

Sperm precedence and homogamy across a hybrid zone in the alpine grasshopper *Podisma pedestris*

G. M. Hewitt,
P. Mason* and
R. A. Nichols

School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K.

Individual virgin females of two races of *Podisma pedestris* were mated sequentially with a male of each race in one or other reciprocal orders, giving four types of double matings. The embryos were karyotyped to determine the extent of each male's paternity. This gave an excess of racial homozygotes as well as showing a predominance of the first male's sperm in fertilization. This confirms the previous indication of assortative fertilization producing homogamy, and reveals first sperm precedence, which is rare in insects. There was some infertility and a biased sex ratio. The implications for the evolution of assortment, reproductive isolation, sperm competition, male–female conflict and mating strategies are discussed.

INTRODUCTION

Hybrid zones have been a subject of research for some time (Wallace, 1887; Murray, 1972) and there is currently considerable interest in them, since they are natural laboratories for the study of a variety of evolutionary questions (e.g., Hewitt, 1988). Thus they can provide information on genome divergence and the evolution of species, and particularly on the factors and characters involved in this. There has been much discussion of whether postzygotic barriers to gene exchange can be reinforced by selection for pre-mating barriers. Can reproductive problems appearing after zygote formation generate selection to produce greater divergence in characters operating before fertilization—such as mate recognition systems? (e.g., Grant, 1975; Paterson, 1978; 1985, Barton and Hewitt, 1981a; Butlin, 1987).

Two parapatric races may have diverged for characters producing some pre or postzygotic isolation and these undergo crossing and recombination in a hybrid zone. For example, the races may have diverged at gene loci controlling embryo development, viability, morphology, mating behaviour, reproductive mechanics and fertilization. The alpine grasshopper *Podisma pedestris*

contains two races which met along the ridges of the Alpes Maritimes of France after the last ice age and now form a narrow hybrid zone there (Hewitt, 1975). These two races have diverged at many loci so that the hybrids are only about half as viable as parental genotypes (Barton and Hewitt, 1981b). They also differ for a centric fusion of the sex chromosome to an autosome—the ancestral XA giving the derived \widehat{XA} . This produces a neoXY male sex chromosome system from the XO male system, and so the races are called XO and XY. Evidence from allozymic, chromosomal, morphological and hybridization studies shows that genes may pass through the hybrid zone; the races are not reproductively isolated (Halliday *et al.*, 1984; Barton and Hewitt, 1988.)

In a previous paper (Hewitt *et al.*, 1987) we reported that mated females taken from the centre of the zone produced embryos containing an excess of chromosomal homozygotes. There is no evidence of assortative mating for karyotype in copulating pairs from the centre of the zone, therefore this homogamy appears to have been caused by assortative fertilization when a female is mated by males of both racial karyotypes.

Podisma pedestris is wingless and so it cannot stridulate like most grasshoppers and Orthoptera. Consequently, this cannot be a component of its courtship, and it has been ranked at the bottom of the grasshopper mating behaviour complexity

* Present address: MAFF, Slough Laboratory, London Road, Slough, SL3 7HJ, U.K.

league (Jacobs, 1953). Therefore, we might not expect its mate recognition system to have become obviously different between the two races. However, observations and filming of mating in the field and in the laboratory reveal considerable antennal and leg vibration accompanied by palpation and mandibulation of the female by the male once mounted. Adult males are brightly coloured and appear to take up obvious vantage points on rocks and plants to bask in the sun and from which they may jump onto approaching females (Hewitt and Mason, unpublished). Clearly the mating behaviour needs careful re-examination.

Even in the absence of complex courtship other prezygotic barriers may be present, since any event up to fertilization of eggs by sperm can be involved. In particular there may be mechanical differences, sperm competition and sperm discrimination by the female. This latter possibility may have interesting consequences for the evolution of genitalia (Eberhard, 1985). In the light of the previous results of homogamy we decided to examine in the laboratory the results of controlled matings involving males of both races with females of each race.

METHODS

Collections

Fourth instar nymphs were collected from two locations near Seyne-les-Alpes, France, known to be examples of the pure XO and pure XY races (Nichols & Hewitt 1986). They were each about 2 km from the centre of the hybrid zone that runs through Col des Tomples near Colet des Fus (XO) and Clottes des Moures (XY). They were separated into males and females and transported rapidly to the laboratory in Norwich, where they were raised to adults in an insectary with a suitably controlled diurnal temperature regime.

