A polygenic basis of hybrid sterility may give rise to spurious localizations of major sterility factors

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A simple model is presented to illustrate how an underlying generalized polygenic basis of hybrid sterility is expected to lead to spurious localizations of factors with major effects, when a conventional experiment of recombination mapping is carried out. The model shows that a major gene will be detected at roughly the same distance from each of the chromosome markers used in the experiment. These expectations are contrasted with the results from several experiments on hybrid male sterility in *Drosophila*, which claimed to have mapped single sterility factors. It is concluded that, except for one report, all the evidence presented so far on the genetic basis of hybrid male sterility in *Drosophila* is in fact compatible with the generalized polygenic model.

Keywords: *Drosophila*, hybrid males, major genes, polygenes, recombination mapping, sterility.

Introduction

One of the main achievements that can be conceived nowadays in the field of evolutionary genetics is perhaps the molecular isolation of the genetic factors that underlie the reproductive barriers among species. As in many other fields of genetical research on eukaryotes, it is in *Drosophila* where the genetic basis of reproductive isolation, particularly of the postmating kind, is best understood (Wu & Palopoli, 1994). In recent years, several experiments have been reported that claimed to have arrived at a precise localization on the *Drosophila* genetic map of some of the factors involved in hybrid male sterility (Coyne & Charlesworth, 1986, 1989; Pantazidis & Zouros, 1988; Orr, 1992; Pantazidis *et al.*, 1993; Perez *et al.*, 1993). These experiments were intended as a first necessary step towards the molecular isolation of the mapped factors. However, that ultimate objective has not been reached in any of the cases referred to. In all those experiments, hybrid male sterility factors were assumed to be genes of major effects on the phenotype (spermatogenesis), i.e. factors whose segregation after having been introgressed in the receptor species should be

easily diagnosed by the fertility of hybrid males. As opposed to this idea, a different paradigm has been emerging, which states the possibility that hybrid male sterility factors are minor genes, or polygenes, with a cumulative effect on the phenotype. That is, the introgression of any single one of these factors is expected to have no phenotypic effect; only after the introgression of a 'sufficient' number of them, is the hybrid male expected to be sterile. This idea is not really new; it was first stated by Pontecorvo (1943), but it has been brought to present attention mostly by the experiments made in recent years with hybrids between *D. buzzatii* and *D. koepferae* (Naveira & Fontdevila, 1986, 1991a,b). With this alternative polygenic hypothesis in mind, new experiments with hybrids between species of the *simulans* clade have been made in the last few years, with the intention of showing whether the results that had seemed to indicate the existence of major genes of hybrid male sterility in these species could result instead from the action of multiple minor genes acting in concert. The results of these experiments have been quite revealing. Thus, although it has not been possible to discard absolutely the existence of the major factors inferred by Coyne & Charlesworth (1986, 1989) close to the loci *white* and *forked* on the X chromosome of *D. mauritiana*, it has been clearly

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shown that the chromosome regions associated with these markers do contain an indeterminate number of interacting minor factors: two introgressions that separately do not cause sterility, frequently give rise to it when they are recombined to be both present in the hybrid male genotype (Naveira, 1992). Also, it has been demonstrated that another formerly reported major factor, also linked to the X chromosome, the gene called *Odysseus* (Perez *et al.*, 1993), is not really enough by itself to produce the sterility of hybrid males when introgressed in a sibling species, but rather that it needs the cointrogression of a chromosome segment bearing an indeterminate series of other factors (Perez & Wu, 1995). Similar results for other X-linked factors have been reported by Wu and coworkers (Cabot *et al.*, 1994; Palopoli & Wu, 1994). Therefore, it may be concluded that the polygenic hypothesis enjoys nowadays a rather convincing body of supportive evidence, and that only two of the reports of major genes of hybrid male sterility have not been seriously challenged so far by a polygenic interpretation. One of them corresponds to the *hms* gene localized by Orr (1992) on the dot chromosome of *D. simulans*, which brings about male sterility when introgressed as a homozygote into the *D. melanogaster* background, and the other relates to the sperm motility factor (SMF) identified in the hybrids between *D. arizonae* and *D. mojavensis* (Pantazidis & Zouros, 1988; Pantazidis *et al.*, 1993). It is the evidence concerning this last factor that we would like to discuss in detail, under the new light of the polygenic hypothesis.

