Patterns of male sterility in a grasshopper hybrid zone imply accumulation of hybrid incompatibilities without selection

David M. Shuker^{*,†}, Karen Underwood, Tania M. King and Roger K. Butlin[‡]

School of Biology, The University of Leeds, Leeds LS2 9JT, UK

It is now widely accepted that post-zygotic reproductive isolation is the result of negative epistatic interactions between derived alleles fixed independently at different loci in diverging populations (the Dobzhansky–Muller model). What is less clear is the nature of the loci involved and whether the derived alleles increase in frequency through genetic drift, or as a result of natural or sexual selection. If incompatible alleles are fixed by selection, transient polymorphisms will be rare and clines for these alleles will be steep where divergent populations meet. If they evolve by drift, populations are expected to harbour substantial genetic variation in compatibility and alleles will introgress across hybrid zones once they recombine onto a genetic background with which they are compatible. Here we show that variation in male sterility in a naturally occurring *Chorthippus parallelus* grasshopper hybrid zone conforms to the neutral expectations. Asymmetrical clines for male sterility have long tails of introgression and populations distant from the zone centre show significant genetic variation for compatibility. Our data contrast with recent observations on ‘speciation genes’ that have diverged as a result of strong natural selection.

Keywords: Dobzhansky–Muller incompatibilities; epistasis; hybrid zone; speciation; hybrid male sterility

1. INTRODUCTION

The evolution of reproductive isolation is central to the process of speciation. Recent theoretical and empirical progress in understanding the genetic basis of reproductive isolation has resulted in a widespread acceptance that post-zygotic reproductive isolation between diverging populations results from the build-up of Dobzhansky–Muller (D–M) incompatibilities (Orr & Turelli 2001; Turelli *et al.* 2001; Coyne & Orr 2004). These genetic incompatibilities are the result of negative epistatic interactions between derived alleles that spread in different populations and reduce hybrid fitness, due to sterility or inviability. They have been observed in a wide range of interspecific crosses (e.g. *Drosophila* flies, Coyne & Orr 2004; *Nasonia* wasps, Breeuwer & Werren 1995; Gadau *et al.* 1999; and *Mimulus* monkeyflowers, Christie & Macnair 1984; Fishman & Willis 2001). What is not clear is the normal function of loci that contribute to D–M incompatibilities, given the only known phenotype is hybrid sterility or inviability, or the reasons for the fixation of the novel alleles at these loci (Coyne & Orr 2004). Do these alleles typically spread via the action of natural or sexual selection or are the new alleles neutral within populations and accumulated by genetic drift? If populations are in contact during divergence, then selection is necessary to overcome incompatibility (Navarro & Barton 2003) but this is not true for allopatric differentiation.

The ‘faster male’ explanation for Haldane’s Rule (the observation that hybrid inviability and sterility are concentrated in the heterogametic sex, see Orr (1997)) suggests that hybrid male sterility might be an incidental side effect of rapid evolution of male reproductive characters resulting from sexual selection (Wu & Davis 1993; Wu *et al.* 1996). There is evidence that this process does contribute to sterility in male-heterogametic taxa (Presgraves & Orr 1998). *OdsH* in *Drosophila mauritiana* is the only hybrid sterility gene so far identified at the molecular level. Sequence comparisons suggest that it has evolved rapidly under positive selection (Ting *et al.* 1998) and the derived allele results in a modest increase in sperm production in young males, which may be the source of this selection (Sun *et al.* 2004). The small number of loci so far identified as contributing to hybrid inviability also show evidence of a history of positive selection (*Xmrk-2* in *Xiphophorus*, Nup96 and *Hmr* in *Drosophila*; Schartl *et al.* 1999; Barbash *et al.* 2003; Presgraves *et al.* 2003). While these observations suggest that reproductive isolation is an incidental consequence of divergence under selection, the sample size is currently very small, biased towards *Drosophila* and based on comparisons after speciation is complete.

If selection leads to the fixation of beneficial alleles within populations that interact negatively with other loci in a hybrid genetic background, we can predict limited genetic variation within populations for D–M incompatibilities. Accumulation of incompatibilities by drift predicts the opposite: populations should typically harbour genetic variation at these ‘synthetic deleterious loci’ (Nei *et al.* 1983; Phillips & Johnson 1998). While the negative fitness effects of such loci will very rarely be felt within populations, they may be expressed in crosses

* Author for correspondence (david.shuker@ed.ac.uk).

† Present address: Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK.

