Genes Genet. Syst. (2001) **76**, p. 345–353

A system of complementary genes in hybrids between *Drosophila koepferae* and *D. buzzatii*: A Markov chain model allows to make inferences about their number and relationships

Antonio R. Carvajal*

Departamento de Biología Celular y Molecular, Facultad de Ciencias, Universidad de A Coruña, Campus da Zapateira S/N, 15071 A Coruña, Spain.

(Received 26 February 2001, accepted 15 September 2001)

In backcrosses between *D. koepferae* and *D. buzzatii*, the disruption of a system of species-specific complementary factors brings about hybrid male inviability. This system consists of a lethal factor, *hmi-1*, linked to the X chromosome of *D. koepferae*, and several conspecific autosomal suppressors. However, *hmi-1* hybrid males can also be rescued by factors present in some strains of *D. buzzatii*. The present work aims to estimate the number of *hmi-1* suppressors in one of these strains by means of Markov chains. The obtained results allow discarding models with one or more chromosomes having independent suppressor effect. On the other hand, models having n chromosomes that interact in groups of r, being $1 < r \leq n$, to produce rescue effect, provide good approximations to the observed results. The best fit to the data is obtained with four or five chromosomes with suppressor effect, interacting epistatically in groups of three to rescue the viability of *hmi-1* males.

INTRODUCTION

The genetic study of postzygotic reproductive isolation has frequently revealed an underlying complex genetic basis. Hence, the information accumulated in the past few years suggests a more complex genetics basis for postzygotic reproductive isolation than had been initially supposed (Wu & Palopoli, 1994; Nei & Zhang, 1998).

In hybrids between the sibling species *D. buzzatii* and *D. koepferae*, both sexes are more or less equally viable in the F_1. However, in backcrosses to *D. buzzatii*, hybrid males are frequently inviable, apparently because of interespecific genetic incompatibilities that are cryptic in the F_1. This is the classical pattern of F_2 breakdown associated with coadapted gene complexes (Carson & Templeton, 1984). In a previous work (Carvajal *et al.*, 1996) a cytological region called *hmi-1* was localised in the X chromosome of *D. koepferae* that, when introgressed in heterozygous condition in *D. buzzatii*, produces male inviability. Autosomal regions from *D. koepferae* were also localised that suppressed the inviability effect associated with *hmi-1* when cointrogressed. These results apparently unveil a system of species-specific complementary factors involved in a X-autosome interaction in *D. koepferae* that, when disrupted in backcross hybrids by recombination with the genome of its sibling *D. buzzatii*, brings about hybrid male inviability. However, there is still the possibility that some populations of *D. buzzatii* may harbour hybrid male rescue factors, similarly to what has been found in the melanogaster complex (Hutter *et al.*, 1990). The present work shows the results of the introgression of *hmi-1* into several *D. buzzatii* strains, and demonstrates that there is indeed a polymorphism for hybrid male rescue factors (suppressors of *hmi-1*). The number of suppressors and their mode of action in one of these strains are assessed by means of Markov chain models. As we will see, this kind of models allows making inferences about the expected proportion of backcross lines with viability rescue at any generation, and hence the proportion of rescued males. In order to do such predictions it is necessary to know both, the initial distribution of suppressors and the transition probability matrix i.e. the matrix of probabilities for shift from one state to another. In our case the unknown factors will be just these, the different types of initial distributions and their transition probability matrix corresponding to different models of suppressors which could explain the observed data.

Edited by Masa-Toshi Yamamoto

* Corresponding author. E-mail: acraaj@mail2.udc.es

MATERIAL AND METHODS

Drosophila *species* *D. buzzatii* and *D. koepferae* are two sibling species of the *repleta* group that coexist in many of the arid and semiarid zones of Andean Bolivia and Northwest Argentina (Ruiz, Fontdevila & Wasserman, 1982; Fontdevila *et al.*, 1988). Both species have the standard *D. repleta* group polytene karyotype consisting in five rod-like chromosomes and a tiny dot-like chromosome. Number 1 corresponds to the X, numbers 2–5 to the long acrocentric autosomes, and number 6 to the dot.

Stocks Three different kinds of stocks were used in the experiment described in this paper. (1) Five strains of *D. buzzatii* San Luis (buzSL): buzSL-5 and buzSL-6 (Naveira & Fontdevila, 1991), founded by single wild-inseminated females collected at Serra San Luis (Argentina) and maintained by mass culturing; buzSL-101, with a *white* (*w*) mutant fixed in homozygous condition in a genetic background of buzzatii San Luis (Naveira & Fontdevila, 1991); buzSL-In(4)s, fixed for 4*s*, a natural population inversion of chromosome 4 (Ruiz & Fontdevila, 1981); and buzSL-In(5)1, fixed for an inversion of chromosome 5 produced in the laboratory (Naveira & Fontdevila, 1985). (2) A hybrid strain, buzSL-Xd[*hmi-1*, w^+] (Carvajal *et al.*, 1996), with *hmi-1* introgressed in heterozygous condition together with the wild-type allele of *white* (w^+) from *D. koepferae*, on the genetic background of buzSL-101. (3) A set of *D. buzzatii* strains coming from the collection of Universidad Autónoma de Barcelona: buzDF, Dean Funes (Argentina); buzC, Carboneras (Spain); buzP, Plasencia (Spain); buzPR, Puebla del Río (Spain); and buzM, Mazarrón (Spain). All of them founded by single wild-inseminated females collected at the respective localities and maintained by mass culturing.