It is known that males with the Y chromosome of *D. arizonae* in a *D. mojavensis* background are sterile, unless a chromosome IV from *D. arizonae* is cointrogressed. Zouros and coworkers assigned this rescuing effect to a single Mendelian factor, the SMF gene. They mapped SMF to a position equidistant from two chromosome markers, located at the ends of chromosome IV. But we think that we can show that this is exactly what is expected under a generalized polygenic model.

The model

One of the main observations that supports a polygenic model is the association of hybrid male sterility to the size of the introgressed chromosome segments. Males are sterile when the size of the chromosome regions received from the donor species exceeds a critical threshold (Naveira & Fontdevila, 1986, 1991b); what is more, the abnormalities observed in the spermatogenesis of hybrid males are

correlated with the size of the introgressions (Naveira & Fontdevila, 1991a). This generalized polygenic model explains these findings by postulating: (i) the existence of a very large number of factors randomly dispersed all over the chromosome; (ii) that each factor makes a relatively minor contribution to sterility; and (iii) that the factors from two different species are incompatible.

Now, let us assume that we have two marker loci, *A*, with alleles *A* and *a*, and *B*, with alleles *B* and *b*, located at each of the two ends of a chromosome whose total map length, in real map units, is *R*. Furthermore, let us assume that crossing-over can occur with equal probability along the chromosome. As an initial simplification, let us allow no more than one single crossover per meiosis for that chromosome pair. Finally, let us say that hybrid male sterility will be produced not by a discrete gene of relatively large effect, but by any chromosome section of at least *X* map units (Fig. 1). Now let us see what would happen if we applied the conventional experimental design (test cross) to map a putative gene for hybrid male sterility located somewhere in between *A* and *B*. One of the two species will be homozygous dominant (wild-type, *AB/AB*), the other homozygous recessive (mutant, *ab/ab*). In the *F*₁ (*AB/ab*) the males will be sterile, but the females will be fertile and will be backcrossed to the homozygous recessive parent (*ab/ab*). Finally, the backcross male offspring will be screened for recombinants between the marker loci and the hybrid sterility character. Hybrid male sterility will be produced whenever the contributing gamete bears more than *X* map units of the *AB* chromosome. According to this scenario (Fig. 1, for $R > 2X$), the following gametes are expected (*fer* = fertile; *ste* = sterile):

if crossing-over in interval (0, *X*): *A fer b*, *a ste B*;
 if crossing-over in interval (*X*, $R - X$): *A ste b*, *a ste B*;
 if crossing-over in interval ($R - X$, *R*): *A ste b*, *a fer B*;
 no crossover (parental types): *A ste B*, *a fer b*.

Now we can proceed to compute four different map distances from our marker alleles to a putative sterility gene, which are a function of corresponding conditional probabilities:

- 1 distance from *A* to *ste* = $f[P(A \text{ fer}/A)] = X$;
- 2 distance from *a* to *ste* = $f[P(a \text{ ste}/a)]$
 $= X + (R - 2X) = R - X$;
- 3 distance from *B* to *ste* = $f[P(B \text{ fer}/B)] = X$;
- 4 distance from *b* to *ste* = $f[P(b \text{ ste}/b)] = R - X$.

That is, distance (1) would be equal to distance (3), and distance (2) equal to distance (4). Therefore,

Fig. 1 Representation of the male sterility or fertility outcomes from crossing-over in the meiosis of a female hybrid, heterozygous for two marker loci, A/a and B/b , under a generalized polygenic model. Each rectangle represents a chromosome, with the black filling designating the introgression from the donor species, marked by the dominant alleles. Distance along the chromosome is indicated in the hybrid female by a scale from 0 to R real map units (total distance separating the two marker loci). Assuming the female is back-crossed with males of the species marked by the mutant alleles, offspring males will be sterile whenever the gamete contributed by the female bears at least X map units of the AB chromosome (black portion of the rectangles). Only single crossovers are considered.

two putative 'genes' for hybrid sterility (which, in fact, we know do not exist) would be, firstly, spuriously located at the same distance from locus A and locus B . Secondly, the distances would be different if we considered the dominant or the recessive alleles. And thirdly the distances would provide a direct estimation of the minimum size of the introgressed chromosome segment that is actually producing the hybrid male sterility.

From equations (1) to (4) it is clear that the first result is independent of the minimum size for hybrid sterility. It can also be shown to remain valid after dropping the restriction of allowing at most one single crossover, because double crossing-over between the same two chromatids does not produce recombination between the marker loci, and the results of any other kind of multiple crossing-over can be reduced to a combination of these two simple cases. The validity of the second result is somewhat more restricted: it depends on the assumptions about the minimum size for hybrid sterility.