Matings

A few days after eclosing as adults, females become more ready to mate. After one week each female was placed individually with a single male in a small rearing cage with suitable herbaceous food plants. These single pair matings were of all four combinations of XO and XY males and females (sample sizes in table 1). They were observed every half an hour until a clear copulation was observed. When sperm and spermatophore transfer occurs the genitalia of the pair are closely engaged and the coupling usually lasts for several hours. After

such a clear copulation and long coupling had been observed the first male was removed from the cage and the female was given a second male of the other karyotypic race on the following day. These pairs were observed until they had mated and coupled fully. Those females not mating readily the second time were presented with males on subsequent days. The result of karyotyping show that both matings transferred sperm. Those that did not mate a second time were excluded. No egg pods were laid between matings.

Progeny

Each twice mated female was kept alone and provided with food and with a suitable mixture of moist sand and peat in which to oviposit. Egg pods were collected, each containing 5–20 eggs, kept in moist peat and sand at 26°C and dissected out 15 days after laying, *i.e.*, before they entered their obligate winter diapause. Each embryo was cultured in 0.05 per cent colchicine in slightly hypotonic insect saline for 30 minutes at 37°C and fixed in 3:1 ethanol/ethanoic acid. This fixed material was stored in the refrigerator at 0°C. Preparations were made by squashing in lactopropionic orcein and the karyotypes were scored under a light microscope.

RESULTS

Preferential fertilization

There are four classes of sequential crossing, females of both races mated first with a male of one race and then with one of the other, *viz.*

Classes of Cross I–IV		
First male	$\widehat{X}A.A$	$X.A.A$
Second male	$X.A.A$	$\widehat{X}A.A$
<hr/>		
Female	$\widehat{X}A.\widehat{X}A$	I
	$X.A.XA$	II
		III
		IV
<hr/>		

The results of the progeny karyotype analysis are given in table 1 for each successful double cross. With a sex-linked character the male offspring give no information on the relative frequency of fertilization by the two possible fathers ($\widehat{X}A$) and (XA). However, the fathers can be determined for female offspring (table 1). The proportion of fertilizations by $\widehat{X}A$ sperm varies between the four classes of cross. The differences between classes of cross account for a significant proportion of the residual deviance ($F_{3/19} = 7.51$; $P < 0.01$).

Table 1 The numbers of progeny embryos of different sex chromosome karyotypes from the four classes of crosses. The data from each female in each class of cross is given for homozygous and heterozygous female progeny, which should be equal if both males have an equal chance of fertilization, and for the numbers of male and female embryos produced. H/D and ND are the numbers of Haplo/Diplo embryos and non-developing eggs

Progeny	Females			Males		Total	Fertilization proportion $\widehat{XA}/\widehat{XA}+XA$	Sex ratio	Infertile eggs		
	$(\widehat{XA}\widehat{XA})$	$(\widehat{XA}XA)$	$(XAXA)$	$(\widehat{XA}.A)$	$XA.A)$				H/D	ND	Total
Cross Class I											
Females											
1	31	5	—	36	—	72	0.86	1.00	8	19	27
2	12	0	—	10	—	22	1.00	0.83	2	3	5
3	9	0	—	6	—	15	1.00	0.67	0	0	0
4	11	5	—	17	—	33	0.69	1.06	2	12	14
5	3	1	—	2	—	6	0.75	0.33	2	9	11
6	23	0	—	17	—	40	1.00	0.74	1	3	4
Total	89	11	—	88	—	188	0.89	0.88	15	46	61
Cross Class II											
1	5	6	—	7	—	18	0.45	0.64	8	0	8
2	2	10	—	11	—	23	0.17	0.92	0	1	1
3	8	0	—	9	—	17	1.00	1.13	0	1	1
4	1	0	—	0	—	1	1.00	0.00	6	1	7
5	1	12	—	4	—	17	0.08	0.31	3	9	12
Total	17	28	—	31	—	76	0.38	0.69	17	12	29
Cross Class III											
1	—	0	4	—	0	4	0.00	0.00	3	1	4
2	—	5	2	—	6	13	0.71	0.86	6	6	12
3	—	3	5	—	8	16	0.38	0.50	1	6	7
4	—	4	5	—	4	13	0.44	0.44	8	1	9
5	—	3	2	—	5	10	0.60	1.00	1	2	3
6	—	9	7	—	5	21	0.56	0.31	7	4	11
Total	—	24	25	—	28	77	0.49	0.57	26	20	46
Cross Class IV											
1	—	4	8	—	6	18	0.33	0.50	3	0	3
2	—	2	1	—	0	3	0.67	0.00	8	1	9
3	—	6	9	—	6	21	0.40	0.40	1	5	6
4	—	2	12	—	8	22	0.14	0.57	6	5	11
5	—	1	0	—	1	2	1.00	1.00	2	2	4
6	—	0	7	—	0	7	0.00	0.00	6	2	8
7	—	0	0	—	1	1	—	—	1	2	3
Total	—	15	37	—	22	74	0.29	0.42	27	17	44

I $(\widehat{XA}\widehat{XA})\varnothing$ mated to $1(\widehat{XA}.A)\delta$ then $2(XA.A)\delta$.

