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Postzygotic isolation and Haldane's rule in a grasshopper

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Hybrid dysfunction was examined in reciprocal F_1 and backcross hybrids between parapatric subspecies of the meadow grasshopper *Chorthippus parallelus*. Haldane's rule applies and dysfunction is restricted to the XO males which are sterile. The degree of disruption differs between reciprocal F_1s. Males whose mothers were *C. p. parallelus* have poorer testes, a consequence of either the *parallelus* X chromosome, which carries a nucleolar organizer region (NOR), or a cytoplasmic factor. There is considerable variation in phenotype amongst F_1 families when compared to parental families, which reflects hybrid breakdown. Sterility is probably caused by detrimental epistatic interactions between the single X and the mixed autosomes.

Significant differences in testis morphology and survivorship among backcrosses could be attributed to cytoplasmically transmitted factors. An interaction between the cytoplasm and the region of the X chromosome carrying the NOR accounts for variation among backcross individuals. There is no incompatibility between the X and autosomes in backcrosses. This raises questions about the mechanism underlying sterility in the F_1.

Hybrid dysfunction in this species appears to be polygenically determined. These data emphasize the importance of cytoplasmic influences, as well as the role of the X chromosome, in postzygotic isolation.

Keywords: hybrid zone, nucleolar organizer region, postzygotic isolation, sex chromosomes, sterility, testis dysfunction.

Introduction

Reproductive isolation can arise in a number of ways. Pre-mating isolation may be effected through ecological or ethological divergence and post-mating (prezygotic) isolation can be attributed to mechanical or gametic incompatibilities. Postzygotic isolation is usually caused by F_1 inviability, F_1 sterility, and F_2 or backcross breakdown (Grant, 1963; Templeton, 1981). Typically two species will be separated by a combination of some of these.

These barriers to gene flow are considered to arise through pleiotropy during divergence, as two independent gene pools undergo adaptive and stochastic change following geographical separation. In the study of evolution it is important to know the type and extent of divergence required for speciation (e.g. Coyne, 1992). Different levels and forms of divergence are believed to be associated with the many proposed origins of new species (Templeton, 1981; Carson & Templeton, 1984; Barton & Charlesworth, 1984). For example, divergence at many loci, each of small effect, may be necessary to effect behavioural isolation (Zouros, 1990), while selection under stressful environments has been proposed to favour tolerant phenotypes that depend upon only a few major loci with epistatic modifiers (Templeton, 1981). While it is clear that individual cases must be investigated in detail before generalizations may be made, there are few experimental studies that provide data to assess these proposals.

This paper investigates the genetic architecture of postzygotic isolation between two naturally hybridizing taxa of the grasshopper *Chorthippus parallelus* whose ranges meet along the Pyrenees (Butlin & Hewitt, 1985; Virdee & Hewitt, 1990).

Haldane's rule

This rule states that 'when in the F_1 offspring of a cross between two animal species or races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex' (Haldane, 1922). There is widespread

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adherence to this pattern among mammals, birds and insects (*Drosophila*, Lepidoptera, Orthoptera) (Charlesworth *et al.*, 1987). Recently there has been much debate about the genetic mechanisms underlying Haldane's rule and whether it is an important phenomenon (Coyne *et al.*, 1991; Frank, 1991a and b; Hurst & Pomiankowski, 1991; Read & Nee, 1991). In the extreme, it has been described as a nearly obligatory first step in reproductive isolation and of significance to speciation (Coyne & Orr, 1989a and b). Some of the mechanisms proposed to explain Haldane's rule are discussed later.

Chorthippus parallelus and Haldane's rule

Two subspecies of the European meadow grasshopper, *C. parallelus parallelus* and *C. p. erythropus*, have distinct ranges meeting below 2100 m in a narrow, extensive hybrid zone that follows the Pyrenean ridge. F_1 male hybrids produced in the laboratory are sterile, with extremely reduced testes, while their sisters are apparently fully fertile (Hewitt *et al.*, 1987). This extreme dysfunction is not found in natural hybrid males from the centre of the hybrid zone. Modification or the erosion of linkage between blocks of genes responsible for dysfunction may account for this observation (Ritchie & Hewitt, 1992; S. R. Virdee & G. M. Hewitt, submitted).

In *C. parallelus*, as in most grasshoppers, there is no Y chromosome, the male is XO and the female XX; consequently sterility cannot be the result of X/Y incompatibility. F_1 males have either a *parellelus* X or an *erythropus* X, together with one set of autosomes from each subspecies. In both cases the X must function with a half foreign autosomal material. This X-autosome imbalance has been suggested as the cause of hybrid sterility (Hewitt *et al.*, 1988; Gosálvez *et al.*, 1988; Bella *et al.*, 1990).

There is evidence for X chromosome divergence and a large effect of the X on meiosis in hybrids (Bella *et al.*, 1990). One chromosomal region on the X, coding for the nucleolar organizer region (NOR), may be involved in hybrid sterility. The two subspecies differ in their complement of NORs; in *C. p. parallelus* there are three NORs, two on autosomes and one on the X, while *C. p. erythropus* possesses only the two autosomal NORs (Gosálvez *et al.*, 1988). Hybrids show irregular NOR expression and sperm abnormalities (Bella *et al.*, 1990). In addition, the NOR cline is very narrow (Ferris, *et al.*, in preparation), which is indicative of selection on this character.

Hewitt *et al.* (1987) found testis follicle length in backcross males to be intermediate between that of pure and F_1 hybrid individuals, which may reflect the influence of recombinant X chromosomes or the involvement of autosomal genes. In addition, there was some indication of differences between the various backcrosses in the degree of dysfunction, implying maternal effects or sex-linkage. Few individuals were scored in this initial study and the aim of the work described here was to further investigate the genetic basis of hybrid sterility, using reciprocal F_1s and backcrosses.

Materials and methods

The testes of *C. parallelus* consist of two sets of ~40 sperm-producing follicles, leading from the two branches of the *vas deferens*. Normally, follicles are elongated with pointed apices, tapering to slender stalks that feed into the *vas deferens* (Fig. 1). Spermiogenesis takes place in the follicles; mitotic cells from the follicle tip divide several times to produce cysts of synchronous spermatogonia which then undergo meiosis and begin to elongate to form spermatids. These travel into the lumen of the follicle and begin to coil together into ordered sperm bundles, one bundle per mitotic cyst. The mature, tightly coiled sperm bundles pass into the *vas deferens* and to the sperm storage organ.

In hybrid males the follicles are severely reduced in size, containing no normal sperm bundles and often no, or little, sperm (Fig. 2). Between these two extremes a continuum of follicle size and organization can be observed, especially in backcrosses (Fig. 3).

