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Meiotic imbalance in laboratory-produced hybrid males of *Chorthippus parallelus parallelus* and *Chorthippus parallelus erythropus*

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Summary

The grasshopper *Chorthippus parallelus* has two quite distinct subspecies, which meet along the Pyrenees forming a hybrid zone. Using silver staining we show that on the French side *Cp. parallelus* has three nucleolar organizer regions, on the L_2, L_3 and X chromosomes, while on the Spanish side *Cp. erythropus* has only two NORs, on the L_2 and L_3. Laboratory F1 hybrid males show reciprocal differences in the expression of NORs. When a *Cp. erythropus* is female parent the male progeny show four active NORs in mitotic cells and two silver precipitates in meiotic cells, as expected. But when a *Cp. parallelus* female donates the X with a NOR, her male offspring have a variable disrupted nucleolar expression. Some NORs are not expressed and extra sites of cryptic rDNA are revealed. Meiosis is more disturbed in this latter F1 cross with higher levels of polyploidy, but both F1s show around 90% spermatid abnormality. Such variation in rDNA expression is also found in individuals collected from the hybrid zone, and the role of this disturbance in affecting fitness is discussed.

1. Introduction

Under an allopatric model of speciation populations diverge genetically during a period of isolation. This may well occur during a contraction in the range of the species. Populations which have experienced some such divergence in isolation may then expand because of changes in the environmental conditions and meet to form hybrid zones. Because of the genetic divergence, pre- or post-zygotic barriers to gene flow may be present at this secondary contact. These barriers may involve differences at many loci, and these will have interactions between them which may affect fitness in a number of ways. Thus many aspects of phenotype can be involved from nuclear and mitochondrial DNA, through chromosomal, enzymatic and morphological traits to behavioural characters such as song. Differences in population structure and individual movement may also be important for the structure and evolution of these zones (Barton & Hewitt, 1989; Hewitt, 1988 for review). In some cases these barriers may be asymmetric so that some genes pass further in one direction. It is therefore necessary to understand any particular system in some detail in order to develop its general significance.

Two distinct subspecies of the meadow grasshopper *Chorthippus parallelus* (*Cp.*): *Cp. parallelus* and *Cp. erythropus* form a hybrid zone in the Pyrenees, which can be interpreted in terms of population movements during the Quaternary period. Because of the recent ice ages the European distribution retreated to different southern refugia and evolved differently in the absence of gene flow. As the glaciers retreated these populations expanded across Spain and France up the Pyrenean valleys and met (Hewitt, 1989). Such secondary contacts are found in a series of cols along the Pyrenees where hybrid populations between both subspecies can be found. A series of papers have reported differences between *Cp. parallelus* and *Cp. erythropus* in morphological, behavioural, electrophoretic and chromosomal characters along with male sterility in F1 hybrids (Butlin & Hewitt, 1985a, b; Hewitt *et al.* 1987, 1988; Gosálvez *et al.* 1988; Ritchie *et al.* 1989). These many differences reflect the divergence that has occurred between these two subspecies. It is clearly important to investigate the cytogenetic basis of the F1 male sterility, so that we may better understand its effect on the structure, maintenance and origin of the contact zones in different cols of the Pyrenees. This should also reveal something of the causes of hybrid dysgenesis observed in the hybrid males.

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One of the main differences between *Cp. parallelus* and *Cp. erythropus* is the localization of active rDNAs (Gosálvez *et al.* 1988; Hewitt *et al.* 1988). In both subspecies autosomes L2 and L3 have active rDNA, but in *Cp. parallelus* the X chromosome also has active ribosomal cistrons which are absent from *Cp. erythropus*. We can produce two types of hybrids, one using pure female *Cp. parallelus* with a pure male *Cp. erythropus* (*a*) the other using a pure female *Cp. erythropus* with a pure male *Cp. parallelus* (*b*). Given that sex determination in this species is XX female/XO male we expect crosses from (*a*) to give rise to hybrid males carrying a presumably active NOR, while in crosses of type (*b*) we must expect male progeny without an active NOR on the X chromosome. This experimental approach has been used to examine NOR expression in F1 males and to investigate the influence that this may have on the process of meiosis.