II $(\widehat{XA}\widehat{XA})\varnothing$ mated to $1(XA.A)\delta$ then $2(\widehat{XA}.A)\delta$.

III $(XA.XA)\varnothing$ mated to $1(\widehat{XA}.A)\delta$ then $2(XA.A)\delta$.

IV $(XA.XA)\varnothing$ mated to $1(XA.A)\delta$ then $2(\widehat{XA}.A)\delta$.

Females have a highly significant tendency to be fertilized by males of their own karyotype, *i.e.*, there is homogamy (table 2, test A). Females also tend to be fertilized by the first male to mate, *i.e.* there is precedence (table 2, test B). When this precedence is taken into account the homogamy is still significant (table 2, test C). The proportion of homogamous fertilizations does not differ significantly between female karyotypes (table 3, test A). The advantage gained by the first male to mate

does not differ significantly between karyotypes either (table 3, test C).

Sex ratio

The sex ratio (male/female) is clearly biased in favour of female embryos in all four classes of cross (table 1)—in total 169:246 giving a 0.69 sex ratio overall. There is clear evidence for a difference between the races ($F_{1/21} = 5.41$; $P <$

Table 2 Analysis of deviance of the proportion of fertilizations by XA sperm (logit link function)

	Degrees of freedom	Residual deviance	Mean deviance ratio
Initial model:			
Constant frequency between classes	22	135.953	—
Successive changes to the model (in the order A, B, C, D)			
A +MOTHER	21	106.415	9.02**
B -MOTHER			
+ORDER	21	90.626	13.85**
C +MOTHER	20	70.340	6.20**
D +MOTHER.ORDER	19	62.195	2.94 ns

The crosses can be divided in two ways: they can be divided according to the karyotype of the mother (MOTHER); alternatively they can be divided according to the order in which the males mated with the mother (ORDER).

** $P < 0.01$; * $P < 0.05$; ns = not significant.

Table 3 Analysis of deviance of the proportion of fertilizations by homogametic sperm (logit link function)

	Degrees of freedom	Residual deviance	Mean deviance ratio
Initial model:			
Constant frequency between classes	22	110.162	—
Successive changes to the model (in the order A, B, C)			
A +MOTHER	21	106.415	1.14 ns
B -MOTHER			
+ORDER	21	71.178	11.91**
C +MOTHER.ORDER	19	62.195	1.37 ns

The crosses can be divided in two ways: they can be divided according to the karyotype of the mother (MOTHER); alternatively they can be divided according to the order in which the males mated with the mother (ORDER).

** $P < 0.01$; ns = not significant.

0.05) with females of the XY race producing nearer to the expected equality in the numbers of male embryos than the XO race females. Precedence has no detectable effect on sex ratio (F 2/19 = 0.78).

Infertile eggs and homogamy

A substantial number of the eggs produced by these crosses when analysed proved to contain either no visible developing cell mass (ND) or else a Haplo-Diplo embryo (HD) (table 1). The eggs were dissected two weeks after laying by which

time a large embryo ~2 mm has normally formed. Normal diploid karyotypes and also polyploid, aneuploid and interchanged ones develop through to this stage (Hewitt & East, 1978). However, unfertilized eggs can occasionally develop as haploid embryos and patches of diploid cells can occur in these, presumably by nondisjunction and restitution. These embryos vary in size; we are able to type embryos larger than ~1000 cells. Indeed, tytoparthenogenesis can occur at a low frequency when one of these autodiploidised embryos hatches (Hewitt, 1979).

Beside its own intrinsic interest this phenomenon could be involved in the sperm precedence and homogamy revealed just previously. Clearly if diploid tytoparthenogenic embryos are produced they will be homokaryotypic and contribute to the homogamy totals. However, it is unlikely that the homogamy totals were distorted significantly by this effect for the following reasons.