Grasshoppers were collected from the region of the Col du Pourtalet in the western Pyrenees (0°25′W, 42°50′N) during August 1987. Adult females were maintained caged in the field and provided with males, fresh grass as food and moist sand into which to lay their eggs (see Kelly-Stebbings & Hewitt, 1972, for details of grasshopper rearing). Egg pods were collected and stored in sterile, moist sand for transport back to Norwich where they were allowed to enter diapause. Diapause was broken by a period of three months at 4°C. Egg pods were hatched under controlled insectary conditions in the following spring.

F_1s and backcrosses

F_1s were produced between the reference populations GA (Gabas), which is *C. p. parallelus*, and SG (Sallent de Gallego), *C. p. erythropus*. Crosses involving individual male and female pairs and also bulk crosses were carried out. The individual crosses resulted in eight reciprocal families of F_1 offspring. However, because they require less time and space, bulk crosses (~20 females and ~15 males in one cage) were

Fig. 1 Testis follicles of a normal male of *Chorthippus parallelus*. (a) Showing healthy follicle arrangement, ×47 mag. (b) Normal sperm bundles in follicle lumen, ×200 mag.

employed for the most part, especially for the backcrosses.

In principle, common rearing effects may confound the results when comparing the offspring of bulk crosses. It was assumed, however, that the major component of variation in dysfunction between crosses was genetic. This seemed reasonable as extensive experience has shown that it is not possible to induce any reduction in testis size or disruption of spermiogenesis in a population from which dysfunction is absent (e.g. GA and SG), by crowding or underfeeding. As a precaution the cage density was maintained constant among crosses by the use of extra cages where necessary. All grasshoppers were kept supplied with fresh food and the cage was regularly rotated.

Measurement of testes dysfunction

Egg pods were hatched in standard laboratory conditions and reared to adulthood. The testes of 2–3 day-old males were fixed in 3:1 ethanol:acetic acid and stored at 4°C. Dissected follicles were hydrolysed in 1 N HCl (5 min ~60°C) and stained with Feulgen (30 min in the dark) (Hewitt *et al.*, 1986 and 1987). Under light microscopy, six follicles from each individual were examined in detail. The parameters recorded for F_1 males were the total number of follicles, follicle length and width, and a qualitative sperm quality/organization score (Table 1). These measures were chosen from many previously examined (Hewitt & East, 1978) because they are reliable indices of dysfunction and relatively easy to score. Follicle width turned out to be highly correlated with follicle length ($r^2 = 77.3$ per cent; d.f. = 1,82; F278.9; $P < 0.0001$) and, consequently, was not scored in the backcross generation. A more informative parameter, the number of sperm bundles per follicle, was introduced.

Results

No effect of male size (as measured by femur length) on the mean follicle length of individual males was detected, rendering correction for male size unnecessary [$r^2 = 1.62$ per cent; d.f. = 1,39; $F = 0.64$; ns. Data from laboratory-reared males originating from a transect spanning the hybrid zone (Virdee, 1991)].

F_1 laboratory crosses

As previously reported (Hewitt *et al.*, 1987) F_1 hybrids between *C. p. parallelus* and *C. p. erythropus* have very poorly developed testes relative to the parental populations (Fig. 2). Although they will mate readily and their mates lay superficially normal egg pods, these males are completely sterile (Table 2). This restriction of sterility to the heterogametic sex is consistent with Haldane's rule. There is some indication that pure females have lower fecundity than the F_1. This may result from inbreeding depression in the pure laboratory stocks, which had been maintained for two generations.

Reciprocal crosses differ in meiotic behaviour. One might, therefore, predict that males from reciprocal crosses, *parallelus* females × *erythropus* males (*p.e*) and vice versa (*e.p*), would also differ in their testis morphology. With respect to variation between families, there is no effect of the cross on the best indicator of testis quality, follicle length (hierarchical analysis of variance, d.f. = 1,6; $F_{\text{cross/(cross.family)}} = 0.09$; ns. Range of family means: (*p.e*) 0.43–1.39 mm; (*e.p*) 0.42–1.14 mm). In this small sample of four families

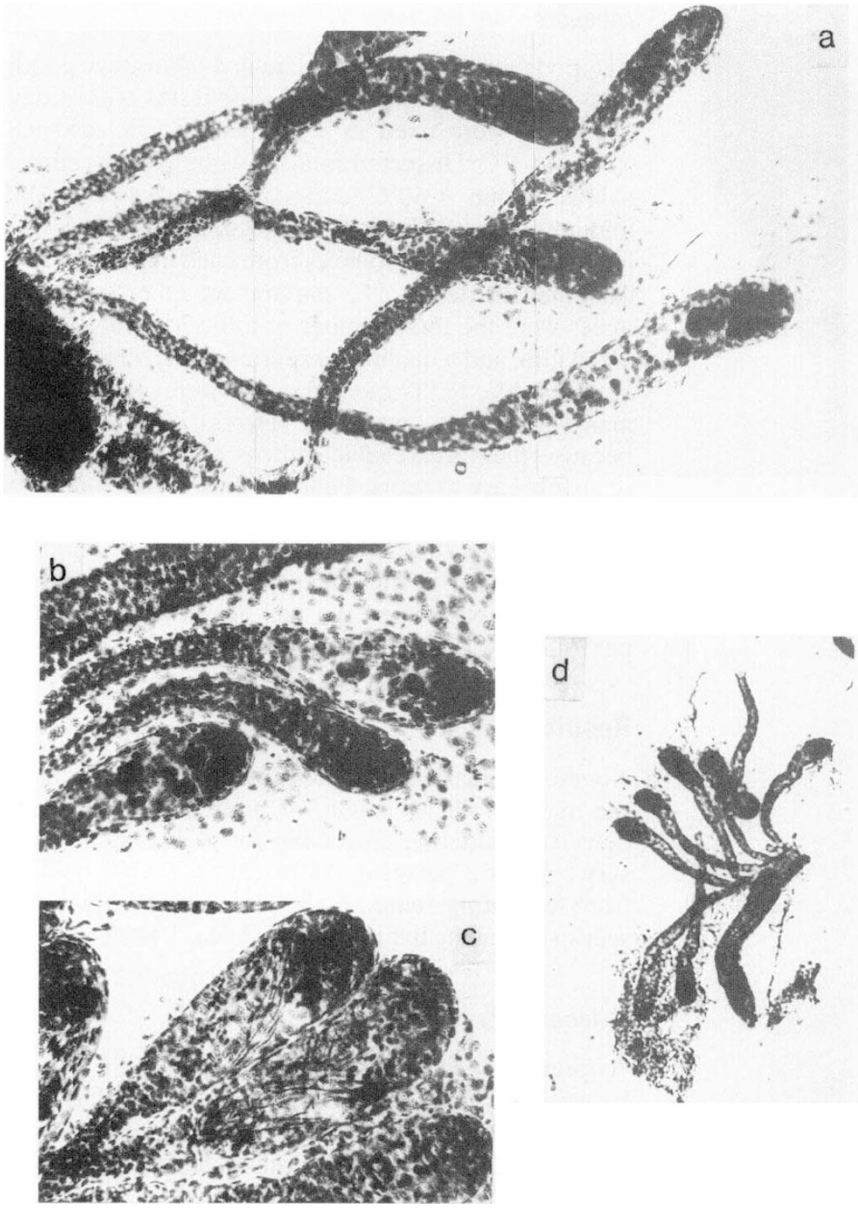

Fig. 2 Testes of F_1 males. (a) Follicles with no meiosis and no sperm, ×218. (b) Showing condensed chromatin, ×214. (c) Small follicles with some stray sperm present, ×214. (d) General follicle arrangement, ×55.