Strain buzSL-Xd[*hmi-1*, w^+] is maintained by individual crosses of hybrid females (wild-type, red eyes) with buzSL-101 males (white eyes), and selection of the adult offspring from those females that, according to the analysis of polytene chromosomes in third instar larvae (Naveira *et al.*, 1986), still contained the *hmi-1*-w^+ gametic association. Recombination distance between *hmi-1* and *white* is 4 cM (Carvajal *et al.*, 1996). Therefore, 96% of w^+ male descendants from these crosses are expected to be carriers of *hmi-1*. This factor behaves as a fully penetrant lethal for males during pupal stages, so that the expected percentage of adult w^+ males with respect to all w^+ individuals is approximately 4% (0.04/1.04) in these maintenance crosses (Carvajal *et al.*, 1996).

Crossing protocol Different F_1 offsprings were obtained by crossing individual w^+ buzSL-Xd[*hmi-1*, w^+] females with males from different *buzzatii* strains (table 1). Some of these crosses produced unusually high frequencies of w^+ males (see Results and table 1), therefore evidencing a rescue activity of *hmi-1* hybrids by factors from respective strains. As will be shown in Results, crosses with strain buzSL-5 consistently produced high rescue frequencies, and were then chosen to carry out backcrosses in order to assess the maximum number of chromosomes with *hmi-1* suppressors effect present in this strain (Fig. 1).

Table 1. Values of $\overline{m}$ (mean of the frequency of w^+ hybrid males relative to w^+ hybrid males plus females) from individual crosses between w^+ buzSL-Xd[*hmi-1*, w^+] females and males from different *buzzatii* strains. Each kind of cross is designated with the name of the *buzzatii* male strain. Homogeneity (λ_H) and goodness of fit chi square with respect to the null hypothesis $\mu = 0.04$ ($\lambda_{0.04}$) are also given. Sample size is given in parenthesis in the column of.

Cross	$\overline{m}$ (sample size)	λ_H	df	$\lambda_{0.04}$
buzSL-101	0.05 (168)	18.95 ns	21	0.49 ns
buzSL-5 (1)	0.41 (787)	30 ns	20	4096***
buzSL-5 (2)	0.38 (177)	30.6***	8	274.6***
buzSL-6	0.05 (99)	3.58 ns	6	0.27 ns
buzSL-In(4)s (1)	0.14 (80)	19.5**	6	17.15***
buzSL-In(4)s (2)	0.05 (231)	11.7 ns	16	0.84 ns
buzSL-In(5)1	0.03 (262)	25.6 ns	22	0.61 ns
buzDF	0.12 (150)	15.39 ns	10	24.17**
buzC	0.08 (72)	13.27 ns	8	3.52 ns
buzP	0.07 (71)	7.99 ns	6	1.71 ns
buzPR	0.065 (46)	8.05 ns	6	0.76 ns
buzM	0.1 (104)	12.6 ns	10	8.54**

ns: $P > 0.05$; *: $0.05 > P > 0.01$; **: $0.01 > P > 0.001$; ***: $P < 0.001$.

In our model, the genotype of buzSL-Xd[*hmi-1*, w^+] females will be represented as L/L^-; $n(Su^-)/n(Su^-)$, and that of buzSL-5 males as L^-/Y; $n(Su)/n(Su)$. In this notation, *L* represents the lethal *hmi-1*, *Su* is a suppressor allele of the *hmi-1* effect, *n* represents the total number of suppressor loci, superscript - indicates absence of the corresponding allele, and Y denotes the Y chromosome. Crosses between these individuals would give rise to F_1 males of genotype either L^-/Y; $n(Su)/n(Su^-)$ or L/Y; $n(Su)/n(Su^-)$, where L^- males (lacking *hmi-1*) would be identified by their *white* phenotype.

Application of Markov chain models requires performing at least two consecutive backcrosses (BC1 and BC2) of L^- males (*white* males) with buzSL-Xd[*hmi-1*, w^+] females (Fig. 2). For BC1, several individual crosses (1×1), hereafter referred to as "lines", involving *white* males taken from the F_1's displaying the highest records of w^+ male frequency were carried out. Again, L^- male offspring from these crosses can be genotypically described as L^-/Y; $x(Su)/x(Su^-)$. The variable *x* refers to the presence of an unknown number of suppressors in the male, $x \in (0, n)$. The same as before, several BC2 lines were obtained using *white* males selected from BC1 offsprings with the highest records of w^+ male frequency.