First, it is rare for a dividing haploid egg to autodiploidise early enough in development so that a whole diploid embryo can be produced—usually various mixtures of haploid and diploid cells are seen. This is true of eggs from unmated females—about 1 in 50 eggs from virgin females (Hewitt and East, unpublished)—but in itself it does not rule out the possibility that a handful of embryos scored as homokaryotypic were autodiploid. Secondly, autodiploidy will produce an equivalent shift in the sex ratio since it generates female homogenetic embryos only. In Class I the shift in sex ratio is not sufficient, since the sex ratio is 88♂, 100♀ while homogamy is 11 Het: 89 Hom females. The same is true for Class IV, although not so dramatically, where the sex ratio is 22♂: 52♀ while homogamy is 15 Het: 37 Hom females. The data from the other classes which involve heterokaryotypic precedence do not exclude this possibility numerically. Thirdly, if apparent homogamy was being produced by autodiploidised unfertilized eggs then one might expect a correlation between the two, a high level of unfertilized eggs producing a high level of homokaryotypic embryos. A careful scrutiny of the data provides no support for this either (fig. 1). High levels of infertile eggs are found in pods showing both complete homogamy and complete heterogamy and there is a spread of homogamy levels in pods showing all levels of infertility. An analysis of deviance shows no effect of infertility on homogamy (F 1/21 = 1.47) but there is an effect of the class of cross (F 3/19 = 4.88 $P < 0.025$). This is particularly pertinent since each class comprises a different combination of female genotype and order of sperm intromission, which

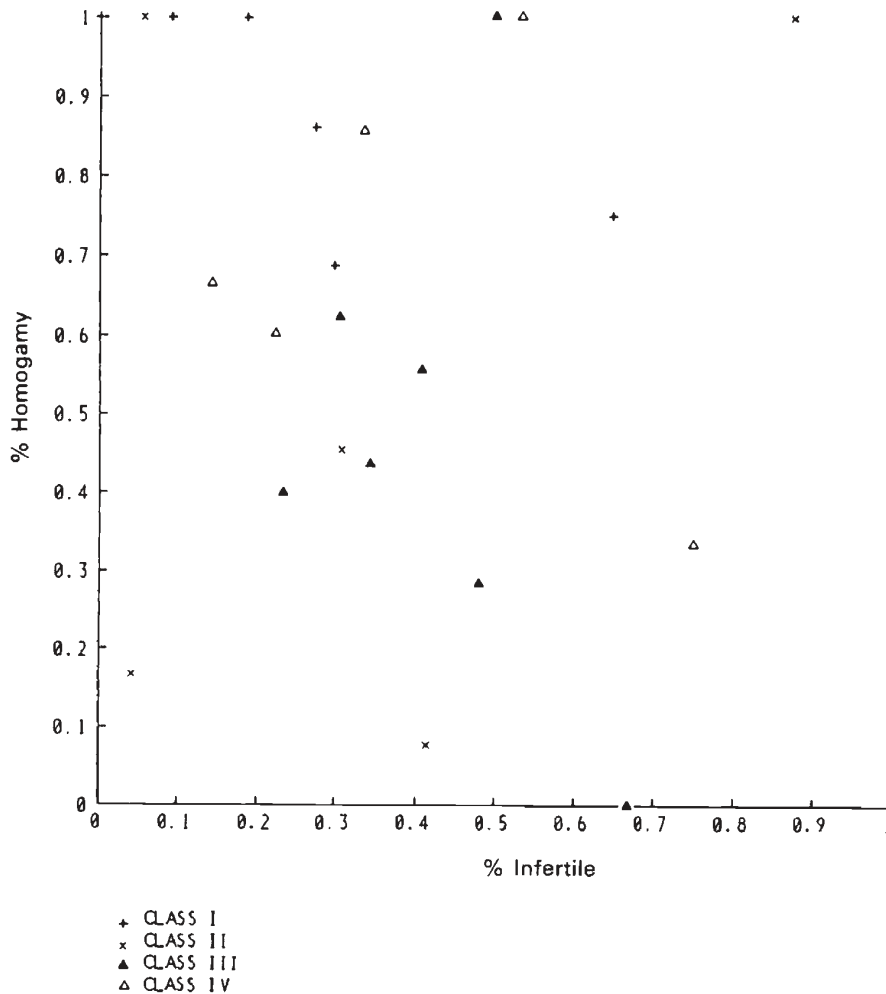


Figure 1 The percentage Homogamy and percentage Infertility observed in the offspring of each female. The four different symbols distinguish females from each class of cross.

may produce different levels of fertility. Class I shows high homogamy with a range of infertility, Class III and IV show a range of homogamy at intermediate infertility levels and Class II females are very disparate (fig. 1). The general clustering of points for pods showing complete and very high levels of homokaryotypes also demonstrates visually the extent of the homogamy.

Explanations of infertility

The high frequency of infertile eggs in these experiments contrasts with the lower level observed in gravid females taken from the field in the hybrid zone (Hewitt *et al.*, 1987). It is also high compared with most experiments in *Podisma* for which com-

parable data are available (table 4). This difference could have a variety of causes. First, the insects producing the present data were reared in the laboratory through their last moult, during mating and laying, whilst those in the previous homogamy work (Hewitt *et al.*, 1987) only spent their laying days in the laboratory. Such differences in conditions may affect fertility, but just how is not obvious. It is possible that the male's fertility is very sensitive to some factor on the journey from the Alps, although mortality on such journeys is regularly about 1 per cent over 2 days. Food is in plentiful supply in the laboratory and it seems unlikely that conditions of excess food and controlled diurnal temperatures would reduce male fertility or reduce the females' ability to store sperm.