2. Material and methods

Adult F1 hybrid males between *Cp. parallelus* and *Cp. erythropus* were obtained from reciprocal crosses by using pure *Cp. parallelus* and *Cp. erythropus* individuals collected in the field (both sides of Col de Portalet, France–Spain). Testes were removed, fixed in 3:1 ethanol–acetic acid and stored under refrigeration. When needed they were disrupted and squashed in 45% acetic acid, and liquid nitrogen was used for freezing cover slips before removal with a razor blade. The slides were air-dried overnight.

For silver staining a 50% solution of NO_3Ag in distilled water (adjusted to pH 3 with formic acid) was used. Slides were incubated with a drop of this solution in a moist chamber at 60 °C for 5 min, then subsequently washed in distilled water and counterstained with a 1% solution of Giemsa in phosphate buffer (pH 6·8) before mounting. Observations were carried out in a Zeiss Photo III microscope and photographs taken with Kodak Plus-X film.

3. Results

The pattern of nucleolar activity in the pure races revealed by silver staining is as follows. In *Cp. erythropus* four silver precipitates are observed in gonial interphase, and two in early meiotic prophase (Fig. 1 *a*, *b*). *Cp. parallelus* shows five silver precipitates in gonial interphase and three in meiotic prophase (Fig. 1 c, d). Such observations correspond to unpaired (gonial interphase) or paired (meiotic) chromosomes. Pure individuals produce between 4–6% of abnormal sperm (Fig. 1 *e*).

The NOR activity observed is quite different in the two types of crosses, and furthermore is different from that expected when the female is a pure *Cp. parallelus*.

In detail, F1 males from *Cp. erythropus* females have four active NORs in all cells; these are visible as four silver precipitates in gonial interphase cells (Fig. 2*a*), or as two silver precipitates in meiotic prophase (Fig. 2*b*). The proportion of abnormal sperm in all the individuals is around 90% (Fig. 2*c*). Results of F1 hybrid males from crosses using a female *Cp. parallelus* are hard to disentangle, there being no common pattern of nucleolar expression. In interphase cells there is large variation in the number of silver precipitates, ranging from three to eight (Fig. 3*a*). Five, the number expected from the known number of NORs in diploid cells, was rarely found. Cells with less than five nucleoli implies that some of them are not being expressed, whilst a higher number implies that extra active NORs are present. This apparently anarchic nucleolar expression in gonial cells is also observed in meiosis. In general the meiocytes of these F1 hybrids show higher levels of ploidy than the normal rate observed in pure *Cp. parallelus* and *Cp. erythropus* individuals, which is not higher than 1%.

The level of ploidy can be quantified by the size of nuclei and also by the number of heteropycnotic X chromosomes per cell (Fig. 3*b*). However, the number of Xs per cell is not always clear because of their irregular allocyclic behaviour. We found extreme cases where no heteropycnotic X chromosomes were observed (Fig. 3*e*). For each level of ploidy we expect a certain number of nucleoli, but the number observed often does not correspond to the number expected of silver precipitates. Most of cells show a variable number of nucleoli ranging from none to five. In general, whatever the level of ploidy of the cell, meiotic prophases have smaller amounts of ribosomal RNAs than expected in such cells, and in some cases only remnants of one active nucleolus are clearly detectable (Fig. 3*e*). The number of abnormal spermatids in these hybrids is high around 90%, similar to the previous reciprocal cross.

4. Discussion

A comparison of the results of these experiments shows clear differences in the meiotic behaviour of the hybrid genomes depending on which subspecies was used as the female parent. However, the fitness of both types of hybrids seems to be similar, as measured by their production of dysfunctional sperm. We are reminded of the results obtained by Dobzhansky (1934) and Dobzhansky & Boche (1933) in interspecific hybrids between *Drosophila pseudoobscura* and *D. persimilis*. In those experiments, the use of a

Fig. 1. NOR activity in pure *Cp. erythropus* (*a*, *b*) and *Cp. parallelus* (*c*, *d*) males. (*a*) and (*c*) are gonial cells at interphase showing four (*Cp. erythropus*) and five (*Cp. parallelus*) active and unpaired nucleoli (arrows). (*b*) and (*d*), early meiotic prophases showing two (*Cp. erythropus*) and three (*Cp. parallelus*) fused nucleoli as effect of the chromosome pairing (arrows). The open arrow shows the X chromosome. (*e*) Normal sperm from a *Cp. parallelus* showing a polyploid spermatid (arrow).