‡ Present address: Department of Animal and Plant Sciences, The University of Sheffield, Sheffield S10 2TN, UK.

between divergent populations that harbour incompatible alleles at high frequency. Early work suggested that standing variation for the strength of reproductive isolation was common (Coyne & Orr 2004, p. 271) but this has only rarely been subjected to systematic study (Wade *et al.* 1997; Reed & Markow 2004; Wade & Johnson 1994). Determining the nature of loci involved in the first stages of the evolution of reproductive isolation by this approach is an important complement to the analysis of interspecific crosses.

Hybrid zones open a window on an intermediate stage of divergence where the behaviour of incompatibilities accumulated in a period of allopatry and then brought into secondary contact can be followed under natural conditions (Harrison 1993; Shuker *et al.* 2005). Gavrilets (1997) has modelled the behaviour of D–M loci in a hybrid zone. Asymmetrical clines in allele frequency are expected to form: steep on the side where the allele is in a foreign genetic background and so contributes to inviability or sterility, but shallow on the other side where the derived and ancestral alleles are competing in a genetic background with which they are both compatible. Therefore, when dispersal is known, cline widths can be used to estimate the strength of selection in both genetic backgrounds under natural conditions (Barton & Hewitt 1985), providing a test for the role of selection in the spread of the derived D–M alleles.

Here, we consider the quantitative genetic basis of hybrid incompatibility in a hybrid zone between two subspecies of the meadow grasshopper, *Chorthippus parallelus parallelus* (CPP) and *Chorthippus parallelus erythropus* (CPE) (Butlin 1998). Specifically, we explore patterns of genetic variation within and between the two subspecies for hybrid male sterility. We consider clines in testis dysfunction, as measured by testis follicle length, through crosses of males from reference populations of each subspecies with hybrid females collected through a well documented transect across the hybrid zone. F1 hybrid males are completely sterile but males collected from the hybrid zone are fertile, as predicted if recombination reconstructs compatible ancestral genotypes (Gavrilets 1997; Butlin 1998; Shuker *et al.* 2005). For comparison, we also consider patterns of genetic variation in a putatively neutral morphological character that varies between the subspecies: male stridulatory peg number. If positive selection has acted on genes that underlie the hybrid male sterility in crosses between CPP and CPE parents, then we predict: (1) genetic variation within distant populations will be low or absent and (2) clines will be steep relative to dispersal on both sides of the zone.

2. METHODS

(a) *The parallelus–erythropus hybrid zone*

The two subspecies meet and form hybrids in passes through the Pyrenees Mountains between France and Spain. Molecular data suggest that these two subspecies have been diverging for about 0.5 M years, having been restricted to separate refuges during recent ice ages (Cooper *et al.* 1995; Lunt *et al.* 1998). The two subspecies differ in a number of morphological, behavioural and genomic traits (Butlin 1998; Shuker *et al.* 2005), with the morphological character that best resolves the two subspecies being male stridulatory peg

number (Butlin & Hewitt 1985; Butlin *et al.* 1991). In the field, hybrids of both sexes are fully viable and fully fertile. However, crosses between ‘pure’ individuals from either side of the hybrid zone yield sterile males, characterized by severe testis dysfunction (an example of Haldane’s Rule as males are XO: Hewitt *et al.* 1987; Bella *et al.* 1990).

(b) *Experimental crosses and cline analysis*

Grasshoppers were collected during July and August 1994, 2000 and 2001 from 28 sites through the Col de la Quillane, in the eastern Pyrenees, following the transect of Butlin *et al.* (1991). Reference populations of the two pure subspecies were collected from Aunat, France (CPP) and Greixer or Bellver, Spain (CPE). See the electronic supplementary material for rearing details. All distances through the hybrid zone were measured from Aunat as the sum of straight-line distances between adjacent sites.

We made crosses between reference population males (collected as either nymphs or adults) and virgin females from sites across the zone, to produce male offspring with differing genetic constitutions. By doing so, we expressed the incompatibilities that exist between the pure subspecies and visualized how the alleles at D–M loci penetrate the hybrid zone as clines in male sterility. For example, Aunat (CPP) reference males that were crossed to females from the most distant site on the CPE side of the hybrid zone produced male offspring with F1 genotypes: a CPE X chromosome and one set of autosomes from each of the subspecies. These offspring were expected to be sterile. A CPP male crossed to a female from a nearby population on the CPP side of the hybrid zone was expected to produce fertile, CPP-genotype offspring. However, a CPP male crossed to a female from the middle of the cline for sterility-causing alleles yielded male offspring with a recombined X chromosome, one set of pure CPP autosomes and one set of recombined autosomes. Thus, measurements of offspring testes reveal the positions of clines for alleles at D–M genes if they are X-linked or involved in dominant genetic incompatibilities.