When the minimum size is near 50 per cent of the AB chromosome, the distances from the dominant alleles approach the distances from the recessive alleles, and a single sterility 'gene' would be located just in the middle of the chromosome, between the two marker loci. This result can be shown to be in fact much more general. Linkage map distance to a marker locus is generally not calculated separately for wild-type and mutant alleles, but by averaging

the distance to both alleles. If we do this, a single sterility 'gene' would be always spuriously located exactly in between the two marker loci of the model. From equations (1) to (4):

$$\begin{aligned} \text{distance from locus } A \text{ to sterility gene} \\ &= \frac{1}{2}(X) + \frac{1}{2}(R-X) = R/2; \\ \text{also, distance from locus } B \text{ to sterility gene} &= R/2. \end{aligned}$$

Discussion and conclusions

The results of Pantazidis & Zouros (1988) place the SMF almost exactly in the middle between the two markers, with the distances from the alleles marking the *D. mojavensis* chromosome segments (equivalent to the wild-type alleles in our model) being slightly smaller than the distances computed from the *D. arizonae* markers (equivalent to the mutant alleles). This is exactly what would be expected according to equations (1) to (4) for a minimum size for hybrid sterility somewhat smaller than 50 per cent of the chromosome. Our model is slightly different from the experimental design of Pantazidis & Zouros because they crossed hybrid females with hybrid males. Nevertheless, as pointed out by themselves (p. 301, part B of Table 1), the genotypic classes they used to compute the map distances do indeed correspond to a test cross, similar to the one modelled here. The conclusions we have derived are entirely valid for their case, assuming only that in

their hybrids the sterility would be produced by the introgression of a large enough segment of the IV_{mojavensis} chromosome in the genetic background IV_{mojavensis}, Y_{arizonae}. One possible criticism to our interpretation of these results would be that only chromosome IV appears to be involved in this interaction with the heterospecific Y, whereas the generalized polygenic model would predict a contribution from all autosomes. However, it has been found that in *buzzatii/koepferae* hybrids there may be a certain heterogeneity among chromosomes in what we called the threshold-size for sterility (Naveira & Fontdevila, 1991a), which is also manifest in the degree of spermatogenic abnormalities associated with each size-interval of the introgressed chromosome segments (Naveira & Fontdevila, 1991b); particularly noteworthy in this respect is that also in these hybrids the chromosome IV (homologous to the IV in *mojavensis/arizonae*) seems to make a higher relative contribution to sterility, in terms of size, than any of the other studied autosomes. If our interpretation is correct, the fertility of hybrid males introgressed with the Y_{arizonae} should be affected by other *mojavensis* autosomes, besides number IV, being in the homozygous state. Former results by Vigneault & Zouros (1986) show that this may be exactly the case (sperm motility of these hybrids very often requires both chromosomes III and IV to be in the heterozygous state). Therefore, although it is still possible that after all SMF is a 'good' major factor of hybrid male sterility, it should be admitted that the evidence so far presented has remarkable coincidences with the expectations from a generalized polygenic model.

Other predictions of this model are verified in the case of the hybrids between *D. simulans* and *D. mauritiana* or *D. sechellia*. Coyne & Charlesworth (1989) claimed to have localized major genes of hybrid male sterility closely linked to each of the species-specific X chromosome markers they used. Estimates of recombination frequencies between each marker and the putative sterility locus lying close by were very similar within each species pair (their Table 2). This is precisely what is expected under a generalized polygenic model: a major gene of hybrid sterility would be spuriously located at the same distance from each of the chromosome markers used in the mapping experiment. It can be argued that the results of Coyne & Charlesworth were derived from repetitively backcrossed hybrids, whereas the model we have presented is formally equivalent just to a first backcross. However, the length of intact chromosome segments around a locus held heterozygous during successive backcrosses

decreases rather slowly after the initial generations (Naveira & Barbadilla, 1992); for example, after ten backcrosses, the number of generations examined by Coyne & Charlesworth, the average intact size (i.e. the length of the chromosome segment from the donor species) is still of slightly more than 20 map units, meaning that more than a quarter of the X chromosome around the marker locus is still from the donor species in this generation. Provided that the intact size exceeds the minimum size for hybrid sterility, the conclusions of our model will be applicable also to backcrosses.

To try to reconcile our own results with the evidence provided by other workers in the same field, making an effort to see the implications of the different hypotheses, is an important scientific task. Only in that way we will be able to advance our knowledge of the living world.

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