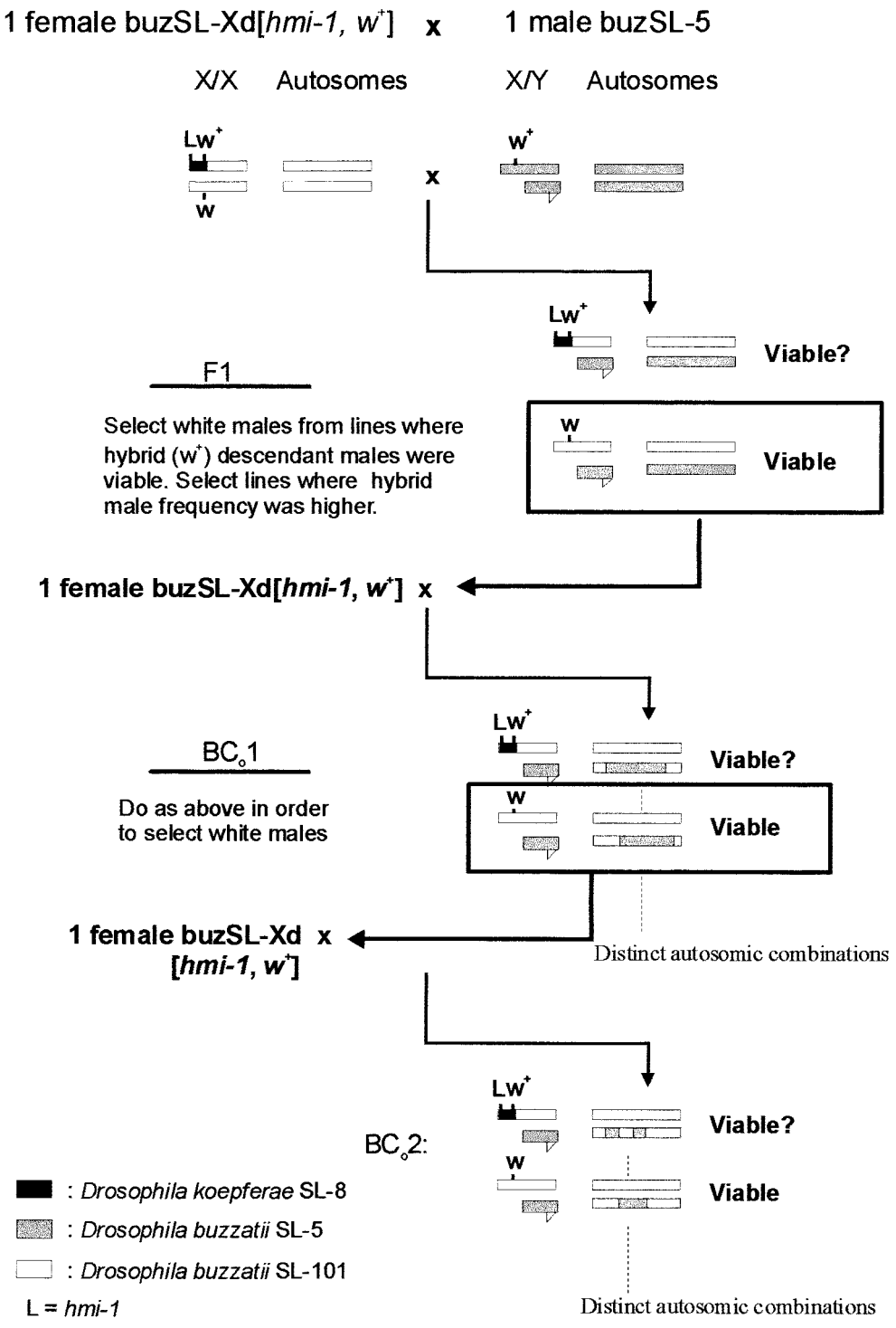

Fig. 1. Crossing protocol for application of Markov chain models to assess the number of buzSL-5 autosomes that are segregating to suppress the lethal effect of *hmi-1*. BC_o: offspring from backcrosses (1 and 2).

The model Consider Markov chain models $\{E_0, ..., E_n\}$ where state E_i refers to i suppressors in heterozygous condition ($0 \leq i \leq n$) in the (BC1 or BC2) male progenitor. Note that the number of suppressors that are to be estimated actually corresponds to the number of autosomes carrying the suppressor effect, since the X chromosome is always from the Xd[*hmi-1*, w^+] strain, and recombination between suppressors in the same chromosome is not possible with the crossing protocol described above since there is no crossing-over in male meiotic cells in these Drosophila species.