Follicle length was used as a measure of male sterility as it is strongly correlated with other measures of testis function, including sperm production (Hewitt *et al.* 1987; Virdee & Hewitt 1992, 1994; Tregenza *et al.* 2002). Six follicles were randomly chosen and measured under a dissecting microscope with an eyepiece graticule following the method of Hewitt *et al.* (1987) and the mean follicle length calculated for each male. We confirmed the positive relationship between follicle length and the presence of sperm bundles in a sub-sample of 433 males using logistic regression (logistic regression coefficient $\beta = 8.24 \pm 1.02$, $\chi^2_1 = 366.1$, $p < 0.0001$). Male stridulatory peg numbers were counted by removing the left rear leg after death and counting the pegs on the femur under a compound microscope.

(c) *Statistical analysis*

We estimated the partitioning of variance among sites along the transect and among families within sites, using hierarchical analysis of variance (for the 2000 and 2001 data only since families were not separated in 1994). We did this separately for the sets of crosses to CPP and CPE males and from either side of the hybrid zone, omitting sites in the transitional area (less than 0.5 cline widths from the estimated zone centre). This yielded four analyses: within each subspecies (i.e. between pure CPP males and females from the CPP side of the zone centre; the same for CPE) and the

Table 1. Variance component analyses for mean follicle length and stridulatory peg number of male offspring in crosses between reference males and females from the CPP or CPE side of the hybrid zone (CPP, *C. p. parallelus*, CPE, *C. p. erythropus*).

trait	male	female	level	var component	F (d.f.)	p
follicle length	CPP	CPP	sites	0.00025	0.95(6,21)	NS
			families	0.01528	3.04(21,97)	<0.001
			residual	0.0306		
		CPE	sites	0.1059	1.89(1,8)	0.21
			families	0.1318	5.24(8,66)	<0.001
			residual	0.229		
	CPE	CPP	sites	0.0170	0.16(5,49)	NS
			families	0.1612	4.35(49,141)	<0.001
			residual	0.1370		
		CPE	sites	0 (bound)	—	—
			families	0.02919	3.80(9,26)	0.004
			residual	0.0380		
peg number	CPP	CPP	sites	1.85	0.85(2,8)	NS
			families	63.04	3.82(8,41)	0.002
			residual	83.34		
		CPE	sites	2.7	0.002(1,9)	NS
			families	163.1	10.32(9,67)	<0.001
			residual	106.4		
	CPE	CPP	sites	85.62	0.67(1,9)	NS
			families	76.16	5.55(9,37)	<0.001
			residual	75.07		
		CPE	sites	— (only one site)		
			families	201.3	11.87(5,22)	<0.001
			residual	73.25		

two reciprocal F1 crosses (between CPP males and females from the CPE side of the zone and vice versa). Residuals did not deviate significantly from a normal distribution for either stridulatory peg number or mean follicle length.

Clines were fitted using an extension of the method applied by Butlin *et al.* (1991). See the electronic supplementary material for further details. No differences were detected between years so all data were combined. To account for the asymmetry predicted by Gavrilets (1997), the zone width was allowed to differ on either side of the centre in some models. The within site variance was allowed to differ between the ends of the transect (i.e. between pure and F1-like crosses) and was assumed to be a linear function of the proportion of 'foreign' alleles. In some models, we added a quadratic term since segregation and linkage disequilibrium are expected to elevate the variance in the centre of a hybrid zone (Barton & Gale 1993).

Models were fitted by calculating the log-likelihood of the observed data for a given set of parameter values, assuming a normal distribution of mean follicle lengths (or peg numbers) and searching for the maximum likelihood parameter combination using a Metropolis algorithm (Tregenza *et al.* 2000). Nested models were compared by testing twice the difference in log-likelihood against a χ^2 distribution, with the degrees of freedom equal to the difference in number of parameters in the models.

3. RESULTS

We analysed follicle length data from a total of 964 males, 527 from crosses to CPP reference males and 437 from crosses to CPE. The females came from 28 sites with 1 to 103 offspring per site, distributed across 1 to 14 families. Stridulatory peg number data were obtained for 776 of the offspring.

