PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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Phil. Trans. R. Soc. Lond. B 1991 **334**, 297-308
doi: 10.1098/rstb.1991.0119

Comparisons among morphological characters and between localities in the *Chorthippus parallelus* hybrid zone (Orthoptera: Acrididae)

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SUMMARY

Two subspecies of the grasshopper, *Chorthippus parallelus*, meet and hybridize in the Pyrenees. The hybrid zone between the two taxa is believed to have formed following range expansion at the end of the last glaciation and to be maintained by a balance between gene flow and selection against hybrids. Laboratory F1 hybrid males are sterile. We have examined morphological characters in two cols about 200 km apart and compared the positions and widths of clines for these characters both within and between cols. Clines are neither coincident (centred in the same position), nor concordant (equal in width) in either col. Furthermore clines are wider and more dispersed in the western col. The lack of concordance can be explained on several models for the maintenance of the hybrid zone and is a common observation. There are many fewer examples of lack of coincidence and this result is more difficult to interpret. We suggest that it is due to a difference in population structure between the cols: a patchy distribution of grasshoppers in the western col produces wider, more dispersed clines while a sharp break in distribution in the eastern col tends to concentrate morphological change over a shorter distance.

1. INTRODUCTION

Hybrid zones are widespread features of both animal and plant species that offer important opportunities for the study of evolutionary processes (Barton & Hewitt 1985, 1989). A common characteristic of the many zones now described is the involvement of numerous traits – electrophoretic loci, chromosomal markers, morphological or behavioural characters – with coincident clines. This is compatible with a secondary origin for the hybrid zones and maintenance by a balance between dispersal and selection against hybrids. In such zones coincidence of the clines for different characters may be brought about by several processes: (i) it may reflect the point of contact of the expanding ranges of the interacting taxa after a period of divergence in allopatry; (ii) features of the population structure such as density troughs or range constrictions may 'trap' clines in characters subject to selection against hybrids; or (iii) overlapping clines with hybrid disadvantage may attract one another (Barton & Hewitt 1985). In many cases these effects may operate together but, although secondary contact may determine the general position of a zone, the influence of population structure is likely to be the strongest on a local scale. By contrast, coincidence of

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Phil. Trans. R. Soc. Lond. B (1991) **334**, 297–308
Printed in Great Britain

Vol. 334. B1271 21 [Published 30 December 1991

clines is not expected if their positions are determined by environmental gradients alone as the points of equal fitness are likely to differ among characters (Hewitt 1988). Moore (1977) has argued that a hybrid zone may be maintained by 'hybrid superiority'; here one or a few hybrid genotypes would be favoured in suitable environmental patches and smooth coincident clines across many characters would not be expected (Hewitt 1988).

Although the maintenance of hybrid zones by dispersal–selection balance leads to the expectation of coincident cline centres, it does not predict constancy of width or shape (i.e. concordance). Clines in different characters at one locality may vary in width because they are subject to different intensities of selection, because of differences in their genetic determination (especially the number of loci involved), or because of their linkage relations to other selected loci. Variation in cline width among localities may be explained by differing environmental gradients, by variation in population structure, by genetic differentiation within the interacting taxa or as a result of 'modification'. Modification may involve either reduction in hybrid disadvantage (see Searle 1986) or reduction in the production of hybrids through assortative mating (i.e. reinforcement; Butlin 1987). Variations in width both among characters and among localities are common features of hybrid zones (Barton & Hewitt 1985). Analysis of this variation may help in understanding the origin, maintenance, and fate of a hybrid zone by facilitating tests for the influence of environmental variables, population structure, and modification. Examples of this approach include: *Bombina* (Szymura & Barton 1986), *Caledia* (Marchant *et al.* 1988), *Heliconius* (Mallet & Barton 1989), *Mus* (Vanlerberghe *et al.* 1988), and *Podisma* (Nichols & Hewitt 1986).