Table 4 A summary of the proportions of eggs containing a Haplo-Diplo embryo (HD) or Not Developing (ND)—together giving the level of Infertility from a series of experiments using laboratory and field mated females from pure and Hybrid Zone (HZ) populations. The occasional females that laid no fertilized eggs at all were excluded. All females laid fertile eggs in the present precedence data (1). In field mated gravid females (2, 3, 4) nearly half the total infertility is accounted for by 4 per cent of females

Experiment	Eggs	HD	ND	Inf
(1) 1986 Precedence (lab. pure nymphs crosses)	595	0.143	0.160	0.303
(2) 1985 Homogamy (field gravid HZ)	1331	0.036	0.102	0.138
(3) 1983 (Field gravid HZ)	473	0.029	0.203	0.232
(4) 1982 (Field gravid HZ)	540	0.028	0.234	0.262
(5) 1979 (Lab. nymphs pure)	211	0.033	0.199	0.242
(6) 1979 (Lab. nymphs pure crosses)	138	0.072	0.616	0.688
(7) 1979 (Lab. nymphs HZ)	532	0.015	0.226	0.241

If the latter occurred then one would expect later laid pods to show more infertile eggs. This is not the case in this experiment: the distribution of HD or ND eggs through time shows no consistent trend (table 5). Adult fertilized female grasshoppers kept alone for several weeks in the insectary do tend after a while to lay small mostly infertile pods but most of the females in this experiment laid two or three pods and two-thirds were laid within one week.

Secondly, this spread of infertile eggs through the laying period also indicates that the female is not running short of sperm for fertilizing later pods because she was only allowed to mate with two males. In fact, in the field and in field enclosures

Table 5 The number of pods laid and collected on successive dates in August and September over the experiment with the Haplo-Diplo embryos found in each sample of pods

Date	22	25	29	3	7	11	15	21
Pods	8	16	12	0	7	2	5	4
HD embryos	8	30	27	0	3	3	11	3

females mate once or twice in a period of a week (Hewitt *et al.*, 1987; Mason, in preparation). However, the matings in the insectary were arranged whilst those of the gravid females were by choice in the field.

Thirdly, these present crosses involve genotypes of two races, so that each mated female contains sperm from both XO and XY males that differ for many genes (Barton and Hewitt, 1981*b*). It is possible that these interact in some way to reduce the chance of an egg being fertilized. Crosses between individuals from the same race or population, even within the zone, will not differ at so many loci. It is also possible that some hybrid embryos are not developing which would increase both the infertility and the homogamy. However, one cannot account for the number of homokaryotypic female embryos from the number of non-developing embryos in Class I and Class IV, unless the effect of precedence is massive. Also it has already been pointed out that homogamy is not correlated with infertility (fig. 1, $F_{1/21} = 1.47$).

A comparison of the frequency of infertile eggs produced by a female with the sex ratio of her embryos suggests that fewer males are produced when there are more infertile eggs (table 1, fig. 2). An analysis of deviance confirms this ($F_{1/21} = 4.51$, $P < 0.05$). However, it is not possible to disentangle the effects of class of cross and infertility on sex ratio from these data. It was noted that there is a significant difference for sex ratio between the two types of females, and whilst there is a similar trend in the proportion of infertile eggs they produce—XY females Class I 0.245, Class II 0.276; XO females Class III 0.374, Class IV 0.373—this is not significant. ($F_{1/21} = 2.16$).

DISCUSSION

These present controlled mating experiments were prompted by the previous finding of an excess of homokaryotypic progeny from wild mated females collected from within a hybrid zone (Hewitt *et al.*, 1987). This homogamy could not be attributed to assortative mating, since copulating pairs occurred in karyotypic frequencies consistent with random mating. These new results where each female is definitely mated by a male of each chromosomal race clearly demonstrate that her eggs tend to be fertilised by the sperm from the male of the same race as herself—there is homogamy again. Furthermore, it is of the same order of magnitude in both.