(a) Genetic variation in hybrid male sterility and stridulatory peg number

Whichever reference population was used, the total variance among offspring in mean follicle length was much greater in crosses that spanned the hybrid zone, producing F1-like genotypes, than in crosses within subspecies (table 1). There was significant variation among full-sib families within sites in all cases, but no significant variation among sites from which female parents were derived. The among-family component of variance was highest for crosses between CPE males and CPP females, where it exceeded 50% of the total variance. Effects of rearing environment were minimized by splitting families and randomizing positions in the controlled temperature room. Maternal effects will be included in the among-family variance but are unlikely to explain this effect on adult male offspring and cannot explain the large difference in variance between pure and F1-type crosses. The family effects therefore indicate significant genetic variation for follicle length segregating within populations of each subspecies. That they increase in inter-subspecific crosses indicates epistatic interactions between the loci contributing to the additive genetic variance within populations and the hybrid genetic background, as expected under the D-M model.

The absence of a significant component of variation among sites suggests that this genetic variation is unlikely to be due to introgression of alleles into sites close to the hybrid zone. The family component of variance remains significant when crosses between the most extreme populations alone are considered (more than 60 km apart, s.d. of parent-offspring distances < 30 m per generation: (Virdee & Hewitt 1990). Inspection of the follicle length distributions for offspring from these

Figure 1. The clines in mean male offspring follicle length (mm) for crosses between (a) CPP and (b) CPE reference males and females from the transect. Data shown are individual male offspring. The solid lines are the fitted maximum likelihood clines and the dotted lines provide an indication of the fitted variance (± 1 s.d.).

extreme crosses shows some evidence of bimodality, with some individuals (9/69 in crosses to CPP males, 4/45 in the reciprocal) having apparently normal testes with sperm production (figure 1). This pattern suggests that there are, segregating within populations, one or more loci of relatively large effect contributing to F1 sterility.

The stridulatory peg number data reveal a very similar pattern (table 1). Variation among families was significant in all cases but variation among sites was not detected. The total variance was markedly higher in hybrid than in pure crosses. Peg number distributions were not bimodal and peg number was not correlated with follicle length among offspring from the same maternal site (e.g. $r=0.03$ and -0.15 , $p>0.2$, for the two reciprocal crosses between the ends of the transect).

(b) Clines in hybrid male sterility and stridulatory peg number

Asymmetrical clines provided a better fit to the mean follicle length data than symmetrical clines for both sets of crosses when analysed separately (crosses to CPP males, $\chi^2_1 = 40.11$, $p<0.001$; crosses to CPE males, $\chi^2_1 = 4.44$, $p=0.035$). However, the asymmetry was much more marked for CPP crosses than for CPE crosses (figure 1). When the two data sets were analysed together, the best fitting model had a common cline centre and width on the steep, non-hybrid side but separate parameters for the two directions of cross for all other parameters (table 2). This model was a significant improvement over a model with the same width on the hybrid side ($\chi^2_1 = 10.1$, $p<0.01$) and the asymmetrical model was a much better fit than the equivalent symmetrical model ($\chi^2_1 = 30.9$, $p<0.001$). The fully independent clines model did not provide a significant improvement over the asymmetrical model with common centre and hybrid-side width ($\chi^2_1 = 4.44$, $p>0.1$). The fitted parameters confirm the substantial difference in variance between the pure and hybrid

crosses, in both directions, but adding a quadratic term did not improve the fit of any of the models.

For stridulatory peg number, we detected a difference between reciprocal crosses suggesting sex linkage. This has been observed previously in *C. parallelus* (Butlin & Hewitt 1988a) but not in a related species pair (*C. brunneus* and *C. jacobsi*; Saldamando *et al.* 2005). A model with common parameters for both directions of cross and including a term for sex linkage, was fitted to the data (table 2). This model includes a significant difference in variance between pure and hybrid crosses, as expected from the variance components analysis, but adding a quadratic term did not improve the fit. The fit was also not improved by allowing differences between directions of cross for any of the parameters. The position of the cline centre is close to that estimated previously but the width is greater, being closer to the value obtained for a transect in the western Pyrenees (Butlin *et al.* 1991).

4. DISCUSSION

Our data on hybrid sterility in *C. parallelus* grasshoppers do not conform to the expectations if positive selection were responsible for the spread of D–M incompatibility alleles. Populations at the extremes of our transect are polymorphic for alleles that contribute to sterility in inter-subspecific crosses, paralleling recent observations in *Drosophila mojavensis* (Reed & Markow 2004). Clines for sterility are asymmetric, as predicted (Gavrilets 1997), and cline widths indicate little or no advantage to derived alleles in compatible backgrounds. As in the case of *D. mojavensis*, our system allows us to study early events in speciation: the spread of alleles that contribute to reproductive isolation before it is complete rather than those that may have accumulated after speciation. The hybrid zone provides the added advantage that the behaviour of these alleles when in contact with an incompatible genetic background can be observed under natural conditions.