A hybrid zone in the grasshopper, *Chorthippus parallelus*, occurs in the Pyrenees where an Iberian subspecies, *C. p. erythropus*, meets the nominate subspecies, *C. p. parallelus*. The zone is known to follow approximately the Pyrenean watershed but to be limited to cols below about 2000 m in altitude (Butlin & Hewitt 1985*a*; Hewitt *et al.* 1988; Ritchie 1988; Hewitt 1990). The subspecies differ in many characters, including morphological (Butlin & Hewitt 1985*a*), behavioural (Butlin & Hewitt 1985*b*; Butlin 1989; Butlin & Ritchie 1991), allozymic (Butlin & Hewitt 1985*b*; M. Duijm, personal communication) and chromosomal (Gosalvez *et al.* 1988). In the laboratory, F1 hybrid males are almost completely sterile but hybrid females are fertile (Hewitt *et al.* 1987). In this paper we examine the clines in morphological characters at two widely separated cols in the Pyrenees and compare their positions and widths both within and between localities.

2. METHODS

(*a*) *Collections*

Grasshoppers were collected in the summers of 1984, 1985 and 1986 from transects in the Col de la Quillane, near Mont Louis, Pyrénées-Orientales and about 200 km to the west in the Col du Pourtalet, Pyrénées-Atlantiques. At each locality collections were made on a principal transect (35 collections from 22 sites, 559 males and 545 females, at the Col du Pourtalet, and 20 collections from 15 sites, 247 males and 258 females, at the Col de la Quillane, some sites being visited in more than one year) and at subsidiary sites on either side of the main transect in the region of the zone centre (figure 1).

(*b*) *Characters*

Morphological characters were measured from dried, pinned adult grasshoppers using an eyepiece graticule. The characters measured were prozona length, metazona length, forewing length, hindwing length, femur length, and femur width in both sexes, stridulatory peg row length and number of stridulatory pegs in males, and lengths of the lateral and apical sclerites of the ovipositor in females. All characters were defined following Butlin & Hewitt (1985*a*). From these measurements the following characters were derived: pronotum length (sum of prozona and metazona), pronotum ratio (prozona/metazona), wing ratio (forewing/hindwing), and femur ratio (length/width) for both sexes, stridulatory peg density for males, and ovipositor length (sum of lateral and apical sclerites) for females.

All of these characters were measured for specimens from the two main transects, although females were omitted from a few collections. For the specimens from the additional 'off transect' collections only the stridulatory peg number was measured as this is the most discriminating single character (Butlin & Hewitt 1985*a*; and below).

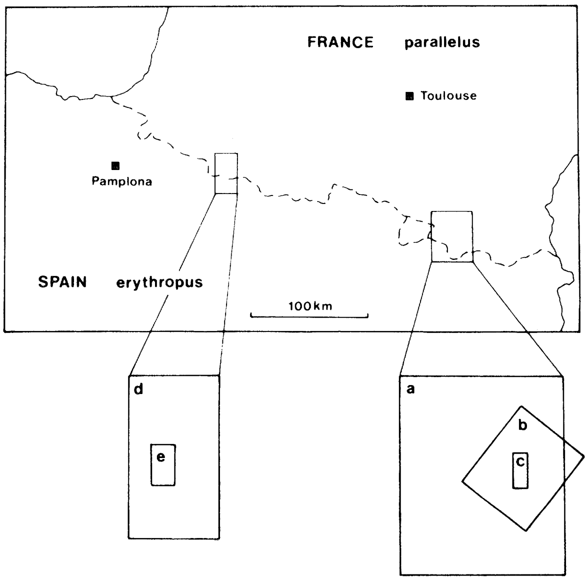

Figure 1. Location of sample sites: (*a–c*) Col de la Quillane, (*d*, *e*) Col du Pourtalet. Mean peg numbers are indicated. Main transect samples are indicated by squares, supplementary collections by points. North is at the top of the map except in (*b*) where the orientation is indicated. The continuous line is the 2000 m contour, open shading indicates woodland and heavy shading lakes. Woodland is omitted from (*a*) and (*d*) for clarity.