It is obvious that the genotypes of the insects involved in these two sets of experiments are very

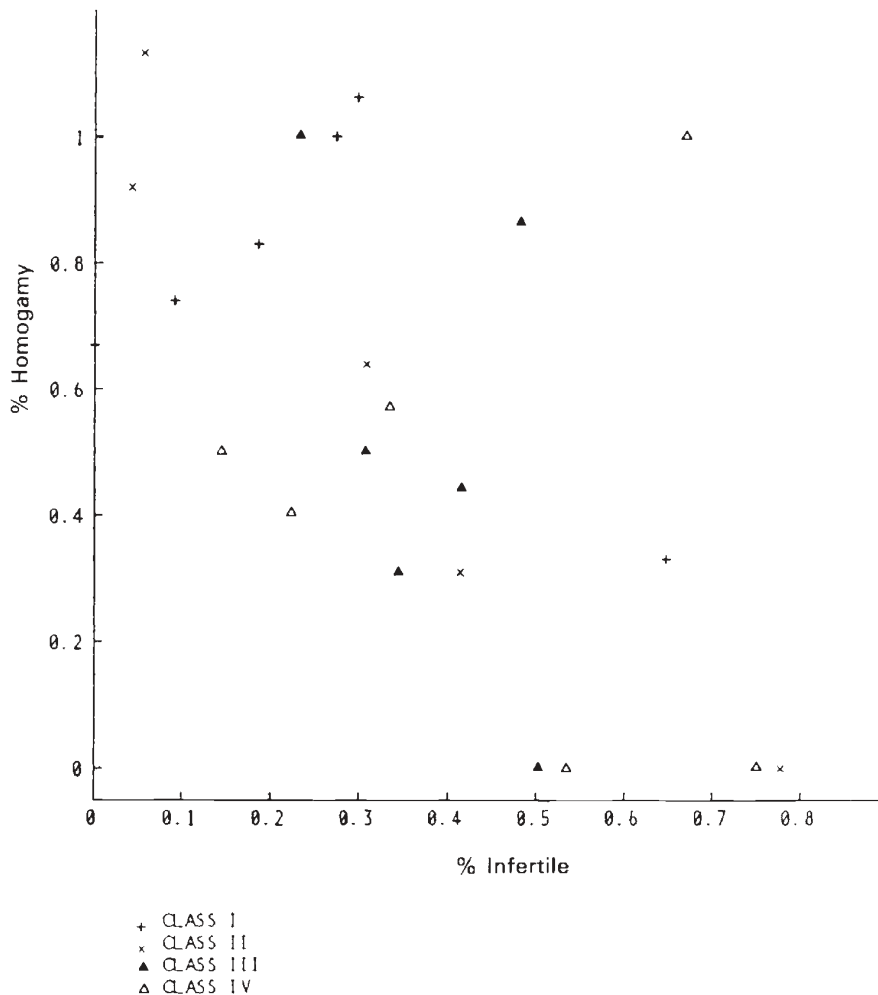


Figure 2 The sex ratio and infertility observed in the offspring of each female. The four different symbols distinguish females from each class of cross.

different. In the former they came from the centre of a 800 m wide hybrid zone at Tende and since the insects' dispersal in this area is <20 m per generation the chromosomes will have undergone many generations of segregation and recombination in diffusing from a pure race to the middle. This process has probably been occurring since the end of the last ice age in the Alps when the two expanding races colonised and met in this region—*i.e.*, in excess of 8000 generations. On the other hand the insects in the present crosses came some 2 km from the centre of the zone at Seyne and thus had essentially pure racial genomes.

Progeny from crosses spanning the hybrid zone show considerably reduced survival that is attributable to differences between the two races at about

150 genes spread through the genome (Barton and Hewitt, 1981*b*). Consequently the sex chromosome fusion is just one small component of the racial differences causing hybrid unfitness and maintaining this hybrid zone. Despite divergence between the two races no fixed allozymic differences have been found and both races show similar polymorphisms (Halliday *et al.*, 1984). This could be because of gene flow and introgression through the zone, or that the two races simply did not diverge at the enzyme loci investigated.

In the light of these considerations it appears that the genomes producing homogamy in the present experiments differ at many loci including the karyotypic change, while in the previous experiments (Hewitt *et al.*, 1987) the karyo-

typic difference may well have been stripped by recombination of many of these associated racial differences—and yet still retains the major causes of homogamy.

The reason for the persistence of this assortment for the sex chromosome after many generations of recombination between the racial genomes in the hybrid zone is unlikely to be a simple pleiotropic effect of a centric fusion. However, chromosome banding techniques indicate that the rearrangement producing the neo XY race is not a simple fusion, but involves the rearrangement of paracentric chromosome regions (Westerman and Hewitt, 1985). Consequently some genes may be permanently linked with the fusion. Furthermore, some normally homologous proximal genes may remain linked to the fusion for some time since chiasmata are reduced in this region (Hewitt and John, 1972). It may be that disequilibrium for the fusion and genes for the assortment is maintained in the zone not only by the assortment itself but also by dispersal of parental sequences of genes from the flanking regions assisted by some linkage and selection against recombinant genotypes.

