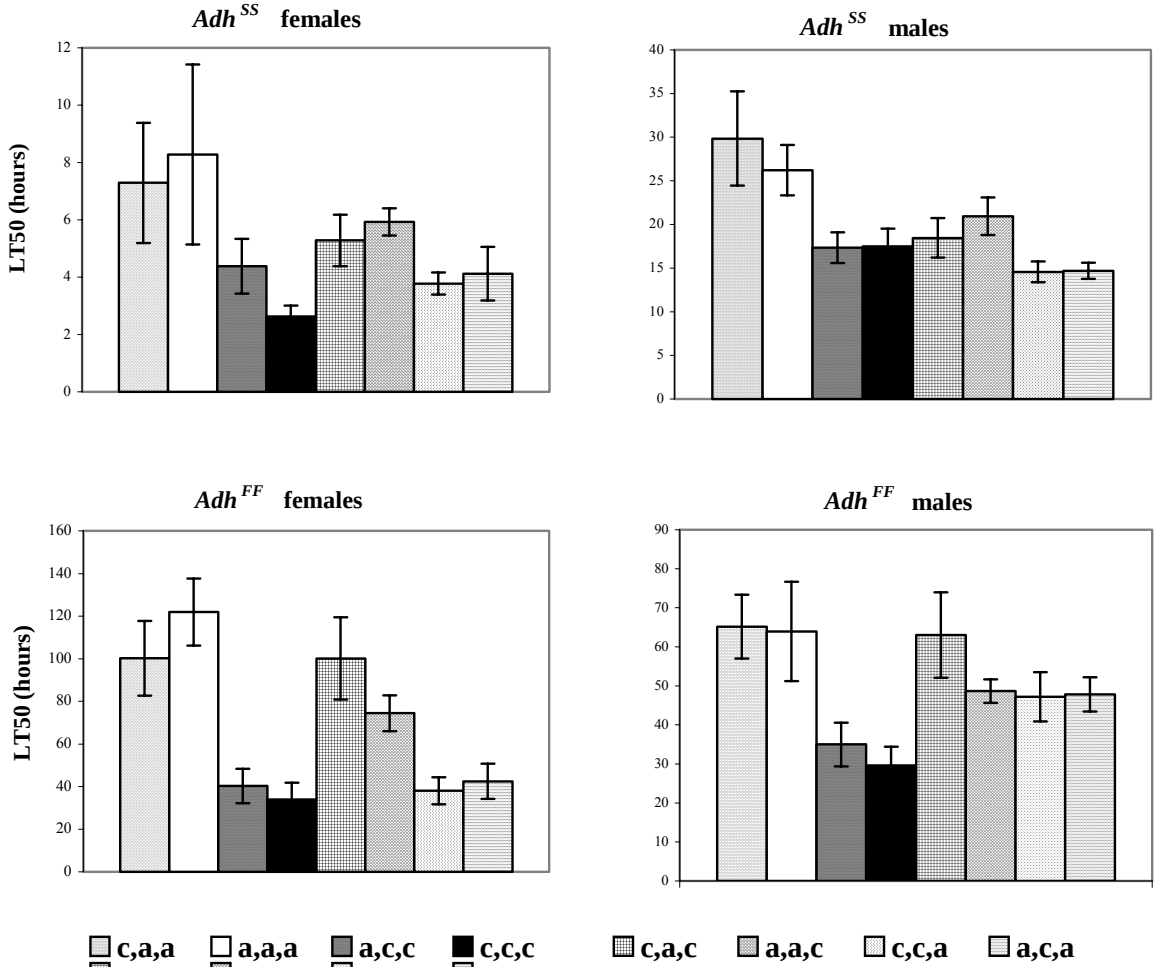

Figure 3: Adult survival on 30% ethanol medium for all chromosomal exchange genotypes, for both *Adh* genotypes and both sexes. Lethal time 50 (LT50) is the time in hours at which 50% of the flies are dead. The three letters signalling each genotype indicate the provenance of X, second and third chromosomes respectively. A "c" indicates a chromosome from the control line (CON-SS in Adh^{SS} lines and CON-FF in Adh^{FF} lines), and an "a" indicates a chromosome from the adapted line (LAR-SS in Adh^{SS} lines and ADU-FF in Adh^{FF} lines). Vertical bars indicate the standard errors.

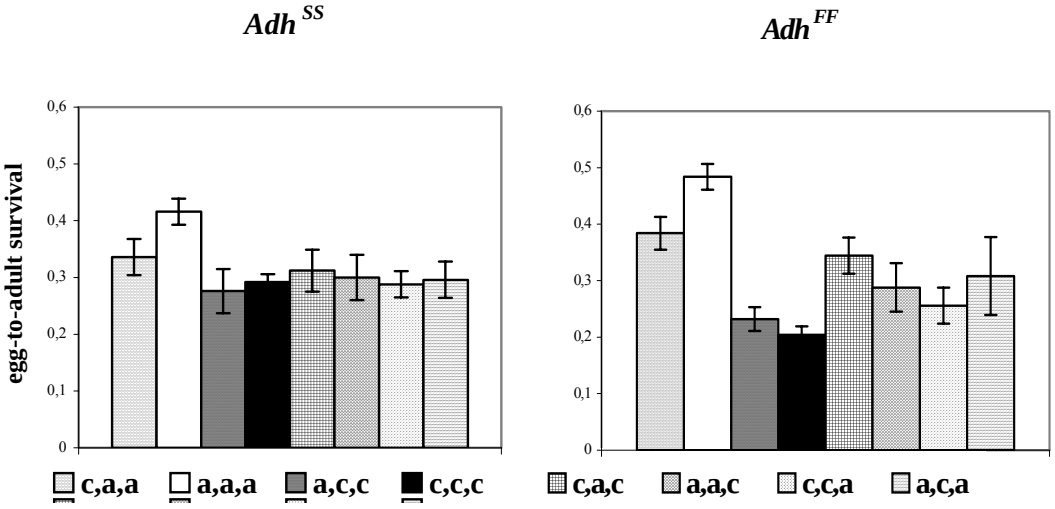

Figure 4: Egg-to-adult survival on 21% ethanol medium for all chromosomal exchange genotypes, for both *Adh* genotypes. Egg-to-adult survival is the proportion of adults emerging from eggs placed on ethanol medium. The three letters signalling each genotype indicate the provenance of X, second and third chromosomes respectively. A "c" indicates a chromosome from the control line (CON-SS in Adh^{SS} lines and CON-FF in Adh^{FF} lines), and an "a" indicates a chromosome from the adapted line (LAR-SS in Adh^{SS} lines and ADU-FF in Adh^{FF} lines). Vertical bars indicate the standard errors.