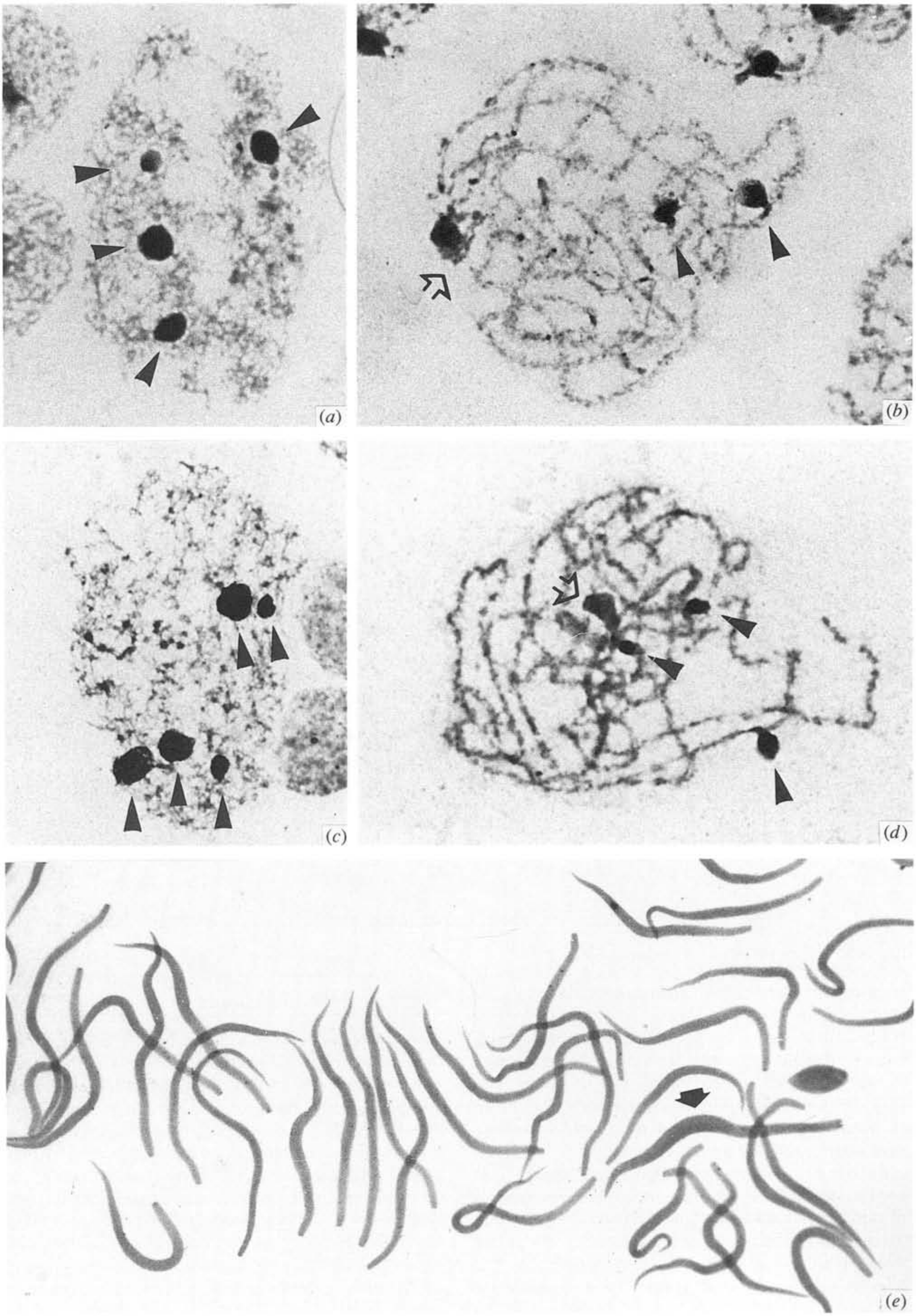

Fig. 1. For legend see opposite.