Transition probabilities between states i and j are given

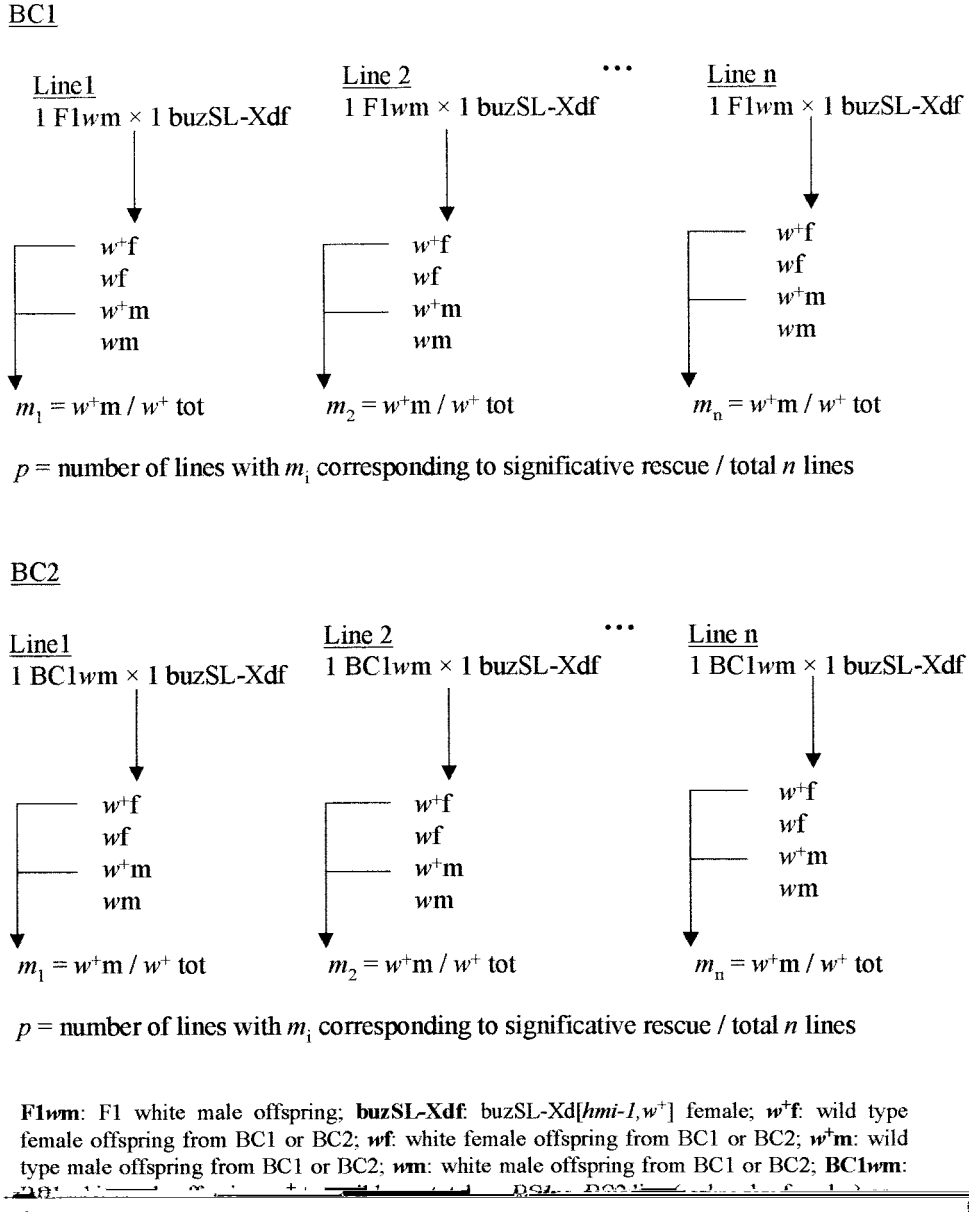

Fig. 2. Crossing scheme showing how measures m and p are obtained both for BC_01 and BC_02.

by

$$\gamma_{ij} = \binom{i}{j}(1/2)^i, \text{ if } i \geq j, \text{ and } \gamma_{ij} = 0, \text{if } i < j;\ i, j \in (0, n). \quad (1)$$

And the state distribution $\gamma^{(t)} = (\gamma_0^{(t)}, \ldots, \gamma_n^{(t)})$ at any generation t is

$$\gamma^{(t)} = \gamma^{(0)}\ \wp^t, \quad (2)$$

where $\gamma_i^{(t)}$ is the probability of state i at generation t, $\gamma^{(0)}$ is the initial distribution of states, and $\wp = (\gamma_{ij})$ is the matrix of transition probabilities.

We are interested in analysing two types of observed frequencies, which we will denote as m and p (Fig. 2). Let us define m as the frequency of w^+ (i.e., hybrid) males with respect to total w^+ offspring (males + females). Let us define p as the frequency of (BC1 or BC2) lines where rescue of male viability is observed. Lines with male viability rescue are considered to be those where the relative frequency of w^+ male offspring (m) is significantly greater than 0.04. In order to obtain the corresponding expected values (parametric), μ and π, for any given number of suppressors, the possibility of epistasis must be considered. No epistasis means that any one suppressor, i.e. one chromosome, is enough to rescue *hmi-1* males, so that the effect of the chromosome on the fitness of the male does not depend on the presence of other chromosomes; on the other hand, epistasis on fitness implies that one chromo-

some requires the presence of some other chromosomes of the same strain in order to carry out the zygotic rescue of *hmi-1* males.

