DEVELOPMENTAL DISRUPTIONS IN HYBRIDS BETWEEN *D. BUZZATII* AND *D. KOEPFERAE*

Antonio CARVAJAL
Departamento de Biología Celular y Molecular, Facultad de Ciencias, Universidad de La Coruña, Spain

In hybrids between the sibling species *D.buzzatii* and *D. koepferae*, both sexes are viable in the F1. However, backcross males to *D.buzzatii* are frecuently inviable, apparently because of interespecific genetic incompatibilities that are cryptic in the F1. That is the classical pattern of F2 breakdown associated with coadapted gene complexes (Carson & Templeton, 1984). In a previous work (Carvajal, Gandarela & Naveira, 1996) a cytologic region called hmi-1 was localized in the X chromosome of *D. koepferae,* that when introgressed in heterozigosity in *D. buzzatii* produces male inviability. Two autosomal regions (4 D1-D3 and the chromosome 6 or "dot") were also localized that when cointrogressed they suppressed the inviability effect associated to hmi-1. These three regions of *D. koepferae* conform to a system of species-specific complementary factors, involved in an X-autosome interaction, that when disrupted in backcross hybrids by recombination with the genome of its sibling *D. buzzatii*, brings about hybrid male inviability. This pattern is similar to that called type II architecture, using Templeton terminology (Templeton 1981). Hmi-1 hybrid males die during the pupal stage, mostly in three periods, namely P1-P2 (before bubble prepupa, 34% of deaths), P7-P8 (yellowing pigmentation of the eye, 23% of deaths), and P15(i) (ptilinium expansion, 37% of deaths). It is possible that these phenotypes were due to the absence of correct hormonal stimuli. On this respect, Madhavan (for reference see Carvajal, Gandarela & Naveira ,1996) observed that the application of juvenile hormone (JH) to pharate pupae of Drosophila blocked adult emergence, so changes in the levels of JH and the ecdysone hormones could explain the lethal effects of hmi-1.

Recent investigations show that the asynapsis (incomplete pairing) of the dot chromosome was not marking introgressed material but some type of distorsion that provokes the excision of a dot homologue or an unusual chromatinization of a portion of the polytenic chromosomes of the larvae. This effect of abnormal chromatinization is possibly related to the regulation processes of nucleolar organizers (Bicudo, 1981).

So, the system consists of an X linked factor of *D. koepferae* (hmi-1) and at least an autosomic factor of the same species that rescues hmi-1 hybrid males as sterile adults. This rescue is observed after cointrogressing 4D1-D5 in heterozygosis. One of the two factors, either hmi-1 or 4D1-D5, acts as a hybrid-specific lethal, and the other one as its conspecific suppressor.

The next step whose results are the subject of this comunication, consisted in assesing the effect produced when the autosome factor of *D. koepferae* was made homozygous in otherwise *D. buzzatii* background. This factor in heterozygous condition seems to have no other phenotypic effect but the rescue of otherwise inviable adult hmi-1 males.

The technique of cytogenetic mapping based on the asynapsis (incomplete pairing) of homologous chromosomes in hybrids of *D. koepferae* and *D. buzzatii* (Naveira, Pla & Fontdevila, 1986) allows to determine, in a precise manner, heterozygous introgressed chromosome regions of one specie into another. Thus, any part of the genome of a fly, that undergoes polytenization in the third instar larvae salivary glands can be diagnosed as introgressed or not, according to the pairing pattern with the homolog (Fig. 1).

In order to make homozygous the region D1-D5 of the chromosome 4 of *D. koepferae,* introgressed previously in heterozygosity, we used a *D. buzzatii* strain called 4s, fixed for inversion 4s, which includes the region D1-D5 (Fig. 1).

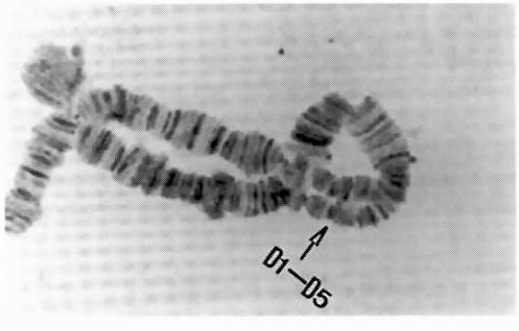

Fig 1: Microphotography of polytene asynapsis in a 4s/D1-D5 fly

Crossing design: female 4s/4s D.b x male 4s D.b/4st D.k --------> female 4s/4s D.b x male 4s D.b/4st D.k --------> female 4s D.b/4st D.k x male 4s D.b/4st D.k --------> female 4st/4st D.k x male 4st/4st D.k --------> 4st/4st *D.koepferae* introgressed in homozygous in *D. buzzatii*. Then we could distinguish between hybrids carrying the region 4D1-D5 of *D. koepferae* in homozygous condition and the normal individuals carrying the homologous region of *D. buzzatii*.

Results: It was observed that both males and females bearing the 4 D1-D5 region of *D. koepferae* introgressed in homozygous condition in *D. buzzatii* were viable. In conclusion, 4D1-D5 must harbor, not the hybrid-specific lethal, but its suppressor, the lethal being then confined to hmi-1.