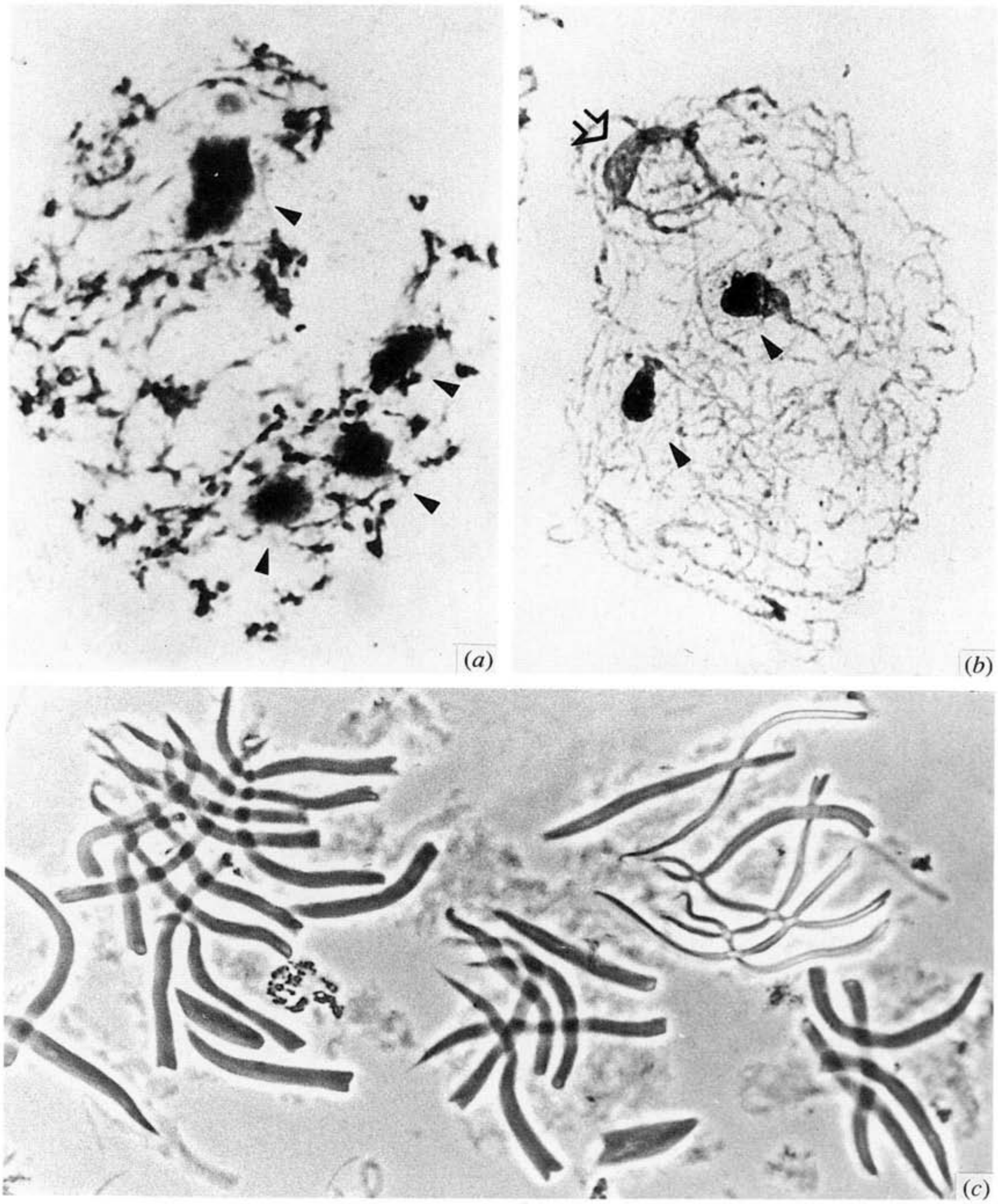

Fig. 2. F1 hybrid male from a cross between a *Cp. erythropus* female and a *Cp. parallelus* male. Four nucleoli are present in gonial cells (*a*) and two in meiocytes (*b*). The open arrow shows the X chromosomes. (*c*) A group of elongated spermatids showing abnormal shape for almost all nuclei.