per cross, any variation between reciprocal crosses is obscured by interfamily differences. It was possible, however, to repeat this test on the bulk-reared F_1 males. In this case there is a significant difference in the length of the follicles [$(e.p)$ $\bar{x}=0.93$ mm, s.d. = 0.40, $n=16$; $(p.e)$ $\bar{x}=0.55$ mm, s.d. = 0.16, $n=14$; $t=2.18$, d.f. = 10, $P<0.05$ single-tailed]. Both sets of males had equally poor sperm organization [$(e.p)$ $\bar{x}=0.51$, s.d. = 0.09; $(p.e)$ $\bar{x}=0.58$, s.d. = 0.04] and no sperm bundles were present in males from either cross. This result points toward either a disruptive effect of the *parallelus* X chromosome (X^P) or a *parallelus* maternal effect, influencing testis morphology as well as meiosis.

Interestingly, although the degree of dysfunction in males from the four F_1 families varies significantly (HIERANOVA d.f. = 6,18; $F^{(\text{cross. family})/}_{(\text{cross. family. indv})}=3.39$; $P<0.025$) there is no such heterogeneity present among families of pure *parallelus* and *erythropus* individuals, produced under the same conditions (HIERANOVA d.f. = 4,14; $F^{(\text{popn. family})/}_{(\text{popn. family. indv})}=0.41$, ns). Variation among F_1 families could result from environmental effects, which may have a disproportionate influence on a disrupted genome. Alternatively, polymorphism for the genes responsible for dysfunction could be expressed in the F_1.

Backcrosses

Four backcrosses (BX) are possible, using the fertile female F_1 hybrids; each of the two reciprocal F_1s [$(p.e)$ and $(e.p)$], crossed to both parental types, p and e [i.e.

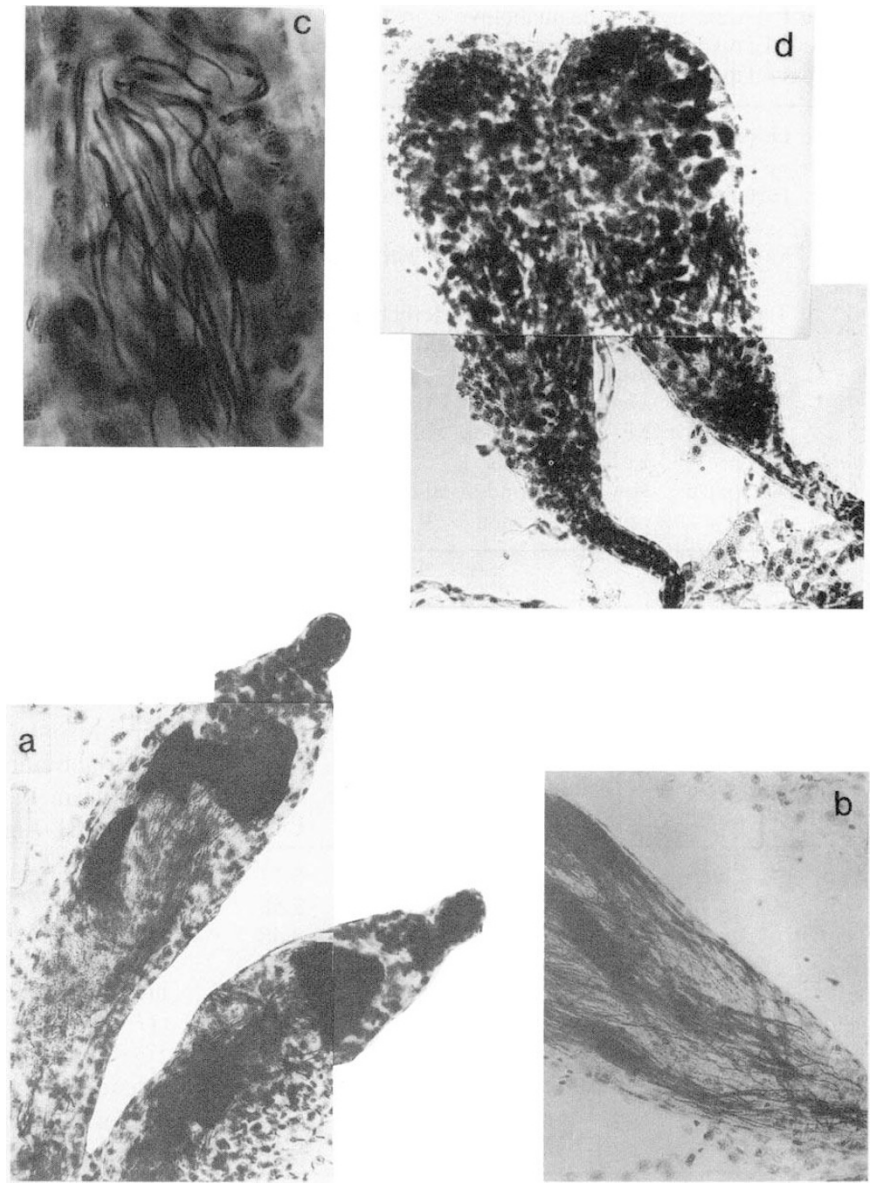

Fig. 3 Backcross follicles. (a) Follicles with aberrant 'pinched' ends and some sperm bundles, × 225. (b) Tangled sperm inside BX follicle × 193. (c) × 814. (d) Follicle with no sperm, × 214.

(*p.e*)*p*; (*p.e*)*e*; (*e.p*)*p*; (*e.p*)*e*]. Testis dysfunction and viability were measured on these individuals.