It is of interest that the homogametic assortment for the sex chromosome rearrangement in the zone is on the same order of magnitude as that between racial genomes from outside the zone. Most probably the causes of the homogamy were present in the two genomes when they met after the last ice age and in the view of many authors this partial reproductive isolation should have been reinforced by selection, since the hybrids have reduced fitness. If this had occurred we would perhaps expect greater homogamy in the zone than between pure samples some distance away. Whilst such reinforcement is perfectly possible there are a number of problems with its development, (Crosby, 1970; Coyne, 1974; Caisse and Antonovics, 1978; Felsenstein, 1981; Spencer *et al.*, 1986). These include the integrity of the existing mating system and swamping by gene flow (Paterson, 1978; Barton and Hewitt, 1981*a*). The development of stronger prezygotic isolation to reinforce postzygotic isolation also depends on major gene effects on assortment, strong selection against loci producing hybrid unfitness and linkage between them; these are somewhat restrictive conditions. Furthermore, there is little, if any, evidence from studies on hybrid zones that such reinforcement has occurred (Barton and Hewitt, 1981*a*; Butlin, 1987), and the multigenic structure of this zone in *P. pedestris* makes it unlikely here (Hewitt *et al.*, 1987). It may be that selection over the last

8000 generations has increased homogamy by a modest amount in this zone, but it will require considerable effort to test this involving much larger numbers of crosses through the zone.

The second major phenomenon revealed by this series of crosses, in addition to homogamy, is that of sperm precedence; the eggs of a female are more likely to be fertilized by the sperm of the first male to mate. This is somewhat unusual in insects where most show a predominance of sperm of the last male to mate, brought about in some cases by physical displacement (Parker, 1970; Waage, 1979; Gwynne, 1984). Only a few investigations have been reported in the Orthoptera and these show a variety of predominance relations. In *Locusta migratoria* when a male mates he blocks effective sperm transfer of a subsequent male by the remains of his spermatheca thus giving him precedence of fertilization. Normally the female does not mate again until after she lays (Mika, 1959). When she lays an egg pod this impediment is ejected and she may mate fully again. The new male achieves 90 per cent fertilization (Parker and Smith, 1975). This may be regarded as single male fertilization rather than first or last predominance. On the other hand, in *Gryllus bimaculatus* sperm appear to be used proportionate to their frequency in the spermatheca (Simmons, 1987). The male who has mated most often, and presumably provided most sperm, fertilizes most eggs. Clearly this involves the mixing of sperm from successive matings, and mixing has been reported in two other crickets *G. integer* (Backus and Cade, 1986) and *Gryllodes supplicans* (Sakaluk, 1986). The catantopine grasshopper *P. pedestris* also demonstrates sperm mixing but with strong first male precedence. Preliminary data from the gomphocerine grasshopper *Chorthippus parallelus* using allozyme markers similarly indicates mixing and precedence (Dagg, 1986).

Following Parker's (1970) seminal paper on sperm competition in insects as a form of sexual selection there has been much discussion of the possible adaptive and evolutionary implications (see articles in Smith, 1984), the generation of conflicting evolutionary forces being particularly important (Parker, 1984). The opportunity for different sperm to enter the female and fertilize her eggs provides an arena for competition and selection. The ability to displace previous sperm (Lefevre and Jonsson, 1962; Waage, 1979) and to produce blocks to subsequent sperm transfer, including guarding females after mating, may well evolve, and so may the females' ability to choose certain mates, modify sperm displacement and

control fertilization (Walker, 1980; Eberhard, 1985; Simmons, 1987). The advantages of mating, in addition to the basic need of fertilization, may include nutrients from the male which can boost egg production, and protection (Gwynne, 1983, 1984; Butlin *et al.*, 1987). The disadvantages may include loss of feeding time and risk of predation (e.g., Cade, 1979). From such a tangle of often conflicting possibilities it is extremely difficult to elucidate just how the various components interact adaptively, how selection is operating and how the process of evolution has occurred. As a consequence, the topic has produced a number of different simple generalizations. Those relevant to the present results include, e.g., males investing nutrients in the progeny are expected to have a high chance of paternity, females receiving benefits from the male are expected to mate multiply and reward males with paternity through sperm displacement, where remating is a cost to the female she should reduce last male predominance; where a female can find mates easily male parental investment will not be correlated with expenditure on paternity assurance (Gwynne, 1984; Parker, 1984). The number of factors involved and their diversity across groups indicate a real need now for detailed studies examining all these components in particular organisms.