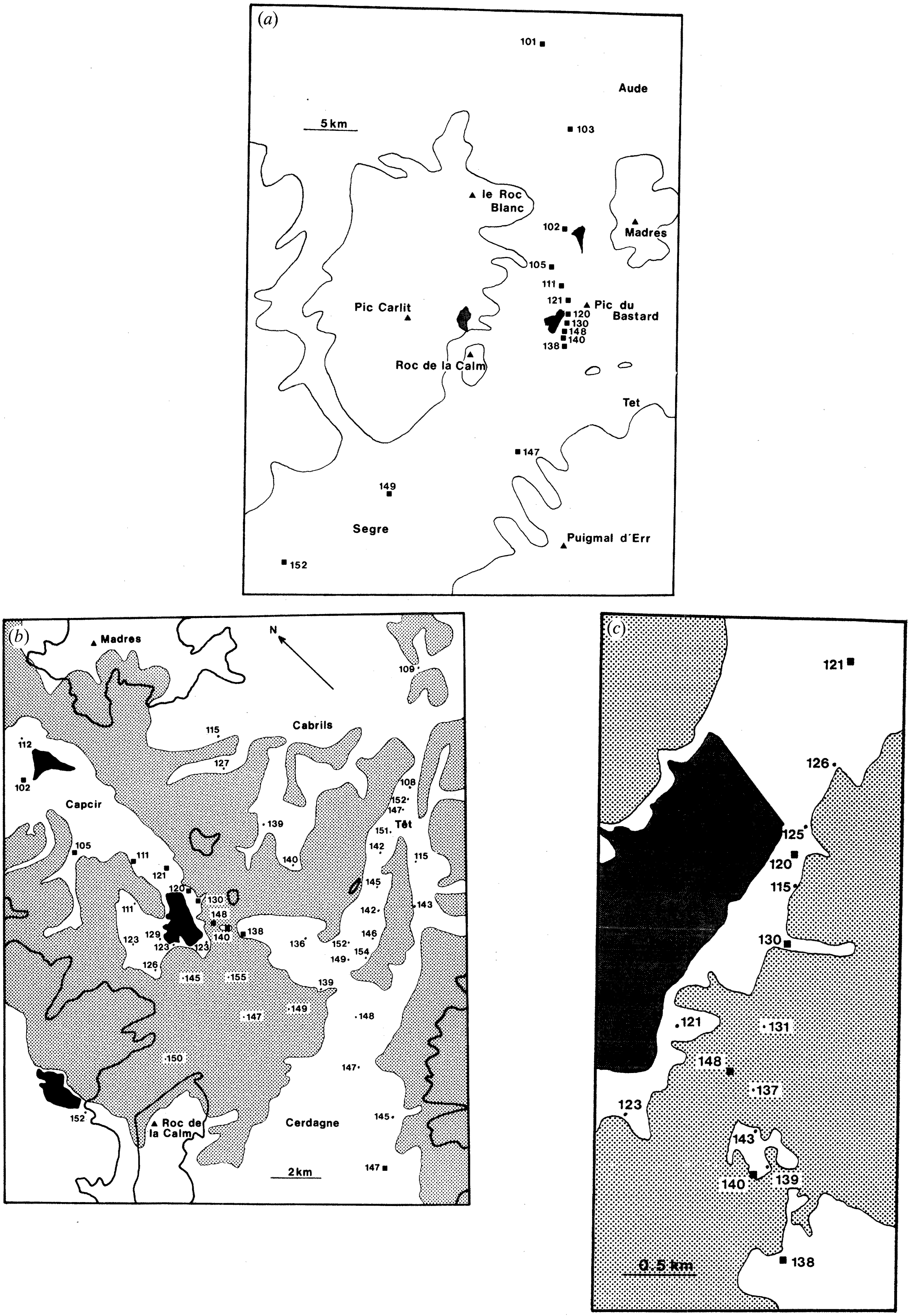

Figure 1. (*a*) The main transect at the Col de la Quillane. (*b*) Supplementary collections around the Col. The summit of the Col de la Quillane is in the centre of the map, at the main transect sample with peg number 138. (*c*) Finer scale sampling near the zone centre.