Our observations on stridulatory peg number provide a useful background against which to interpret the male sterility data. Although the pegs are essential for production of acoustic mating signals, peg number is not tightly correlated with song structure, which is determined primarily by leg movement patterns (von Helversen & Elsner 1977; Butlin & Hewitt 1988b). Therefore, selection on peg number is likely to be weak at most and this is consistent with the wide clines observed here and in previous analyses (Butlin *et al.* 1991). There was no evidence for asymmetry of clines or differences in cline parameters between the two directions of cross, except for the effects of sex linkage. No elevation in variance at the cline centre was detected, suggesting that the number of loci underlying divergence between the two subspecies is large (Barton & Gale 1993).

Our observations on the hybrid male sterility clines are also consistent with the theoretical predictions for D–M incompatibility loci when the derived alleles are neutral or weakly selected within populations, but interact to cause sterility in hybrids. As in the Gavrilets (1997) model, clines are asymmetrical. Introgression on the hybrid side is wide because the ancestral allele, for example from CPP, does not have significantly reduced fitness relative to the derived allele present in CPE and does not cause sterility.

Table 2. Maximum likelihood (ML) estimates and lower and upper support limits (SL) of fitted parameters for the follicle length and stridulatory peg number clines (CPP, *C. p. parallelus*, CPE, *C. p. erythropus*).

trait and parameter	reference male	ML estimate	lower SL	upper SL
<i>mean follicle length</i>				
centre of cline (km)		27.89	27.67	28.02
width on hybrid side (km)	CPP	23.76	14.42	29.78
	CPE	1.899	0.2 (lower bound)	3.719
width on pure side (km)		0.718	0.288	1.108
pure mean (mm)	CPP	2.157	2.137	2.187
	CPE	2.339	2.188	2.496
change in mean (mm)	CPP	-1.044	-1.162	-0.973
	CPE	-1.255	-1.336	-1.154
variance on pure side (mm ²)	CPP	0.048	0.043	0.056
	CPE	0.162	0.215	0.105
change in variance (mm ²)	CPP	0.254	0.153	0.380
	CPE	0.320	0.291	0.361
<i>stridulatory peg number</i>				
centre (km)		28.87	27.37	30.45
width (km)		20.96	15.66	27.93
pure mean		99.75	97.24	102.7
change in mean (autosomal)		39.40	33.99	43.47
change in mean (sex-linked)		12.92	9.180	17.59
variance on pure side		150.7	127.5	179.7
change in variance		94.15	38.41	147.3

This is not what we would expect if the derived CPE allele were appreciably fitter than the ancestral allele (i.e. if its original spread had been driven by positive selection). Introgression on the pure side is much less because the derived allele from CPE contributes to sterility in the CPP genetic background. Introgression on the hybrid side suggests weak selection, at most, favouring derived alleles in both backgrounds (see the electronic supplementary material for a discussion of the strength of selection). The cline is much wider in crosses to CPP than in crosses to CPE but there is no reason to expect them to be equal: derived alleles at different loci are expected to be fixed independently in the two backgrounds (but see below).

We did not observe the displacement of clines reported by Virdee & Hewitt (1994) and predicted by Gavrillets (1997) to arise as a result of recombination generating fit ancestral genotypes with compatible alleles. However, displacement by a distance comparable to the width on the steeper side of the cline would be difficult to detect, given our sample spacing and the variance present within sites. The essential point, that populations in the centre of the hybrid zone can be crossed to either parental subspecies and generate largely fertile hybrid offspring, remains true. The wider clines and greater displacement reported previously (Virdee & Hewitt 1994; Butlin 1998) are probably an artefact of fitting clines to their relatively small data sets, without allowing for a change in variance across the transect and without allowing for asymmetry; fitting symmetrical clines with constant variance to our data generates results similar to those obtained previously.

Hybrid offspring had greater within-site variance in peg number than pure offspring, as might be expected from a reduction in homeostasis in the mixed genetic background and there was significant variance among families, consistent with the high heritability expected for a quantitative trait under weak selection (see Butlin & Hewitt 1986 for a closely related species).