A) Non-epistatic model

If we consider that no epistasis occurs, then the expected frequency π of lines where rescue takes place are derived from:

$$\pi = \sum_{i=1}^{n} \gamma_i^{(t)} = 1 - \gamma_0^{(t)}, 0 \le \pi \le 1 \quad (3)$$

For BC1 offspring, $t = 0$; for BC2, $t = 1$.

With respect to μ, the probability that a male will inherit no suppressors is expressed by the transition probability $\gamma_{i\,0} = (1/2)^i$, i being the number of effective suppressors carried by the father. If $i = 0$, then 96% of w^+ males will be inviable. It follows that

$$\mu = [1 - 0.96(1/2)^i] / [2 - 0.96(1/2)^i]. \quad (4)$$

B) Epistatic model

If we assume that r suppressors ($r > 1$) have to be present for rescue to occur, then:

$$\pi = \sum_{i=r}^{n} \gamma_i^{(t)}, \quad (5)$$

where $n \ge r$ is the total number of suppressors.

The probability that a male will inherit an insufficient number of suppressors is:

$$P_{\text{inv}} = \sum_{j=0}^{r-1} \gamma_{ij} = \sum_{j=0}^{r-1} \binom{i}{j} (1/2)^i, \text{ if } i \ge r; P_{\text{inv}} = 1, \text{if } i < r. \quad (6)$$

Once again, w^+ inviable males correspond to $0.96 \times P_{\text{inv}}$. Then,

$$\mu = [1 - 0.96 \times P_{\text{inv}}] / [2 - 0.96 \times P_{\text{inv}}]. \quad (7)$$

Data homogeneity was tested by means of the Pearson chi-squared goodness of fit statistic. The average value of m was obtained as $\overline{m} = \sum_1^k = m_i / k$, where k is the number of lines examined. The normal approximation to the binomial distribution for large sample sizes was applied for testing hypotheses concerning μ and π. Standard normal deviates (z) were obtained without the correction for continuity (Therefore, e.g., $z = (p - \pi) / (\pi(1-\pi)/k)^{1/2}$). Tables of deviates were analysed by the sequential Bonferroni technique (Holm, 1979; Rice, 1989), under the composite null hypothesis ($\mathbf{H}_{0,c}$) that all the component deviates are taken from a standard normal distribution. Each deviate is replaced by its corresponding P value, and ranked from smallest (P_1) to largest (P_k). If $P_1 \le \alpha/k$, where α is the selected significance level, the corresponding test is declared to indicate significance at the table wide α level. Successively higher P values are similarly examined, until the inequality $P_i \le \alpha/(1 + k - i)$ is not met.

RESULTS AND ANALYSIS

F_1 offsprings from individual crosses between buzSL-Xd[*hmi-1*, w^+] females and males from strains buzSL-5(1) and (2), buzSL-In(4)s, buzDF, and buzM rendered $\overline{m}$ values significantly higher than the expected 0.04 (table 1), Therefore evidencing the presence of suppressors of the *hmi-1* effect.

Different lines from buzSL-5 (1) F_1 crosses were chosen to carry out the analysis by Markov chains, both because the frequency of w^+ males was significantly higher than 0.04 in all the lines examined (data not shown although in table 1 it is shown that such data was homogeneous), and their average was the highest ($\overline{m} = 0.41$ in table 1).

From the F_1 of crosses with buzSL-5 (1), two backcross generations (BC1 and BC2) were obtained (see crossing protocol). The results of the two backcrosses are shown in tables 2 and 4 (BC1 and BC2 respectively) in terms of the frequency m of w^+ males. To build the second backcross, male parents were chosen from the BC1 line dis-

Table 2. Observed frequencies of w^+ males (m) in BC1 offsprings. The male parents coming from the F_1 cross produced by buzSL-5(1) in Table 1. Sample size is the total number of w^+ (i.e., hybrid) offspring recorded. Standard deviates of the normal approximation to the binomial distribution, under different null hypotheses ($\mathbf{H}_{\mu=\mu_0}$), are indicated for each line. * = significant departures from the null hypothesis at the table-wide 0.05 level, after sequential Bonferroni tests.

Line	m (sample size)	$Z_{\mu=0.04}$	$Z_{\mu=0.22}$	$Z_{\mu=0.14}$	$Z_{\mu=0.25}$	$Z_{\mu=0.34}$
1	0.14 (66)	4.15*	–1.57	0.00	–2.06	–3.43*
2	0.12 (93)	3.94*	–2.33	–0.56	–2.89*	–4.48*
3	0.08 (60)	1.58	–2.62*	–1.34	–3.04*	–4.25*
4	0.23 (40)	6.13*	0.15	1.64	–0.29	–1.47
5	0.26 (39)	7.01*	0.60	2.16	0.14	–1.05
6	0.20 (66)	6.63*	–0.39	1.40	–0.94	–2.40*
7	0.18 (99)	7.11*	–0.96	1.15	–1.61	–3.36*
8	0.38 (16)	6.94*	1.54	2.77*	1.20	0.34
9	0.09 (65)	2.06*	–2.53*	–1.10	–2.98*	–4.25*

playing the highest rescue value (line 8, i.e. BC1-8, in Table 2). Standard normal deviates (z) of the approximation to the binomial distribution, under different null hypotheses, and the results of testing those hypothesis against observed values, are also shown (see later).