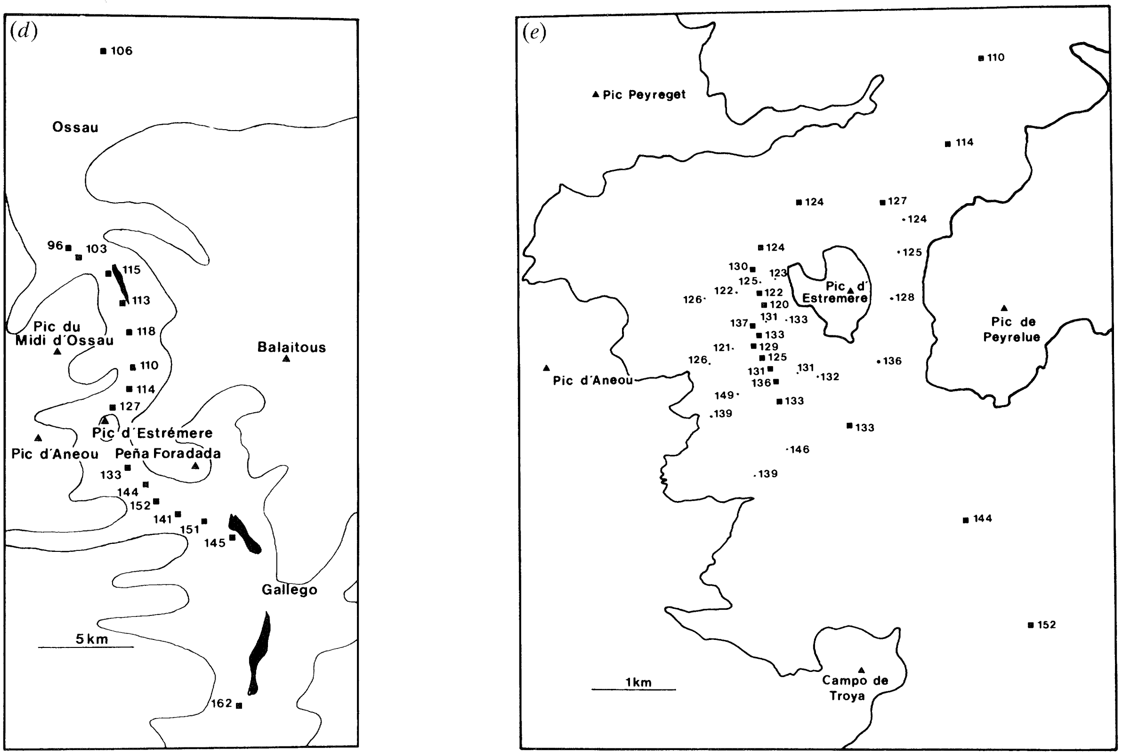

Figure 1. (*d*) The main transect at the Col du Pourtalet, excluding samples close to the Col which is between Pic d'Aneou and Pic d'Estremere. (*e*) Main transect and supplementary collections near the zone centre.

Table 1. *Fitted parameters for male and female characters at the Col de la Quillane*

(The model fitted was:

$x = a + b \cdot \text{altitude} + m \cdot ([1 + \tanh(2[d - d0]/w)]/2) + s_e^2$,

or one of the submodels:

$x = a + s_e^2$,

$x = a + b \cdot \text{altitude} + s_e^2$,

$x = a + m([1 + \tanh(2[d - d0]/w)]/2) + s_e^2$,

where x is the morphometric variable, a is the mean for *C. p. parallelus* (or the intercept if a regression on altitude is included), b is the coefficient of regression on altitude (per 1000 m), m is the estimated difference between subspecies in mean (or intercept), d is the cumulative straight line distance of the sample site from the *C. p. parallelus* (north) end of the transect, $d0$ is the estimated distance of the cline centre from the same point, w is the estimated cline width, and s_e^2 is an error term.

Parameter estimates are only given for the best fitting model. — indicates that a parameter was not included in the best fitting model. The variance ratio tests the improvement of this model over the next simpler model (see Methods): $^*p < 0.05$, $^{**}p < 0.01$. Some standard errors are not defined by the OPTIMIZE program because of the shape of the likelihood surface around the estimated values. The figure in brackets under m is the difference between subspecies in units of the within collection standard deviation. x indicates that a value of $w = 0.2$ was used because otherwise the optimization process tended to ever smaller values. Where the estimated or set value of w was much less than the spacing between samples the standard errors of both w and $d0$ are unreliable and are given in parentheses.)