D. pseudoobscura female parent produced males with testes of normal size, while those from the reciprocal mating were of reduced size. The cytology was very different between these reciprocals, but as in *C. parallelus*, both reciprocal males in *Drosophila* were entirely sterile.

In F1 *C. parallelus* the genomes interact and produce similar levels of abnormal sperm regardless of the female used as parent. However, the fact that the meiotic process is more affected when the parent female is *Cp. parallelus* has further implications. The interactions in a hybrid between the two autosome sets are certainly complex and there are many possible types of epistatic effects between different gene loci that could be occurring. In our case, it is clear that these interactions are primarily dependent on which subspecies sex chromosome is present in the hybrid genome. An X-chromosome-carrying rDNA produces a more disturbed meiotic process, involving amphiplastic responses. But it does not mean that this X chromosome is the only thing responsible for the disturbances. The genetic control of nucleolar activity is a multistep process with many genes involved in its regulation (Flavell & Martini, 1982). The genetic information of a *parallelus*-X affects the production of rRNA in the hybrid cells while an *erythropus*-X does

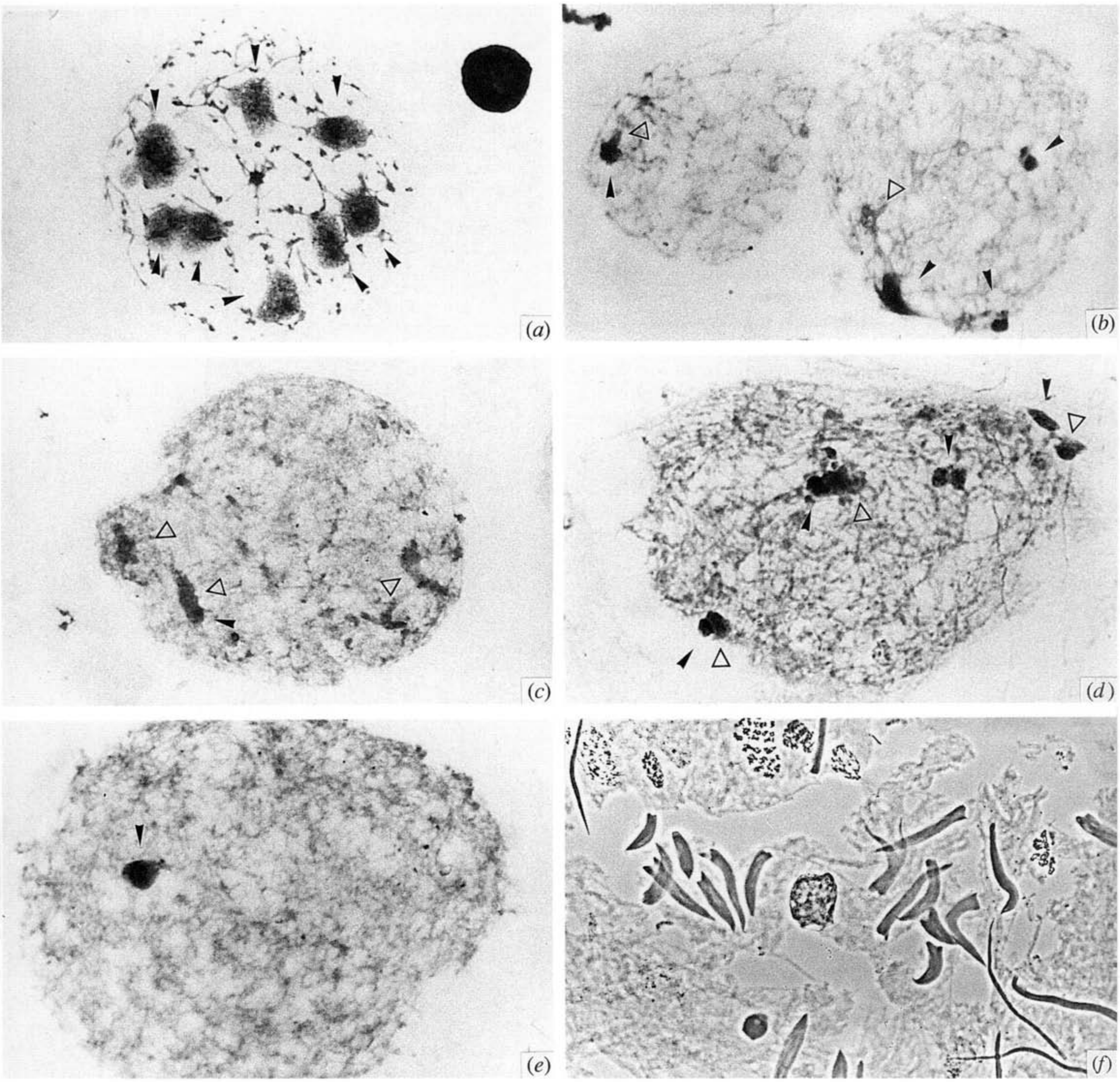

Fig. 3. F1 hybrid male from a cross between a *Cp. parallelus* female and a *Cp. erythropus* male. (*a*) Gonial prophase showing eight nucleoli. (*b*) Two meiotic prophases from the same cyst. The cell on the left is diploid and only shows a nucleolus attached to the X chromosome (open arrow). The cell on the right is probably tetraploid. Three nucleoli and a single heteropycnotic X are showed. (*c*) Polyploid meiocyte showing three heteropycnotic X chromosomes and a single one carrying remnants of the nucleolar activity. Note the absence of silver precipitates in the autosomes. (*d*) Polyploid cell with three heteropycnotic X chromosomes all of them showing nucleolar activity as well as one of the autosomal pairs. (*e*) Polyploid meiocyte showing absence of heteropycnotic X chromosomes. There is just a single rDNA active site in this cell. (*f*) Abnormal sperm.

not apparently disturb normal production. It is worth contrasting this with the amphiplastic situation found in interspecific hybrids from other organisms, especially that concerning NOR expression. There the suppression of NOR activity seems to be quite stable, always affecting the same set of chromosomes in interspecific hybrids (Miller *et al.* 1976; Lacadena *et al.* 1988). In our case, however, the expression varies from one cell to another within the same individual. This, and the fact that the X chromosome varies in number and cycle of pycnosis, produces an unusually complex pattern.