Testis dysfunction. In general, the backcross testes are intermediate between the parental and F_1 phenotypes (Fig. 4). Analysis of variance on canonical variate scores reveals significant differences among crosses (CV1 $F = 9.53$; d.f. = 3,55; $P < 0.0001$. CV2 $F = 2.97$; d.f. = 3,55; $P < 0.05$. Table 3 and Fig. 5). As all backcross males have the same proportion of heterozygous loci, this is suggestive of complex genetic determination. Males from the (*e.p*)*e* cross have longer follicles than the other three crosses and contain more, well organized sperm bundles with few abnormalities (Fig. 5). On average half the X chromosomes in the backcross males will be *parallelus* and half *erythropus*, therefore any difference between the (*e.p*)*e* and (*p.e*)*e* males might be attributed to some cytoplasmic factor. However there is no significant effect of cytoplasm on testis [(*e.p*)*e* and (*e.p*)*p* vs. (*p.e*)*e* and (*p.e*)*p*, $t = 1.82$; d.f. = 57; ns). The nuclear genetic constitution has a significant influence; those males deriving three-quarters of their autosomal genes from *C. p. erythropus* tend to exhibit less dysfunction than those that are a three-quarters *parallelus* ($t = 3.69$; d.f. = 57; $P < 0.001$. Table 3; Fig. 5).

No single character varys significantly among crosses. The distributions of follicle length and sperm

Table 1 Explanation of the qualitative score used to assess the level of disorganization and abnormal sperm production in cross offspring

Score	Description	Explanation
1	Perfect	Good sperm bundles. Few/no free spermatids
2	Good	Loose sperm bundles with some free spermatids
3	Tangled	Spermatids in disorderly bundle arrangement/or poorly developed spermatids in bundles
4	No bundles	Very messy sperm. No organization
5	Little sperm	Most follicles empty of sperm. With or without meiosis
6	No sperm	Few cells, Condensed chromatin often present in lumen

Table 2 The fecundity of F_1 males and females with respect to the number of nymphs and adults produced per egg pod. Data are from density-regulated, bulk-reared grasshoppers. Note that all egg pods fathered by F_1 males are inviable

Parents		Number of egg pods	Nymphs per pod	Adults per pod
Mother	Father			
(e.p)	e	53	6.11	3.11
(p.e)	e	53	3.89	2.58
(e.p)	p	46	5.04	3.48
(p.e)	p	35	3.09	1.63
e	(e.p)	19	0	—
e	(p.e)	34	0	—
p	(e.p)	42	0	—
p	(p.e)	29	0	—
p	p	40	3.18	1.68
e	e	27	2.63	0.82

e = *C. p. erythropus* (SG), p = *C. p. parallelus* (GA). Notation = (female:male). Data from simulataneously reared intrapopulation crosses are shown for comparison.

bundle density combined for all four crosses (Fig. 4) are significantly non-normal (Kolmogorov-Smirnov $DN = 0.177$, $P < 0.05$ follicle length; $DN = 0.180$, $P < 0.05$ bundle number), with two distinct peaks for follicle length corresponding to 'good' and 'bad' males.

The level of dysfunction expressed may depend on an interaction between the two X chromosome types (X^e and X^P) and the backcross environment. This environment has two components, autosomal and cytoplasmic. If the X chromosome type is known, the effect of different origins, or incompatibility, between the X and the autosomes or cytoplasm can be investigated.

For 25 of the backcross males it was possible to stain for the NOR on the X, thus determining the origin of this region of the X chromosome (Bella *et al.*, 1990; J. H. Rubio *et al.*, in preparation). The percentage of abnormal spermatids was also scored. The remaining 35 males did not yield sufficient good testis material for this analysis. This subset of less severely affected males consisted of 11 with the *erythropus* X and 14 with the *parallelus* X (with a NOR). The origin of each male's cytoplasm was known from its grandmother. The autosomal environment will be mixed but autosomal material from one subspecies will predominate (ratio of *parallelus* to *erythropus* of 3:1 or 1:3 depending on the cross).

Interactions between the X chromosome and autosomes do not account for the variation present among backcross individuals in testis quality; the effect of X-autosome compatibility was analysed using the percentage normal sperm (the most discriminating character) with follicle length and bundle density as covariates ($F = 0.74$; d.f. = 1,21; ns. Fig. 6). In contrast, those males which have complementary cytoplasm and X chromosome have significantly longer follicles and fewer abberant spermatids than those possessing X and cytoplasm of different origins ($F = 16.35$; d.f. = 1,21; $P < 0.001$. Fig. 6).

Viability differences. There is significant heterogeneity among backcrosses in fitness indices. The number of adults produced by each cross, corrected to the number of egg pods used, is poorest for the $(p.e)p$ cross ($G = 29.86$; d.f. = 3; $P < 0.001$; Table 2). Other data (C. Ferris *et al.*, in preparation) suggest the order of hatch rate to be $(e.p)p > (e.p)e > (p.e)e > (p.e)p$, which is consistent with the data presented here. In addition, *parallelus* females mated with males from this backcross lay fewer pods ($G = 12.47$; d.f. = 3; $P < 0.01$). There is no significant heterogeneity in fecundity in crosses to *erythropus* females ($G = 3.69$; d.f. = 3; ns), however, the sample size is small.

The indication that the crosses with the poorest testes also suffer in viability may suggest similar genetic determination. However, these viability data are from unreplicated crosses (one bulk cage per cross) and should be treated with some caution as these patterns may reflect environmental factors, e.g. the condition of the mothers, and not genetic traits. Testes and viability results from the backcross and the F_1 are summarized in Table 4.

Discussion

In *Chorthippus parallelus* Haldane's rule applies; the F_1 between French and Spanish subspecies shows