On the other hand, several aberrant phenotypes (1/20 adult males) were recorded (Figure 2) when the region 4D1-D5 was made homozygous. Some individuals appeared with abdominal deformations, changes in the distribution of abdominal bristles in the antero-posterior axis of the ventral part and incomplete tergites and a few cases of individuals without external genitalia. These phenotypes, that affect preferentially the males, also appeared at higher frequences (1/10 adult males) in heterozygotes between the chromosome bearing the 4D1-D5 region of *D. koepferae* and the 4s inversion of *D. buzzatii*. Finally, they increased in degree and frequency (see table) after crosses to obtain flies with the hmi-1 in heterozygous condition and the 4D1-D5 in homozygous condition. These phenotypes are similar to the abnormal abdomen (aa) syndrome (Templeton, 1979) so called because of one of its pleiotropic effects. The aa syndrome is determined by a major X-linked segregating unit that engages in very strong epistatic interactions with X-linked,

Y-linked, and autosomal genes. Moreover there are strong epistatic interactions among the autosomal modifiers. The aa is associated with the preferential amplification in polytene tissues of 28S ribosomal genes (Carson & Templeton, 1984). The aa syndrome displays a wide variety of pleiotropics effects, many of which are consistent with the phenotypes normally associated with ribosomal deficiences in *Drosophila*, and some of which are associated with the effects aa has on juvenile hormone metabolism.

Furthermore when the polytenic kariotypes of descendants of crosses between females bearing hmi-1 in heterozygous condition and males with 4D1-D5 in homozygous condition were analyzed, some cases of unusual frecuency of spontaneus asynapsis in the X chromosome and a dot-like chromatin body were observed. Also it seems interesant to emphasized that till now it has been impossible to find adult females bearing both the hmi-1 and the 4D1-D5 in heterozygous and homozygous condition respectively. There are besides several crosses (see table) that yield offspring with significantly fewer females than males. Thus, it is possible these females were inviable ones while the hmi-1 males bearing the supressor in homozygous condition are now viable ones.

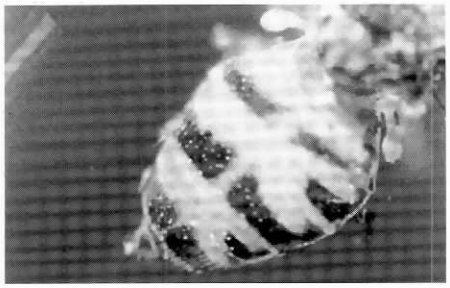

Figure 2: Hybrid abnormal abdomen

Table:

Line	No. of females	No. of males	No. of abnormal females	No. of abnormal males
1	13	18	0	1
2	28	75	1	10
3	22	51	0	11
4	23	31	0	0

It is clear that we know at least two regions of *D. koepferae* that belong to an specific genetic complex that when broken by recombination in the hybrids between *D. koepferae* and *D. buzzatii* yields developmental distorsions that produces in some cases the male death at the pupal stage (incomplete development) and in other cases a cluster of anomalies that to affect preferentially to the abdominal region. It seems too that complex epistatics interactions must be involved in these processes including changes in apriori so distinc systems as the regulatory processes of ribosomal RNA synthesis and the hormones that deal with the metamorphosis of the fly. It has been proposed many times that changes in genetic regulation are of fundamental importance in eucariotic evolution (Hedrick & Mc. Donald, 1980). Take into account then the genetic revolution or better the regulatory revolution concept which assume rapid evolutionary changes and speciation due to the readjustment of coadapted genetic complexes after colonization event of a new environment by a few number of individuals (Templeton, 1979). This "revolution" can quickly alter morphology, development, life history parameters and behavior, and such alterations can be so drastic that a new "species" evolves with pre- and/or post-mating isolating mechanisms. Genes that underlie the "genetic revolution" appear to be at loci having fundamental regulatory roles in the organism (Templeton ,1979). The named aa syndrome seems to be a consequence of a type II architecture. Hybrid inviability between *D. koepferae* and *D. buzzatii* could be a consequence of disruptions in the same type of architecture producing, between others, alterations in abdomen development in such hybrids and drastic developmental alterarions that finally yield lethality.

References

Bicudo, H.E.M.C. (1981). Caryologia 34: 231-253.
Carson, H.L. & Templeton, A.R. (1984). Ann. Rev. Ecol. Syst. 15: 97-131.
Carvajal, A.R., Gandarela, M.R. & Naveira, H.F. (1996). Genetica 98: 1-19.
Hedrik, P.W. & Mc. Donald. (1980). Heredity 45: 83-97.
Naveira, H.F., Fontdevila, A. (1991). Heredity 67: 57-72.
Naveira, H.F., Pla, C. & Fontdevila, A. (1986). Genetics 71: 199-212.
Templeton, A.R. (1979). Genetics 92: 1265-82.
Templeton, A.R. (1981). Ann. Rev. Ecol. Syst. 12: 23-48.