Almost all BC1 lines displayed a significant rescue of adult hybrid males at the table-wide 0.05 level, since all P values (except P_9 corresponding to line 3) of z deviates met the inequality $P_i \leq 0.05/(1 + k - i)$ of the sequential Bonferroni test (Table 2, 3rd column; $k = 9$). With respect to line 3, $P_9 = 0.057$ which is marginally significant. Therefore, we consider that observed frequency of viability lines p belongs to [0.88–1] interval . Observed values for m ranged from 0.08 to 0.38 ($\overline{m} = 0.16$), the heterogeneity among lines being significant ($\chi^2_H = 16.77$*, 8df, $0.05 > P > 0.01$).

Both under non-epistatic and epistatic models, the observed value for p at BC1 means that the frequency of effective suppressor in strain buzSL-5 is high. The expected value π at BC1 depends on distribution of suppressors at F1 and hence at parental strains. If supressors are fixed or almost fixed in parental strains then we expect roughly a value near 1 independently of the model we consider. The observed p value in BC1 seems to sustain this. However, the best fit to observed rescue frequencies within lines (m) with the non-epistatic model is obtained with just 1 suppressor ($\mu = 0.34$), and then still only three out of nine introgression lines (numbers 4, 5, and 8 in Table 2, last column) do not significantly differ from the expected rescue frequency ($\mu = [1 - 0.96(1/2)] / [2 - 0.96(1/2)] = 0.34$, according to (4)), since there are simply too few hybrid males in most lines. Therefore, lines 1,2,6,7,9 in table 2 allow the rejection of all non epistatic models ($\mu \geq 0.34$). Non-redundant epistatic models ($n = r$) provide a much better fit to observed rescue frequencies, both for two ($\mu \cong 0.22$) and three ($\mu \cong 0.14$) interacting supressors (see Table 2, 4th and 5th columns, respectively; see Table 3 for expected frequencies under different epistatic models), although a few lines depart significantly from expectations, reflecting the significant heterogeneity observed in m values among BC1 lines. Therefore, line 9 of table 2 allows the rejection of $\mu = 0.22$ ($n = r = 2$ model) and line 8 of the same table allows the rejection of $\mu = 0.14$ ($n = r = 3$ model). This heterogeneity is easily explained if redundant epistatic models ($n > r$) apply, simply by the expected segregation of supressors among F1 offspring males (i.e. male parents in BC1 cross). Therefore, if five suppressors (the maximum number we can detect with our experimental approach) inter-

Table 3. Expected frequencies of hybrid males (μ) in the offspring of test crosses (BC1 or BC2, see Material and Methods), for different epistatic models. A maximum of 5 autosomal suppressors (one per autosome) of the *hmi-1* lethal factor is assumed to be in heterozygous condition in the tested genotype (always the male sex, according to our protocol); the transimission of any combination of at least r of these factors brings about full rescue of adult *hmi-1* hybrid males in the offspring. E_i states refer to i suppressors in heterozygous condition in the male progenitor.

Epistatic model		μ	Epistatic model		μ
E_i	r		E_i	r	
5	5	0.065	4	3	0.25
5	4	0.18	4	2	0.41
5	3	0.34	3	3	0.14
5	2	0.45	3	2	0.34
4	4	0.09	2	2	0.22

Table 4. Observed frequencies of w^+ males (m) in BC2 offsprings from the BC1 cross produced by line 8 in Table 2. Sample size is the total number of w^+ (i.e., hybrid) offspring recorded. Standard deviates of the normal approximation to the binomial distribution, under different null hypotheses ($\mathbf{H}_{\mu=\mu o}$), are indicated for each line. * = significant departures from the null hypothesis at the table-wide 0.05 level, after sequential Bonferroni tests.

Line	m(sample size)	$Z_{\mu = 0.04}$	$Z_{\mu = 0.22}$	$Z_{\mu = 0.14}$	$Z_{\mu = 0.25}$	$Z_{\mu = 0.34}$
1	0.07 (41)	0.98	–2.32	–1.29	–2.66*	–3.65*
2	0.07 (57)	1.16	–2.73*	–1.52	–3.14*	–4.30*
3	0.00 (43)	–1.34	–3.48*	–2.65*	–3.79*	–4.71*
4	0.07 (82)	1.39	–3.28*	–1.83	–3.76*	–5.16*
5	0.21 (146)	9.87*	–0.29	2.44	–1.12	–3.32*
6	0.14 (37)	3.10*	–1.17	0.00	–1.54	–2.57*
7	0.00 (24)	–1.00	–2.60*	–1.98	–2.83*	–3.52*
8	0.08 (13)	0.73	–1.22	–0.62	–1.41	–1.98
9	0.19 (27)	3.98*	–0.63	0.75	–0.72	–1.64
10	0.17 (29)	3.57*	–0.65	0.47	–0.99	–1.93
11	0.10 (39)	1.91	–1.81	–0.72	–2.16	–3.16*
12	0.30 (45)	8.90*	1.29	3.09*	0.77	–0.57
13	0.27 (56)	8.78*	0.90	2.80*	0.35	–1.33

acting in groups of any three are segregating among F1 offspring males, three different m values (for 5, 4 and 3 suppressors inherited) are expected in BC1 lines (see Table 3, $\mu = 0.34$ for state E_5, $\mu = 0.25$ for E_4, and $\mu = 0.14$ for E_3). Neither of these frequencies alone can explain BC1 results (see Table 2, three last columns, for tests of the corresponding hypotheses), but their combination easily can.