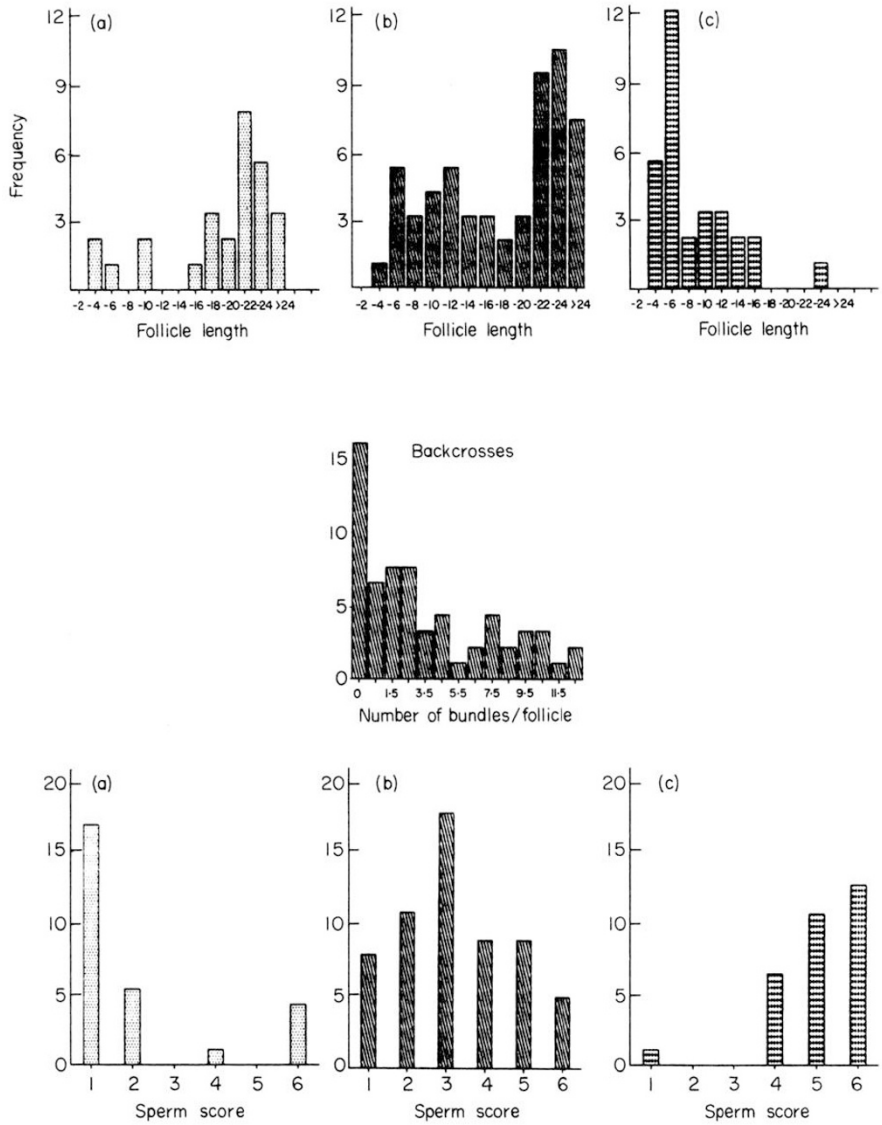

Fig. 4 Frequency distributions of testes characters for (a) pure, (b) backcross and (c) F_1 males respectively. Crosses are between GA and SG. Follicle length (in graticule units) and sperm score data are shown for all crosses, whereas the number of sperm bundles per follicle was only recorded in backcrosses (1 graticule unit = 0.1 mm).

male sterility. These males have normal mating behaviour and healthy accessory glands (Hewitt *et al.*, 1987), only sperm production and testis follicle development is disrupted by hybridization. Some mechanism involving the X chromosome must be invoked to account for the asymmetry between the sexes.

The simplest explanation for Haldane's rule in the F_1 is disruptive epistasis between the males' single X chromosome and one set of foreign autosomes. The F_1 female, as a consequence of possessing an X chromosome from each subspecies, avoids these detrimental interactions. However, epistasis occurring in F_1 hybrids is clearly mediated by maternally transmitted factors. The observation of greater meiotic disruption in (*p.e*) individuals (Bella *et al.*, 1990) implicates the X–NOR itself, genes linked to it or simultaneously inherited *parallelus* cytoplasmic factors in the cause of dysfunction. The NOR shows variable expression within individuals, indicating imperfect control of rDNA synthesis and suggesting that it may itself be directly involved in hybrid sterility. In contrast, autosomal differences among backcross types appear to influence testis. The same may be important in determining viability and fecundity, through the disruption of reproductive and developmental processes.

A particularly revealing result is the effect of the interaction between sex chromosome and cytoplasm on backcross dysfunction as a whole. In crosses where segregation has separated the X from its usual cytoplasmic background, dysfunction is significantly worsened. The autosomal background, however, has no effect on the level of dysfunction. Incompatibility between cytoplasmic binding factors and epigenetic marks on the X could disrupt the chromosome restructuring that occurs during gametogenesis (Jablonka &

Table 3 Follicle data for backcross males

Cross	Follicle length (mm)	Number of bundles	Sperm score	Number of follicles
(e.p)e	2.20	7.33	2.37	72.00
	0.56	1.90	0.97	11.03
(p.e)e	1.78	2.81	3.11	73.00
	0.63	2.88	1.30	8.41
(e.p)p	1.64	3.21	3.47	81.27
	0.84	4.27	1.70	11.45
(p.e)p	1.42	1.86	3.83	77.33
	0.74	2.31	1.46	8.71
Coefficients				
CV1	0.266	0.936	0.259	−0.484
CV2	−0.194	1.125	1.015	0.790

$n = 15$ per cross, means and s.d. shown. Notation: p = *C. p. paralleluls* (GA), e = *C. p. erythropus* (SG). Bracketed letters indicate the origin of hybrid females involved in each cross, i.e. (female:male) male.

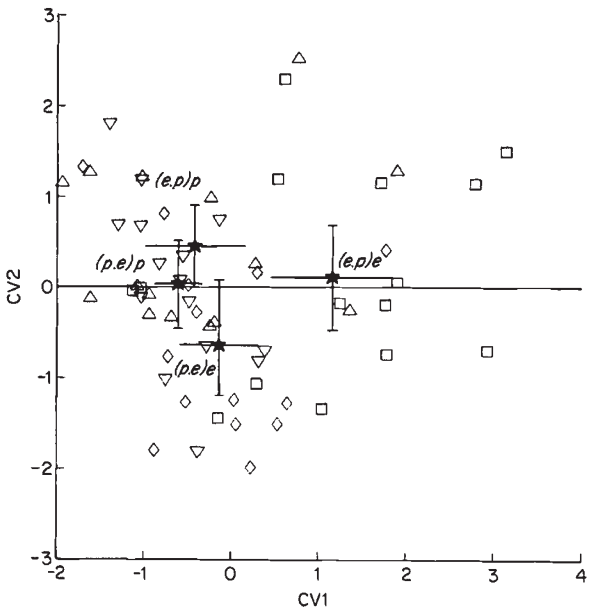

Fig. 5 The first two canonical variate axes for backcross males, based on follicle length, sperm score and bundles per follicle. Ninty-five per cent confidence intervals for CV scores are shown. $n = 15$ per cross. (□) *(e.p)e*, (◇) *(p.e)e*, (△) *(e.p)p*, (▽) *(p.e)p*, (☆) centroids.