Substantial among-family variance in sterility was observed in all types of cross, even in populations remote from the hybrid zone, with the total variance much greater in hybrid than in pure crosses. This is inconsistent with strong selection favouring the derived alleles that cause sterility in hybrid crosses that would lead rapidly to fixation within populations. The simplest explanation for this observation is that alleles responsible for D-M incompatibilities are neutral, or nearly neutral, against their own genetic background (Phillips & Johnson 1998). Balancing selection is a possible alternative explanation for the maintenance of high levels of genetic variation within populations. It is difficult to reject this hypothesis: it would predict steep clines but high variance within populations making cline widths difficult to estimate. Introgression across the zone might also contribute to the observed variation. This is highly unlikely on the CPP side where the distance from the Aunat site to the zone centre is more than 15 times the estimated cline width. However, it is possible on the CPE side where the estimated cline width is much greater. Introgression over this distance might suggest that some of the derived alleles are actually less fit than the ancestral alleles, in which case their original spread most likely involved drift in small populations, perhaps during post-glacial range expansion. The wide cline on this side of the hybrid zone may be related to the known displacement of a cline for an X chromosome marker from CPP into CPE (Ferris *et al.* 1993). An interesting feature of the data is the apparent bimodality, especially in the cross between CPP males and females from the CPE reference site. This suggests segregation of alleles at loci with large effect, most probably on the X chromosome in CPE.

Our results provide a counterpoint to recent evidence that genes contributing to post-zygotic reproductive isolation diverge as a result of selection. The four loci that have been identified in interspecific hybridizations as contributing to D-M incompatibilities and have been

characterized at a molecular level, all show evidence for recent adaptive evolution (Ting *et al.* 1998; Scharl *et al.* 1999; Barbash *et al.* 2003; Presgraves *et al.* 2003). The approach of examining variation within populations that influences fitness of hybrids has been relatively neglected, even though the *Xmrk* locus in platyfish is polymorphic within populations (Scharl 1995) and hybrid rescue loci in *Drosophila* vary among lines (Davis *et al.* 1996). Apart from the current study, there have been only two systematic investigations of variation for compatibility: Wade and collaborators (Wade & Johnson 1994; Wade *et al.* 1997) found substantial segregating variation within *Tribolium castaneum* for hybrid survival and morphological abnormalities when crossed with *T. freemani*, and Reed & Markow (2004) found variation within and between *Drosophila mojavensis* populations for male sterility when crossed to *D. arizonae*. There is variation among widespread European populations of CPP for sterility (follicle length) when hybridizing with CPE (Tregenza *et al.* 2002). Here we observe variation within populations and provide evidence that selection on D–M incompatibility alleles is weak or absent. Further study of this type of variation can contribute significantly to our understanding of speciation.

We are grateful to NERC for funding.