		parameter value ± standard error					
	Character	*a*	*b*	*m*	*d*0/km	*w*/km	variance ratio
Males							
a	pronotum length/mm	2.79 ± 0.011	—	—	—	—	—
b	pronotum ratio	1.02 ± 0.014	0.05	−0.0943 ± 0.0109 (1.49)	20.8(±0.578)	0.324(±13.4)	11.94**
c	forewing length/mm	8.69 ± 0.052	—	—	—	—	—
d	hindwing length/mm	4.64 ± 0.089	—	0.765 ± 0.118 (1.15)	22.8 ± 0.494	2.72 ± 1.64	14.01**
e	wing ratio	1.84 ± 0.020	—	−0.216 ± 0.0264 (1.53)	22.9 ± 0.412	2.70 ± 1.14	24.61**
f	femur length/mm	9.26 ± 1.13	−0.536 ± 0.786	—	—	—	12.48**

g	femur width/mm	1.85±0.0084	—	—	—	—	—
h	femur ratio	4.78±0.035	—	−0.266±0.0434 (1.30)	20.8±1.35	8.38±3.40	5.67**
i	row length/mm	3.33±0.048	—	0.850±0.066 (2.79)	24.2±0.803	16.1±3.72	73.11**
j	peg number	98.3±1.84	—	49.8±2.64 (4.12)	23.2±0.330	8.34±1.14	92.90**
k	peg density/pegs per mm	29.6±0.384	—	6.12±0.507 (2.28)	22.7±0.356	4.22±0.970	27.06**
l	CV1	−3.02±0.175	—	5.22±0.254	22.6±0.258	6.92±0.846	111.64**
m	CV2	1.82±0.416	−1.32±0.286	—	—	—	5.65*
Females							
n	pronotum length/mm	3.39±0.013	—	0.317±0.038 (1.89)	52.0(±0.391)	*x*	4.86**
o	pronotum ratio	1.04±0.0081	—	−0.0650±0.0099 (1.09)	21.5±0.461	2.08±1.65	18.42**
p	forewing length/mm	5.34±0.234	0.715±1.64	—	—	—	9.12**
q	hindwing length/mm	3.56±0.127	0.328±0.96	0.445±0.0827	22.0(±0.284)	0.524(±0.818)	7.03**
r	wing ratio	1.47±0.0067	—	—	—	—	—
s	femur length/mm	10.9±0.122	−0.666±0.852	—	—	—	18.90**
t	femur width/mm	2.11±0.086	—	—	—	—	—
u	femur ratio	4.71±0.015	—	—	—	—	—
v	lateral sclerite/mm	0.358±0.0082	—	0.179±0.0101 (2.79)	22.0±0.11	*x*	71.10**
w	apical sclerite/mm	0.588±0.0057	—	0.118±0.0064 (2.59)	20.8±0.20	0.584±2.24	24.30**
x	ovipositor length/mm	0.903±0.0323	0.039±0.029	0.278±0.0211 (3.13)	20.9±0.039	*x*	58.26**
y	CV1	−4.65	1.9	2.72	21.4	*x*	69.84**
z	CV2	0.247±0.101	—	−2.78	51.3±1.37	3.78±0.920	12.23**

(*c*) *Analysis*

The specimens varied in overall size. A simple correction for size variation was applied by regressing each character on the pronotum length and using the resulting relationship to adjust the character to a pronotum length of 3.0 mm in females or 2.5 mm in males. Such a correction was necessary because size varies with environmental parameters, notably altitude, and tends to obscure differences between subspecies. The simple approach of regressing individual characters on pronotum length was preferred over principal components analysis because of the difficulty of interpreting principal component axes. We used an untransformed rather than a logarithmic scale for the same reason. With the range of size variation involved this made little difference to the results. Regressions were calculated separately for the two cols and for the two sexes. In no case did the slope of the regression vary significantly among collections within a col and sex.

It is likely that correlations among some characters still exist after correction for size. Therefore we have conducted a canonical variates analysis (CVA) to summarize variation in the data in a smaller number of dimensions. The CVA was conducted, separately for each col and sex, on the original measurement data without size correction and excluding the derived characters. However, measurements were expressed on a standard scale to facilitate interpretation of the character loadings, by using the formula: $x' = (x - \text{mean}(x))/s_x$ where x is the original and x' the standard scale measurement and s_x^2 is the within-collection variance of the character derived from an analysis of variance among collections. The input groups for the CVA were collections, thus the analysis searched for variation among collections without reference to subspecific differences. In each of the four analyses CV1 and CV2 together explained more than 75 % of the total variance.