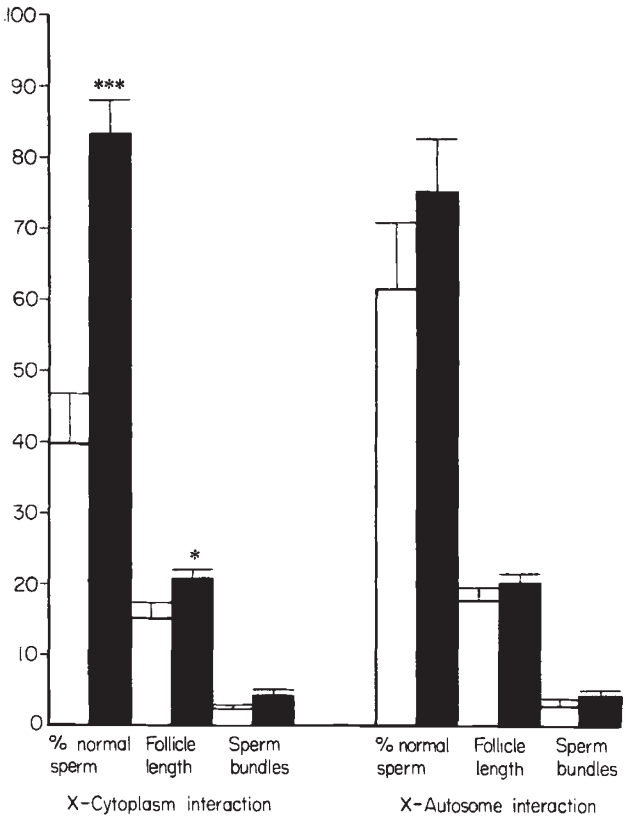

Fig. 6 Mean values and standard errors for percentage of normal spermatids, follicle length (graticule units) and sperm bundles per follicle of backcross males whose X chromosome type could be determined (see text). Comparisons are made between males whose X and cytoplasm/majority of autosomes have the same or different origins, e.g. an *(e.p)p* with X^e has 'compatible' X chromosome and cytoplasm but the X is 'incompatible' with the majority of autosomes. One-way ANOVA, $*P < 0.05$; $***P < 0.0001$. (□) Incompatible, (■) compatible.

Lamb, 1991). In addition to accounting for some of the testis variation between backcross individuals, this process is significant to Haldane's rule: the greatest conformational changes occur in male X chromosomes, making germ line disruption more likely in heterogametic males.

The significant differences observed between backcrosses and the significant autosome effect may reflect the bimodality observed in the backcross phenotype (Fig. 4). The crosses that are significantly different may, by chance, differ in the proportion of 'compatible' and 'incompatible' individuals. With such a distribution the sample sizes used are not sufficient to investigate between cross differences with complete confidence. The comparisons made, irrespective of cross (i.e. those made with respect to the X chromosome), should be the most reliable. These observations may provide some insight into the cause of male sterility in the F_1.

In F_1 males there is no incompatibility between X and cytoplasm as both are inherited together. Thus the mechanism behind dysfunction in the F_1 and the backcrosses may be different. The more normal testes of the backcross may arise simply because the majority (three-quarters) of autosomes are from the same subspecies so the probability of detrimental epistatic interactions among autosomes is less than in the F_1. This

Table 4 Summary of the main results from F_1s and backcrosses

F_1
1 Severe hybrid dysfunction (malformed testis follicles and sterility) expressed in males only
2 Reciprocal differences: [*p.e*] males show more meiotic disruption and have shorter testis follicles than [*e.p*]
Backcross
Testes
1 Intermediate between F_1 and parental
2 The (*e.p*)*e* backcross has significantly better testes than (*p.e*)*e*, (*p.e*)*p* and (*e.p*)*p*, n.s.d. between the latter three crosses
3 Males whose autosomes are 3/4 *erythropus* have significantly better testes than those 3/4 *parallelus*
4 Males with cytoplasm and X chromosome from different subspecies have significantly poorer testes than males with compatible combinations
5 There is no significant X-autosome interaction in backcross males
Viability
1 Pattern consistent with trend in testes data: (*e.p*)*p* > (*p.e*)*p* > (*e.p*)*e* > (*p.e*)*e*

improved balance is disturbed when segregation results in disruptive pairings of X and cytoplasm. One should expect incompatibility between the X chromosome and foreign autosomes in backcrosses, as was assumed in the F_1, but this does not seem to be the case. The dominant effect of the cytoplasm on backcross dysfunction suggests that the determination of F_1 sterility may involve more complex interactions than the simple X-autosome imbalance assumed above. Maternal factors may also be important in mediating interactions between X and autosomes in the F_1.

Staining for a chromosome marker associated with the XNOR (C-banding) in large numbers of individuals provides good evidence for a bias in X chromosome survival (C. Ferris *et al.*, in preparation). Individuals with conspecific X chromosome and cytoplasm predominate. Thus, the chromosome region containing the NOR appears to be under selection. This is confirmed by the steepness of the transition between the *C. p. parallelus* and *erythropus* states, which is very narrow compared to other markers (Hewitt, 1990, 1992). There are also indications of variable nucleolar expression within the transition zone (Bella *et al.*, 1990). Under- or over-expression of rDNA in field hybrids could itself affect fitness (Hewitt *et al.*, 1988; Hewitt, 1992). The behaviour of the single copy of the X–NOR in the disrupted, heterozygous environment of F_1 males may be the proximate cause of sex-limited sterility in *C. parallelus*.

Generally, there is compelling theoretical and empirical evidence for the primary involvement of sex chromosomes in Haldane's rule, although the genetic mechanism underlying the asymmetry remains controversial.

Muller's (1940, 1942) 'hemizygosity hypothesis' contended that preferential sterility or inviability of the heterogametic sex is a direct consequence of interactions between a single X chromosome and a mixed autosomal background. He posited that many alleles causing postzygotic isolation are recessive, such that they are more frequently expressed when on the X. This argument explains Haldane's rule and the large influence of the X chromosome in postzygotic isolation.

The X chromosome, however, has also been shown to play a large role in the fertility and viability of hybrids of the homogametic sex (usually females), which is not predicted by Muller's hypothesis (Orr & Coyne, 1989). Charlesworth *et al.* (1987) argue that genes which ultimately cause postzygotic isolation accumulate on the X chromosome as a result of natural selection. The X can evolve faster than autosomes because advantageous, but partially recessive alleles will be expressed and selected in males if they are X-linked (Coyne & Orr, 1989b). The X chromosome will diverge more rapidly than autosomes and be responsible for incompatibility between hybridizing, divergent taxa.

Coyne and Orr's theories rely on the assumption that mutations causing isolation were originally recessive or underdominant, for which the evidence is scant (Charlesworth *et al.*, 1987). It has also been argued (Hurst & Pomiankowski, 1991) that although the accumulation of advantageous recessives will be faster on the X, using the same logic, mildly deleterious mutations will accumulate more quickly on autosomes. Assuming most mutations are neutral, or near neutral, autosomes should change more quickly than the X (Hurst & Pomiankowski, 1991).

Another complementary explanation of Haldane's rule gives the behaviour of heteromorphic sex chromosomes during development as at least part of the reason why postzygotic isolation involves sex chromosomes more than autosomes (Jablonka & Lamb, 1991). As a result of chromatin structure divergence, chromosome restructuring during gametogenesis is inadequate and causes infertility. The heterogametic sex is most affected because their heteromorphic sex chromosomes undergo most restructuring. This developmental hypothesis requires no assumptions about the nature of the mutations contributing to divergence. However, owing to the paucity of information on how chromosomes carry epigenetic information in their chromatin

structure, this epigenetic hypothesis will not be easy to test.