In contrast to BC1 lines, a significant rescue of adult hybrid males was observed only in 6 out of 13 BC2 offsprings ($p = 0.46$, Table 4, 3rd column). Hybrid male frequencies (m) in these six lines ranged from 0.14 to 0.30 ($\overline{m} = 0.21$). $\chi^2_H = 5.09$, 5 df, $P = 0.4$.

Since all male parents (*white* phenotype) for BC2 crosses came from the same BC1 offspring (BC1-8), there should be no heterogeneity in the initial distribution of states, so that (2):

$$\gamma^{(1)} = \gamma^{(0)}\,\wp^1 = (0,\ldots,0,1)\,\wp = (\gamma_{n0},\ldots\,\gamma_{nr},\ldots\,\gamma_{nn})\,,$$

where n corresponds to the total number of suppressors borne in heterozygosis by the grandfather (the *white* male that fathered BC1-8), and γ_{nj} refers to the transition probabilities from state n to j. If, as before, r designates the minimum number of suppressors that should be present in the genome of a hybrid male for it to be viable as adult, then at BC2 the expected frequency of lines displaying significant rescue activity (5) would be given simply by:

$$\pi = \sum_{j=0}^{n}\gamma_{nj} - \sum_{j=0}^{r-1}\gamma_{nj} = 1 - \sum_{j=0}^{r-1}\gamma_{nj} \qquad (8)$$

Table 5 shows the results of testing different epistatic models against the observed value of p (0.46). First, for non-redundant models, the highest expected frequency of rescue lines is obtained for two interacting chromosomes ($n = r = 2$), namely $\pi = 0.25$, which is slightly significant with respect to the observed value ($z = 1.75$, $P = 0.04$). By other side the expected m value under this model is, from (6) and (7) with $i = 2$, $m = 0.22$ which fits well with all rescue lines in table 4 (lines 5,6,9,10,12,13) but as we saw falls in line 9 (and line 3) of BC1 lines (table 2). Also, lines 12, 13 in table 4 allow the rejection of $\mu = 0.14$ ($n = r = 3$ model) and lines 5,6 in the same table allow the rejection of all non epistatic models ($\mu \geq 0.34$). Thus, although we can not discard a sample size effect with respect the hypothesis $n = r = 2$, a better explanation of our data comes with $n > r$ redundant epistatic systems. Four different cases of combinations of n factors taken $\geq r$ at a time ($\mathbf{C}_{(n,\,\geq r)}$) were not significant at a table-wide 0.05 level, namely (5, ≥3), (4, ≥3), (4,≥2), and (3, ≥2) (see Table 5). As to rescue frequencies within BC2 lines (m), all observations can be explained by any of these combinations (Table 4). However, if lines from BC1 and BC2 are taken altogether, only the expectations from $\mathbf{C}_{(5,\,\geq 3)}$ and $\mathbf{C}_{(4,\,\geq 3)}$ are always met, whereas $\mathbf{C}_{(4,\,\geq 2)}$ and $\mathbf{C}_{(3,\,\geq 2)}$ fail to explain the observations from lines 3 and 9 in BC1.

Table 5. Expected frequencies of viability rescue lines (π) obtained after a test cross (see Material and Methods), for different kinds of redundant epistatic models ($n>r$) models. E_n states refer to n suppressors in heterozygous condition in the tested genotype (the grandfather of all the offsprings examined in the lines); any combination of at least r of these factors brings about full rescue of *hmi-1* hybrid males. The goodness of fit between these expected frequencies and the experimental value obtained from BC2 lines in Table 4 ($p = 0.46$) is also shown.

Epistatic model E_n	r	π_o	$\chi^2_{\pi=\pi_o}$, 1df
5	4	0.19	4.59*
5	3	0.50	≈ 0 ns
5	2	0.81	8.12**
4	3	0.31	0.78 ns
4	2	0.69	2.19 ns
3	2	0.50	≈ 0 ns

ns: $P > 0.05$; *: $0.05 > P > 0.01$; **: $0.01 > P > 0.001$

DISCUSSION

In this work the polymorphism for hybrid male rescue factors has been investigated in a series of *D. buzzatii* stocks, both from Argentina and Spain, after experimental crosses with an introgression line bearing a hybrid-lethal factor from its sibling *D. koepferae*. Among spanish stocks only a single one (buz M, table 1) showed a significant, although slight, viability rescue effect. Stocks from Argentina displayed strong differences in their hybrid male rescue activity. Therefore, buzSL5(1) had the highest effect (all replicates produced hybrid male rescue), buzSL-In(4)s had a partial effect (significant increase of hybrid male viability was detected only in a few replicates), whereas buzSL-In(5)1 and buzSL6 had no effect at all. These differences indicate the probable existence of polymorphism for hybrid male rescue factors in the natural population of San Luis, where the founding flies of these stocks were sampled. This polymorphism may have been lost during colonisation of the Old World by *D. buzzatii* (this species originated in South America, and then was spread to the Old World and Australia; Ruiz *et al,* 1982).