Clines for single loci maintained by a balance between dispersal and selection are expected to follow a characteristic sigmoid shape described by a tanh curve (Bazykin 1969; Endler 1977). Multilocus clines may depart from this shape because linkage disequilibria are generated by gene flow into the cline centre or by selection. Associations between alleles at different loci can induce a step in the cline with long tails of introgression to either side (Barton 1983; Szymura & Barton 1986). However, for morphological characters of uncertain, but probably polygenic genetic determination (Butlin & Hewitt 1988) the simple tanh curve is likely to be a reasonable approximation.

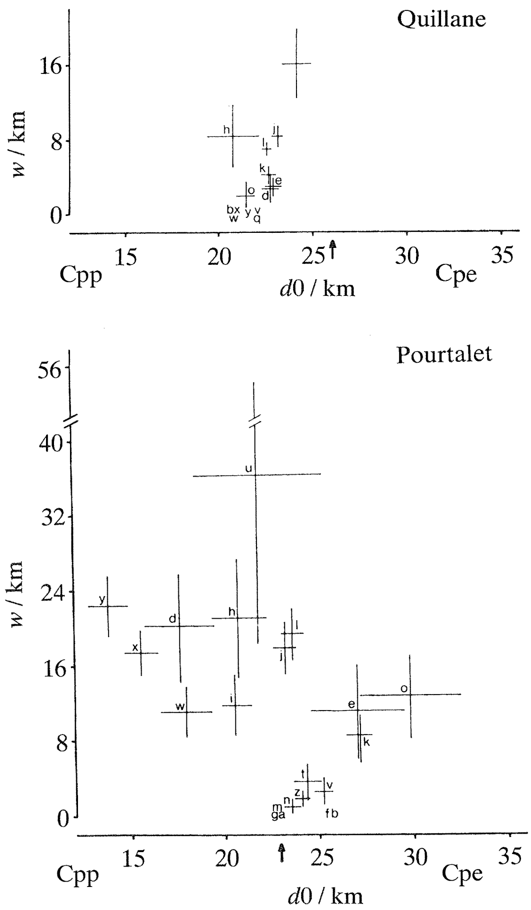

Figure 2. Estimated cline width (w) plotted against estimated position of the cline centre ($d0$). Error bars are ±1 standard error. Letter codes are taken from tables 1 & 3. Arrows on the $d0$ axis indicate the summits of the cols and Cpp and Cpe indicate the *C. p. parallelus* (north) and *C. p. erythropus* ends of the transect respectively.

We have tested each morphological character and the first two canonical variates with a model incorporating a tanh curve. The model includes the parameters of the curve – $d0$, the cline centre, and w, the cline width – and also an estimate of the difference between the subspecies, m. In both transects altitude is likely to be a major environmental variable that is confounded with any clinal variation because the expected position of the cline centre is at the highest point of the transect. Altitude may influence grasshopper size and shape through effects on climate, or indirectly through effects on vegetation (Virdee 1991). Therefore our model also incorporates an effect of altitude, b. The total model has the form:

$$x = a + b \cdot \text{altitude} + m \cdot ([1 + \tanh(2[d - d0]/w)]/2) + s_e^2,$$

where a is the intercept for *C. p. parallelus* (or the mean if there is no significant regression on altitude), d is the distance measured along the transect from the northernmost collection site, and s_e^2 is an error term. The errors were assumed to be normally distributed. An examination of the distribution of residuals indicated no major departures from normality and logarithmic transformation of the original data had little effect on the results. The model was fitted using the OPTIMIZE directive in GENSTAT (copyright Rothamsted Experimental Station, Harpenden, Hertfordshire, U.K.). The significance of the fit was determined by regressing the collection means of the observed values on the collection means of the fitted values. Sub-models containing the regression on altitude alone, or clinal variation alone, were also fitted and comparisons among models were made using the residual sums of squares from the regression of collection means. Note that previous publications (e.g. Butlin 1989; Hewitt 1990) utilized a formula that gave cline width estimates of $w/2$. The formula used here is more in line with general usage where width = 1/(maximum slope) (e.g. Szymura & Barton 1986).