Recently, the divergence of meiotic drive systems, involving segregation distorter and responder alleles, has been implicated in hybrid sterility and inviability (Frank, 1991a; Hurst & Pomiankowski, 1991). These authors explain the important role played by sex chromosomes by their susceptibility to the rapid evolution of meiotic drive, arising from the assumption that they do not recombine with each other. This, and other, assumptions of this argument have been recently challenged (Coyne *et al.*, 1991) and defended (Frank, 1991b). It is proposed that incompatibilities between diverged X and Y drive mechanisms or autosomal modifiers can lead to hybrid sterility or inviability.

In an XO system, such as exists in *Chorthippus parallelus*, interactions between the X chromosome and diverged autosomal modifiers could, in theory, lead to the sterility of hybrids (Frank, 1991a). There is evidence for the occurrence of meiotic drive in XO species (see Hurst & Pomiankowski, 1991), and meiotic drive in general may be more common than previously thought (Frank, 1991a). It remains to be seen whether the meiotic drive hypothesis stands up to scrutiny.

The genetic mechanism underlying Haldane's rule, and in particular the number of genes involved, has been most thoroughly investigated in *Drosophila*. Incompatability between X and Y chromosomes appears to be the proximate causes of Haldane's rule in hybrids of *Drosophila simulans, D. mauritiana*, and *D. sechellia* (Coyne, 1985). A contrasting situation exists in the *D. arizonae/D. mojavensis* pair. Here the effect on sperm motility can be traced to the interaction between a single autosomal locus and the sex chromosomes, with many other loci on this chromosome having a minor additive effect on fertility (Vigneault & Zouros, 1986; Pantazidis & Zouros, 1988; Zouros, 1990). In *Drosophila simulans/mauritiana* hybrids at least five loci are responsible for reproductive isolation, with the X-linked segment making the largest contribution to sterility (Coyne, 1984). Such a polygenic architecture with large effects of the X chromosome has been revealed by analyses of other *Drosophila* hybrids (Dobzhansky, 1936 and 1974; Pontecorvo, 1943; Ehrman, 1961; Heikkinen & Lumme, 1991). In addition, in *Colias* butterflies there are X chromosomal 'supergenes' which exert a large influence on most of the traits that distinguish *C. eurytheme* and *C. philodice*, such that *eurytheme* and *philodice* X chromosomes differ in their degree of compatibility with various hybrid backgrounds (Grula & Taylor, 1980).

Natural hybridization can also yield valuable information about the number of genes responsible for postzygotic isolation. For example, in *Podisma pedestris* there is a 50 per cent reduction in the fitness of inter-racial hybrids compared with parentals which has been attributed to the combined small effects of ~150 loci (Barton & Hewitt, 1981). A similar situation exists in the *Bombina bombina/variegata* hybrid zone, where ~55 loci are involved (Szymura & Barton, 1991).

This type of analysis, to estimate the number of genes involved in dysfunction between *C. p. parallelus* and *C. p. erythropus*, has yet to be done. However, the evidence suggests that most postzygotic barriers are polygenic (Barton & Charlesworth, 1984), with the exception of recently diverged species pairs where a few X-linked genes appear to effect isolation (Vigneault & Zouros, 1986; Orr, 1989; Orr & Coyne, 1989). Laboratory crosses are likely to underestimate the number of loci involved because recombination over the few generations studied in such experiments cannot break up linkages between alleles effectively (Falconer, 1981). Populations that hybridize naturally often differ extensively; for example, an average of 20 per cent of the electrophoretic loci have diverged across a sample of 21 hybrid zones (Barton & Hewitt, 1983). Morphological, behavioural and chromosome differences are also common (Barton & Hewitt, 1985). All this circumstantial evidence points to polygenic determination of postzygotic isolation in *C. parallelus*.

The mechanism that causes sterility or inviability to be restricted to the heterogametic sex is incompletely understood. There is, however, an indisputable bulk of evidence in favour of a central role for the X chromosome. In *C. parallelus*, disruptive epistasis between the X, in particular the X–NOR, and a mixed autosomal background remains the most parsimonious explanation of Haldane's rule, although in this case cytoplasmic factors also have an important influence.

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References

BARTON, N. H. AND CHARLESWORTH, B. 1984. Genetic revolutions, founder effects and speciation. *Ann. Rev. Ecol. Syst.*, **15**, 133–164.

BARTON, N. H. AND HEWITT, G. M. 1981. The genetic basis of hybrid inviability in the grasshopper *Podisma pedestris*. *Heredity*, **47**, 367–383.

BARTON, N. H. AND HEWITT, G. M. 1983. Hybrid zones as barriers to gene flow. In: Oxford, G. S. and Rollinson D. (eds) *Protein Polymorphism: Adaptive and Taxanomic Significance*, Blackwell Scientific Publications Ltd, Oxford, pp. 341–359.

BARTON, N. H. AND HEWITT, G. M. 1985. Analysis of hybrid zones. *Ann. Rev. Ecol. Syst.*, **16**, 113–148.

BELLA, J. L., HEWITT, G. M. AND GOSALVEZ, J. 1990. Meiotic imbalance in laboratory-produced hybrid males of *Chorthippus parallelus parallelus* and *Chorthippus parallelus erythropus*. *Genet. Res. Camb.*, **56**, 43–48.

BUTLIN, R. K. AND HEWITT, G. M. 1985. A hybrid zone between *Chorthippus parallelus parallelus* and *Chorthippus parallelus erythropus* (Orhtoptera: Acrididae): morphological and electrophoretic characters. *Biol. J. Linn. Soc.*, **26**, 269–285.

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.

CHARLESWORTH, B., COYNE, J. A. AND BARTON, N. H. 1987. The relative rates of evolution of sex chromosomes and autosomes. *Am. Nat.*, **130**, 113–146.

COYNE, J. A. 1984. Genetic basis of hybrid sterility between two closely related species of *Drosophila*. *Proc. Natl. Acad. Sci., U.S.A.*, **81**, 4444–4447.

COYNE, J. A. 1985. The genetic basis of Haldane's rule. *Nature*, **314**, 736–738.

COYNE, J. A. 1992. Genetics and speciation. *Nature*, **355**, 511–515.

COYNE, J. A., CHARLESWORTH, B. AND ORR, H. A. 1991. Haldane's rule revisited. *Evolution*, **45**, 1710–1713.

COYNE, J. A. AND ORR, H. A. 1989a. Patterns of speciation in *Drosophila*. *Evolution*, **43**, 362–381.

COYNE, J. A. AND ORR, H. A. 1989b. Two rules of speciation. In: Otte, D. and Endler, J. A. (eds) *Speciation and its Consequences*, Sinaeur Associates, Sunderland, MA, pp. 180–207.