The occasional finding of more than five silver precipitates per diploid cell is of great interest, since this is indicative of cryptic rDNA which is expressed under disturbed or relaxed regulation. This result fits with our previous observations of chromosomes carrying two active NORs per chromosome in individuals collected from natural populations in the contact zone through the Col de Portalet in the Pyrenees (Gosálvez *et al.* 1988). Variation in the level of individual primary NOR expression was also found in individuals of this contact zone. These results indicate that malcontrolled expression of rDNA similar to that reported here is being produced in the hybrid genomes generated in natural zones of contact. Significantly we now have evidence that, within the hybrid zone, those individuals showing abnormalities in NOR expression are carriers of *Cp. parallelus*-like sex chromosomes (Gosálvez *et al.*, in prep.). Individuals in the hybrid zone will possess various levels of heterozygosity, and the genetic interaction occurring in some of them permit higher levels of genetic homeostasis than in the strict F1 hybrids produced in our experiment. The subsequent production of fitter individuals in natural conditions is then expected in this situation.

The composition and dynamics of the hybrid zone between *Cp. parallelus* and *Cp. erythropus* in the Pyrenees is complicated, and this difference we have described in the nucleolar behaviour in reciprocal F1 males adds to this. Other work in progress also points to differences in the reciprocal F1s, which may have their cause in the disturbed nucleolar activity and ultimately may be the cause of certain tantalising aspects of the zones structures and distribution. For example, several morphological and behavioural characters show differences between reciprocal crosses which could be due to sex linked and maternal inheritance and some show signs of dominance (Butlin & Hewitt, 1988). Also we have some results from F1 embryo analysis which show that when *Cp. parallelus* is the female parent the mode of inheritance is disturbed (Bella, Butlin and Hewitt, in prep.). Finally, there is growing evidence from our current detailed collections around Col de Portalet that the *Cp. parallelus* X-chromosome may be asymmetrically distributed (Gosálvez and Hewitt, in prep.). Some of these various features may have a common cause.

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