In grasshoppers a number of factors are already apparent. There is evidence of precedence of first males sperm with some mixing in *P. pedestris* and *C. parallelus* (loc. cit.). Sperm transfer takes about 20 min in *Podisma* and yet pairs in the field or laboratory remain coupled for hours, and even overnight (Mason and Hewitt, unpublished). In *Chorthippus* the whole thing is much quicker (Green, 1987; Ritchie, 1988). After a single mating a female can lay fertile pods for the rest of her life and there was no indication of females running short of sperm in the present experiments. Nutrients are transferred by the male in grasshoppers (Friedel and Gillot, 1977) and can boost egg production in *Chorthippus* (Butlin *et al.*, 1987). Females of *Podisma* may mate several times in the field (Hewitt *et al.*, 1987; Mason and Hewitt, unpublished) and several other gomphocerine species including *Chorthippus* remate readily in the laboratory. Virgin females of two parapatric races of *C. parallelus* show mating preference for males of their own race based on both acoustic and pheromonal signals. This preference decays with age (Ritchie, 1988). Experiments on *C. brunneus* show that the quantity and some components of the male's song influence his mating success. There is evidence for stabilizing selection, and

heritabilities are low (Butlin *et al.*, 1985; Butlin and Hewitt, 1986). In this paper we demonstrate preferential fertilization of eggs by sperm of their own genotype in *Podisma*.

From such a constellation of factors a number of selective interactions are possible. For example, since the first male gains most fertilizations and provides the female with abundant sperm, then in successive matings males will provide nutrient with a low probability of fertilization. So male genotypes which mature early, produce a large initial spermatophore and choose virgin females might be at an advantage. If spermatophore size is related to body size then female genotypes choosing larger males should be advantageous; however larger males may take longer to reach adult (Hassall and Grayson, 1987). If the interaction of sperm and female reproductive tract allows discrimination between which genotype and sperm will actually fertilize the ova, then female genotypes capable of discriminating sperm from compatible males will be advantaged. Besides this present evidence from *Podisma* there is a wealth of experimental data from *Drosophila* that show the necessary genetic variability and discrimination at this stage (Gromko, *et al.*, 1984). Of particular relevance here are the differences in predominance between attached XY and nullisomic sperm in *D. melanogaster* (Olivieri *et al.*, 1970; Johnsen and Zarrow, 1971), while there is clear evidence for female discrimination between sperm differing by a marked reciprocal translocation (Childress and Hartl, 1972). A wide range of predominance ratios have been reported in *D. melanogaster* where the males differ at various loci (reviewed in table III, Gromko *et al.*, 1984) indicating considerable genetic variation for this character. This clearly suggests that continuing evolution of such systems is possible. The genes affecting discrimination and precedence will probably have pleiotropic effects on other characters which will have their own effects on fitness. Consequently some evolutionary modifications may be more easily achieved than others, and conversely some changes in mating and fertilization mechanisms may be determined by selected changes in other aspects of the organism's biology. Adaptive or apparently adaptive characters may be constrained by their genetic architecture.

A third point of interest to emerge from these experiments is that the sex ratio is biased toward female embryos (0.69), and this is more marked with parent females of the XA unfused (XO) race than those of the XA fusion (XY) race. This could be because a poorer performance of male deter-

mining sperm or of very early male embryos: it provides another example of Haldane's Rule (Haldane, 1922; Jones and Barton, 1985; Charlesworth *et al.*, 1987) and it is perhaps significant that once again the sex chromosome is involved. Fewer males are produced when there are more infertile eggs laid, and this could be either sperm or embryo failure. But it is perhaps less likely to be a problem with sperm since one might expect both male and female determining sperm to be present in numbers at fertilization and failure of a male sperm to fertilize will not give an infertile egg. However, not enough is known about the mechanics of sperm storage and fertilization to be at all sure of this. Such a research program is clearly needed to illuminate homogamy and precedence, as well as infertility.

The higher frequency of infertile eggs recorded for these laboratory mated females, as compared with the field mated ones in the previous experiment (Hewitt *et al.*, 1987), may simply be that the males' fertility was more affected by the journey back to Norwich from the high alpine meadows. It could be because different whole racial genomes were involved in the matings and these may interact in some way to produce infertility; in the previous experiments on homogamy males and females came from the same population in the hybrid zone where such a possibility seems less likely. It could also be because the matings were arranged in these current experiments, whereas the females could choose their mates in the field in the previous ones. When given a free choice she may mate with superior and perhaps even with more compatible males. These possibilities suggest a variety of alternative lines of enquiry.

Acknowledgements We are particularly grateful to Marise East for her customary excellent technical assistance, to Lincoln Rickwood and Sim Webb for help collecting insects and to Drs Roger Butlin and Michael Ritchie for comments on the manuscript. The SERC and NERC provided financial assistance and we thank them both.

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