The existence of a polymorphism for rescue factors in *D. buzzatii* means that the effect of the introgression of the hybrid lethal factor *hmi-1*from *D. koepferae* will depend on the genetic background. Examples of conditional viability have long been known in Drosophila, such as can be found in the works by Sturtevant (1929), Watanabe *et al.* (1977) and Lee (1978), where the viability of hybrid females from the cross between *D. melanogaster* females

typically associated to developmental instabilities (Mϕller & Swaddle, 1997), has also been observed in interespecific hybrids (Carvajal, 1996; Wade *et al.*, 1997) and consistently actually appear in several *buzzatii-koepferae* introgression lines (H. Naveira personal communication). It could well be the case that the systems of complementary genes whose disruption brings about hybrid inviability consist of regulators occupying relatively lower levels in the developmental hierarchy, which usually manifest functional redundancy (Krumlauf, 1992).

Finally, it must be stressed that the estimate of the number of lethal-suppressors obtained in this paper should be confirmed by appropriate crosses using chromosome markers. Those results constitute simply an estimate of the minimum number of linkage-groups affecting the character (hybrid male rescue), and do not give information on the nature of the genetic factors involved (either polygenes or major genes). Anyway, it must be remembered that hybrid inviability factors reported so far generally correspond to major genes, whereas most sterility factors behave as polygenes (Maside *et al.*, 1998).

I thank Horacio Naveira for many comments on the original manuscript. My research was supported by a scholarship from Universidade da Coruña, and grants 10305B95, 10304B97 and PGIDT 99BIO10302 from the "Xunta de Galicia" to H. N.

REFERENCES

Carson, H. L., and Templeton, A. R. (1984) Genetic revolutions in relation to speciation phenomena: The founding of new populations. Ann. Rev. Ecol. Syst. **15**, 97–131.

Carvajal, A. R. (1996) Developmental disruptions in hybrids between *D. buzzatii* and *D. koepferae*. Int. J. Dev. Biol. Supplement **1**, 93–94.

745–754.
Møller, A. P., and Swaddle J. P. (1997) Asymmetry, developmental stability, and evolution. Oxford Univ. Press, Oxford, U.K.
Naveira, H. F., and Fontdevila, A. (1985) The evolutionary history of *D. buzzatii* IX. High frequencies of new chromosome rearrangements induced by introgressive hybridization. Chromosoma **91**, 87–94.
Naveira, H. F., and Fontdevila, A. (1991) The evolutionary history of *D. buzzatii* XXII. Chromosomal and genic sterility in male hybrids of *D. buzzatii* and *D. koepferae*. Heredity **66**, 233–239.
Naveira, H. F., Pla, C., and Fontdevila, A. (1986) The evolutionary history of *Drosophila buzzatii* XI. A new method for citogenetic localisation based on asynapsis of polytene chromosomes in interspecific hybrids of *Drosophila*. Genética **71**, 199–212.
Nei, M., and Zhang, J. (1998) Molecular origin of species. Science **282**, 1428–1429.
Rice, W. R. (1989) Analyzing tables of statisticals tests. Evolution **43**, 223–225.
Ruiz, A., and Fontdevila, A. (1981) Two new chromosome arrangements in *Drosophila buzzatii*. DIS **56**, 111–114.
Ruiz, A., Fontdevila, A., and Wasserman, M. (1982) The evolutionary history of *D. buzzatii*. III. Cytogenetic relationships between two sibling species of the *buzzatii* cluster. Genetics **101**, 503–518.
Sturtevant, A. H. (1929) The genetics of *Drosophila simulans*. *Carnegie Inst. Washington Publ*., **399**, 1–62.
Tautz, D. (1992) Redundancies, development and the flow of information. ByoEssays **4**, 263–265.
Wade, M. J. Johnson, N. A. Jones, R. Siguel, V., and Mcnaughton, M. (1997) Genetic variation segregating in natural populations of *Tribolium castaneum* affecting traits observed in hybrids with *T. freemani*. Genetics **147**, 1235–1247.
Watanabe, T. K., Lee, W. H., Inoue, I., and Kawanishi, M. 1977. Genetic variation of the hybrid crossability between *Drosophila melanogaster* and *Drosophila simulans*. Jpn. J. Genet. **52**, 1–8.
Wu, C-I., and Palopoli, M. F. (1994) Genetics of postmating reproductive isolation in animals. Annu. Rev. Genet. **28**, 283–308.