REFERENCES

- Barbash, D. A., Siino, D. F., Tarone, A. M. & Roote, J. 2003 A rapidly evolving MYB-related protein causes species isolation in *Drosophila*. *Proc. Natl Acad. Sci. USA* **100**, 5302–5307. (doi:10.1073/pnas.0836927100.)
- Barton, N. H. & Gale, K. S. 1993 Genetic analysis of hybrid zones. In *Hybrid zones and the evolutionary process* (ed. R. G. Harrison), pp. 13–45. New York, NY: Oxford University Press.
- Barton, N. H. & Hewitt, G. M. 1985 Analysis of Hybrid Zones. *Annu. Rev. Ecol. Syst.* **16**, 113–148. (doi:10.1146/annurev.es.16.110185.000553.)
- Bella, J. L., Hewitt, G. M. & Gosálvez, J. 1990 Meiotic imbalance in laboratory-produced hybrid males of *Chorthippus parallelus parallelus* and *Chorthippus parallelus erythropus*. *Genet. Res.* **56**, 43–48.
- Breeuwer, J. A. J. & Werren, J. H. 1995 Hybrid breakdown between two haplodiploid species: the role of nuclear genes and cytoplasmic genes. *Evolution* **49**, 705–717.
- Butlin, R. K. 1998 What do hybrid zones in general, and the *Chorthippus parallelus* zone in particular, tell us about speciation? In *Endless forms: species and speciation* (ed. D. J. Howard & S. H. Berlocher), pp. 367–378. New York, NY: Oxford University Press.
- Butlin, R. K. & Hewitt, G. M. 1985 A hybrid zone between *Chorthippus parallelus parallelus* and *Chorthippus parallelus erythropus* (Orthoptera: Acrididae)—Morphological and Electrophoretic Characters. *Biol. J. Linn. Soc.* **26**, 269–285.
- Butlin, R. K. & Hewitt, G. M. 1986 Heritability estimates for characters under sexual selection in the grasshopper, *Chorthippus brunneus*. *Anim. Behav.* **34**, 1256–1261.
- Butlin, R. K. & Hewitt, G. M. 1988a Genetics of behavioral and morphological differences between parapatric subspecies of *Chorthippus parallelus* (Orthoptera: Acrididae). *Biol. J. Linn. Soc.* **33**, 233–248.
- Butlin, R. K. & Hewitt, G. M. 1988b The structure of grasshopper song in relation to mating success. *Behaviour* **104**, 152–161.
- Butlin, R. K., Ritchie, M. G. & Hewitt, G. M. 1991 Comparisons among morphological characters and between localities in the *Chorthippus parallelus* hybrid zone (Orthoptera: Acrididae). *Phil. Trans. R. Soc. B* **334**, 297–308.
- Christie, P. & Macnair, M. R. 1984 Complementary lethal factors in 2 North-American populations of the yellow monkey flower. *J. Hered.* **75**, 510–511.
- Cooper, S. J. B., Ibrahim, K. M. & Hewitt, G. M. 1995 Postglacial expansion and genome subdivision in the European grasshopper *Chorthippus parallelus*. *Mol. Ecol.* **4**, 49–60.
- Coyne, J. A. & Orr, H. A. 2004 *Speciation*. Sunderland, MA: Sinauer Associates.
- Davis, A. W., Roote, J., Morley, T., Sawamura, K., Herrmann, S. & Ashburner, M. 1996 Rescue of hybrid sterility in crosses between *D. melanogaster* and *D. simulans*. *Nature* **380**, 157–159. (doi:10.1038/380157a0.)
- Ferris, C., Rubio, J. M., Serrano, L., Gosálvez, J. & Hewitt, G. M. 1993 One-way introgression of a subspecific sex chromosome marker in a hybrid zone. *Heredity* **71**, 119–129.
- Fishman, L. & Willis, J. H. 2001 Evidence for Dobzhansky–Muller incompatibilities contributing to the sterility of hybrids between *Mimulus guttatus* and *M. nasutus*. *Evolution* **55**, 1932–1942.
- Gadau, J., Page, R. E. & Werren, J. H. 1999 Mapping of hybrid incompatibility loci in *Nasonia*. *Genetics* **153**, 1731–1741.
- Gavrilets, S. 1997 Hybrid zones with Dobzhansky-type epistatic selection. *Evolution* **51**, 1027–1035.
- Harrison, R. G. (ed) 1993 *Hybrid zones and the evolutionary process*. Oxford: Oxford University Press.
- Hewitt, G. M., Butlin, R. K. & East, T. M. 1987 Testicular dysfunction in hybrids between parapatric subspecies of the grasshopper *Chorthippus parallelus*. *Biol. J. Linn. Soc.* **31**, 25–34.
- Lunt, D. H., Ibrahim, K. M. & Hewitt, G. M. 1998 MtDNA phylogeography and postglacial patterns of subdivision in the meadow grasshopper *Chorthippus parallelus*. *Heredity* **80**, 633–641. (doi:10.1038/sj.hdy.6883110.)
- Navarro, A. & Barton, N. H. 2003 Accumulating postzygotic isolation genes in parapatry: a new twist on chromosomal speciation. *Evolution* **57**, 447–459.
- Nei, M., Maruyama, T. & Wu, C.-I. 1983 Models of the evolution of reproductive isolation. *Genetics* **103**, 557–579.
- Orr, H. A. 1997 Haldane's Rule. *Annu. Rev. Ecol. Syst.* **28**, 195–218. (doi:10.1146/annurev.ecolsys.28.1.195.)
- Orr, H. A. & Turelli, M. 2001 The evolution of postzygotic isolation: Accumulating Dobzhansky–Muller incompatibilities. *Evolution* **55**, 1085–1094.
- Phillips, P. C. & Johnson, N. A. 1998 The population genetics of synthetic lethals. *Genetics* **150**, 449–458.
- Presgraves, D. C. & Orr, H. A. 1998 Haldane's rule in taxa lacking a hemizygous X. *Science* **282**, 952–954. (doi:10.1126/science.282.5390.952.)
- Presgraves, D. C., Balagopalan, L., Abmayr, S. M. & Orr, H. A. 2003 Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila*. *Nature* **423**, 715–719. (doi:10.1038/nature01679.)
- Reed, L. K. & Markow, T. A. 2004 Early events in speciation: polymorphism for hybrid male sterility in *Drosophila*. *Proc. Natl Acad. Sci. USA* **101**, 9009–9012. (doi:10.1073/pnas.0403106101.)
- Saldamando, C. I., Miyaguchi, S., Tatsuta, H., Kishino, H., Bridle, J. R. & Butlin, R. K. 2005 Inheritance of song and stridulatory peg number divergence between *Chorthippus*