3. RESULTS

(*a*) *Col de la Quillane*

C. p. erythropus differs from *C. p. parallelus* in the following ways (table 1): the median suture of the pronotum is nearer the posterior end, the hindwings are longer, the hind femur is broader relative to its length (at least in males), the stridulatory peg row is longer and more dense, and both sclerites of the ventral valve of the ovipositor are longer.

As expected from previous studies (Butlin & Hewitt 1985*a*; Butlin 1989), the character that differed most between subspecies was the number of stridulatory pegs (table 1). Clinal variation explains 94% of the variance among collections for this character with an estimated width of 8.4 km. The centre of the cline is about 3 km north of the Col de la Quillane itself (figures 2 and 3). However the north side of the Col is wooded (figure 1) and *Chorthippus parallelus* populations are restricted to small clearings in this area. The estimated cline centre is close to the northern edge of the wooded area and is, therefore, located at a point of low population density as well as a constriction of the distribution range. More detailed mapping of grasshopper densities in the area would be necessary to confirm that population structure explains the position of the cline but this hypothesis seems most plausible. The environment on either side of the Col, outside the wooded area, is broadly similar: a patchwork of open grazing land and small meadows.

Several other characters differ between the subspecies and show clinal variation with estimated centres close to the centre for stridulatory peg number (figure 2). However, some characters have estimated centres displaced to the north, *C. p. parallelus*, side (figure 3). Cline widths for these characters are narrow, indeed they are effectively indeterminate for male pronotum ratio, female hindwing length and the characters of the female's ovipositor because they are much less than the spacing of collection sites. The significance of these displacements was assessed by fitting a new cline model for each character in which the cline centre was fixed at the estimated centre of the peg number cline (table 2). The peg number cline was chosen as a reference point because the large difference between subspecies means that the clinal pattern is better defined for this character than any other. This new model explained significantly less of the variance among collections for male pronotum ratio, the characters of the ovipositor,