DOBZHANSKY, TH. 1936. Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. *Genetics*, **21**, 113–135.

DOBZHANSKY, TH. 1974. Genetic analysis of hybrid sterility within the species *Drosophila pseudoobscura*. *Hereditas*, **77**, 81–88.

EHRMAN, L. 1961. The genetics of sexual isolation in *Drosophila paulistorum*. *Proc. Natl. Acad. Sci., U.S.A.*, **49**, 155–158.

FALCONER, D. S. 1981. *An Introduction to Quantitative Genetics*. Longman, London.

FRANK, S. A. 1991a. Divergence of meiotic drive-suppression systems as an explanation for sex-biased hybrid sterility and inviability. *Evolution*, **45**, 262–267.

FRANK, S. A. 1991b. Haldane's rule: A defense of the meiotic drive theory. *Evolution*, **45**, 1714–1716.

GOZALVEZ, J., LOPEZ-FERNANDEZ, C., BELLA, L. J., BUTLIN, R. K. AND HEWITT, G. M. 1988. A hybrid zone between *Chorthippus parallelus parallelus* and *Chorthippus parallelus erythropus* (Orthoptera: Acrididae): Chromosomal differentiation. *Genome*, **30**, 656–663.

GRANT, V. 1963. *The Origin of Adaptations*. Columbia University Press, New York.

GRULA, J. W. AND TAYLOR, O. R. 1980. Some characteristics of hybrids derived from the sulfur butterflies *Colias eurytheme* and *C. philodice*. *Evolution*, **34**, 673–687.

HALDANE, J. B. S. 1992. Sex ratio and unisexual sterility in hybrid animals. *J. Genet.*, **12**, 101–109.

HEIKKINEN, E. AND LUMME, J. 1991. Sterility of male and female hybrids of *Drosophila virilis* and *Drosophila lummei*. *Heredity*, **67**, 1–11.

HEWITT, G. M. 1990. Divergence and speciation as viewed from an insect hybrid zone. *Can. J. Zool.*, **68**, 1701–1715.

HEWITT, G. M. 1992. In press. After the ice-*parallelus* meet *erythropus* in the Pyrenees. In: Harrison, R. G. (ed.) *Hybrid Zones and the Evolutionary Process*, Oxford University Press, Oxford.

HEWITT, G. M., BUTLIN, R. G. AND EAST, T. M. 1987. Testicular dysfunction in the hybrids between parapatric subspecies of the grasshopper *Chorthippus parallelus*. *Biol. J. Linn. Soc.*, **21**, 25–34.

HEWITT, G. M. AND EAST, T. M. 1978. Effects of B chromosomes on development in grasshopper embryos. *Heredity*, **41**, 347–356.

HEWITT, G. M., EAST, T. M. AND SHAW, M. W. 1986. Sperm dysfunction produced by B-chromosomes in the grasshopper *Myrmeleotettix maculatus*. *Heredity*, **158**, 59–68.

HEWITT, G. M., GOSALVEZ, J., LOPEZ-FERNANDEZ, C., RITCHIE, M. G., NICHOLS, W. AND BUTLIN, R. K. 1988. Differences in the nucleolar organisers, sex chromosomes and Haldane's rule in a hybrid zone. In: Brandham, P. E. (ed.) *Kew Chromosome Conference III*, HMSO, London, pp. 109–119.

HURST, L. D. AND POMIANKOWSKI, A. 1991. Causes of sex ratio bias may account for unisexual sterility in hybrids: A new explanation of Haldane's rule and related phenomena. *Genetics*, **128**, 841–858.

JABLONKA, E. AND LAMB. J. L. 1991. Sex chromosomes and speciation. *Proc. R. Soc. Lond. B.*, **243**, 203–208.

KELLY-STEBBINGS, A. F. AND HEWITT, G. M. 1972. The laboratory breeding of British Gomphocerine grasshoppers (Orthoptera, Acrididae). *Acrida*, **1**, 233–245.

MULLER, H. J. 1940. Bearings of the *Drosophila* work on systematics. In: Huxley, J. S. (ed.) *The New Systematics*, Claredon Press, Oxford, pp. 185–268.

MULLER, H. J. 1942. Isolating mechanisms, evolution and temperature. *Biol. Symp.*, **6**, 71–125.

ORR, H. A. 1989. Genetics of sterility in hybrids between two subspecies of *Drosophila*. *Evolution*, **43**, 180–189.

ORR, H. A. AND COYNE, J. A. 1989. The genetics of postzygotic isolation in the *Drosophila virilis* group. *Genetics*, **121**, 527–537.

PANTAZIDIS, A. C. AND ZOUROS, E. 1988. Location of an autosomal factor causing sterility in *Drosophila mojavensis* males carrying the *Drosophila arizonensis* Y chromosome. *Heredity*, **60**, 299–304.

PONTECORVO, G. 1943. Viability interactions between chromosomes of *Drosophila melanogaster* and *Drosophila simu-*

lans. J. Genet., **45**, 51–66.

READ, A. AND NEE, S. 1991. Is Haldane's rule significant? *Evolution*, **45**, 1707–1709.

RITCHIE, M. G. AND HEWITT, G. M. 1992. In press. Outcomes of negative heterosis. In: Lambert, D., Spencer, H. and Masters, J. (eds) *Essays in Honour of H.E.H. Paterson*, Johns Hopkins University Press, Baltimore, VA.

SZYMURA, J. M. AND BARTON, N. H. 1991. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: Comparisons between transects and between loci. *Evolution*, **45**, 237–261.

TEMPLETON, A. 1981. Mechanisms of speciation- a population genetic approach. *Ann. Rev. Ecol. Syst.*, **12**, 23–48.

VIGNEAULT, G. AND ZOUROS, E. 1986. The genetics of asymmetrical male sterility in *Drosophila mojavensis* and *Drosophila arizonensis* hybrids: Interactions between the Y chromosome and autosomes. *Evolution*, **40**, 1160–1170.

VIRDEE, S. R. 1991. *Ecological and genetical components of a Pyrenean hybrid zone in the meadow grasshopper* Chorthippus parallelus *(Orthoptera: Acrididae)*. Ph.D. Thesis, University of East Anglia.

VIRDEE, S. R. AND HEWITT, G. M. 1990. Ecological components of a hybrid zone in the grasshopper *Chorthippus parallelus* (Zetterstedt) (Orthoptera: Acrididae). *Bol. San. Veg. Plagas (Fuera de Serie)*, **20**, 299–309.

ZOUROS, E. 1990. Searching for speciation genes in the species pair *Drosophila mojavensis* and *D. arizonae*. In: Hewitt, G. M., Johnston, A. W. B. and Young, J. P. W. (eds) *Molecular Techniques in Taxonomy*, NATO ASI Series, Springer-Verlag, Berlin, pp. 233–248.