- brunneus* and *C. jacobsi*, two naturally hybridizing grasshopper species (Orthoptera: Acrididae). *J. Evol. Biol.* **18**, 703–712.
- Schartl, M. 1995 Platyfish and swordtails—a genetic system for the analysis of molecular mechanisms in tumor formation. *Trends Genet.* **11**, 185–189. (doi:10.1016/S0168-9525(00)89041-1.)
- Schartl, M., Hornung, U., Gutbrod, H., Volff, J. N. & Wittbrodt, J. 1999 Melanoma loss-of-function mutants in *Xiphophorus* caused by *Xmrk*-oncogene deletion and gene disruption by a transposable element. *Genetics* **153**, 1385–1394.
- Shuker, D. M., King, T. M., Bella, J. L. & Butlin, R. K. 2005 The genetic basis of speciation in a grasshopper hybrid zone. In *Insect evolutionary ecology* (ed. M. D. E. Fellowes, G. J. Holloway & J. Rolff). Wallingford, UK: CABI Publishing.
- Sun, S., Ting, C. T. & Wu, C. I. 2004 The normal function of a speciation gene, *Odysseus*, and its hybrid sterility effect. *Science* **305**, 81–83. (doi:10.1126/science.1093904.)
- Ting, C. T., Tsaui, S. C., Wu, M. L. & Wu, C. I. 1998 A rapidly evolving homeobox at the site of a hybrid sterility gene. *Science* **282**, 1501–1504. (doi:10.1126/science.282.5393.1501.)
- Tregenza, T., Pritchard, V. L. & Butlin, R. K. 2000 The origins of premating reproductive isolation: testing hypotheses in the grasshopper *Chorthippus parallelus*. *Evolution* **54**, 1687–1698.
- Tregenza, T., Pritchard, V. L. & Butlin, R. K. 2002 The origins of postmating reproductive isolation: testing hypotheses in the grasshopper *Chorthippus parallelus*. *Popul. Ecol.* **44**, 137–144. (doi:10.1007/s101440200017.)
- Turelli, M., Barton, N. H. & Coyne, J. A. 2001 Theory and speciation. *Trends Ecol. Evol.* **16**, 330–343. (doi:10.1016/S0169-5347(01)02177-2.)
- Virdee, S. R. & Hewitt, G. M. 1990 Ecological components of a hybrid zone in the grasshopper *Chorthippus parallelus* (Zetterstedt) (Orthoptera: Acrididae). *Boletín de Sanidad Vegetal (Fuera de Serie)* **20**, 299–309.
- Virdee, S. R. & Hewitt, G. M. 1992 Postzygotic isolation and Haldane's rule in a grasshopper. *Heredity* **69**, 527–538.
- Virdee, S. R. & Hewitt, G. M. 1994 Clines for hybrid dysfunction in a grasshopper hybrid zone. *Evolution* **48**, 392–407.
- von Helversen, O. & Elsner, N. 1977 The stridulatory movements of acridid grasshoppers recorded with an opto-electronic device. *J. Comp. Physiol.* **122**, 53–64. (doi:10.1007/BF00611248.)
- Wade, M. J. & Johnson, N. A. 1994 Reproductive isolation between 2 species of flour beetles, *Tribolium castaneum* and *T. freemani*—variation within and among geographical populations of *T. castaneum*. *Heredity* **72**, 155–162.
- Wade, M. J., Johnson, N. A., Jones, R., Siguel, V. & McNaughton, M. 1997 Genetic variation segregating in natural populations of *Tribolium castaneum* affecting traits observed in hybrids with *T. freemani*. *Genetics* **147**, 1235–1247.
- Wu, C. I. & Davis, A. W. 1993 Evolution of postmating reproductive isolation—the composite nature of Haldane's rule and its genetic bases. *Am. Nat.* **142**, 187–212. (doi:10.1086/285534.)
- Wu, C. I., Johnson, N. A. & Palopoli, M. F. 1996 Haldane's rule and its legacy: why are there so many sterile males? *Trends Ecol. Evol.* **11**, 281–284. (doi:10.1016/0169-5347(96)10033-1.)

The electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2005.3242> or via <http://www.journals.royalsoc.ac.uk>.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.