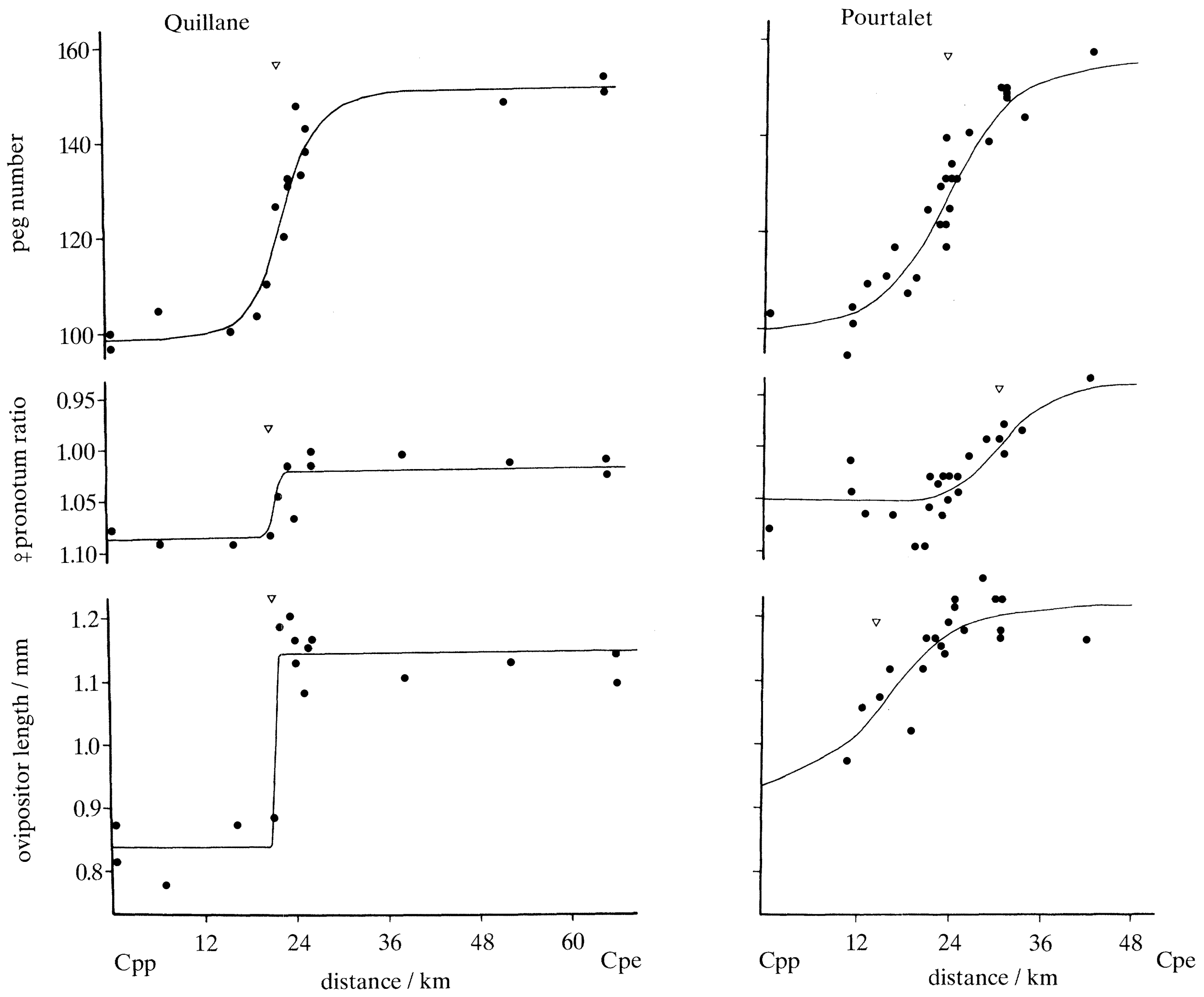

Figure 3. Examples of fitted clines to show the variation in position and width within and between cols. Points are sample means. Triangles indicate cline centres and Cpp and Cpe indicate the *C. p. parallelus* (north) and *C. p. erythropus* ends of the transect respectively.

and CV1 for females while no fit could be obtained for female hindwing length. Thus there appears to be a real displacement for these characters which have cline widths of less than 1 km and centres within the broad area of suitable habitat to the north of the Col de la Quillane (the area known as Capcir, figure 1).

Female pronotum length and CV2 for females do not appear to change in a clinal pattern. The fits obtained with centres well south of the Col de la Quillane probably reflect a size difference for the two most remote *C. p. erythropus* populations that is not explained by variation with altitude.

(*b*) *Col du Pourtalet*

In this transect the two subspecies differ in the same ways as they do at the Col de la Quillane (table 3). In addition *C. p. erythropus* has a larger pronotum (independent of altitude) but shorter hind femur (at least in males) than does *C. p. parallelus*. The environment at the Col du Pourtalet is not complicated by extensive wooded areas close to the Col. Instead there is fairly continuous grazed grassland, interrupted by rocky outcrops, and broadly suitable for *Chorthippus parallelus* up to an altitude of about 2000 m. The Col du Pourtalet itself is a narrow pass approached by a single river valley on either side. Just to the east is another pass, the Col de Peyrelue, which is higher and narrower but nevertheless contains grasshopper populations (figure 1).

As at the Col de la Quillane, stridulatory peg number is the most divergent character between the subspecies and a simple cline explains a high proportion of the variation among collections (91 %, table 3). The estimated cline centre is very close to the Col (figures 2 and 3) and so is clearly at a constriction in the grasshopper's range. The estimated width of 18 km is more than twice the width at the Col de la Quillane.

Figure 2 clearly shows that cline centres are much more dispersed at the Col du Pourtalet than at the Col de la Quillane and that the clines are generally wider. Of 16 characters that differ between the subspecies, significantly less variation is explained by a cline coincident with the peg number cline for 9 characters (table 4). As at the Col de la Quillane, this includes ovipositor characters and male pronotum ratio, but the similarity between transects is limited. Male pronotum ratio changes abruptly in both Cols but is displaced to the north at Quillane and to the south at Pourtalet. Apical sclerite and ovipositor lengths are displaced to

Table 2. *Estimated cline widths when the cline centre is assumed equal to the estimated centre for peg number (23.2 km) at Col de la Quillane*

	males		females	
character	w	F^a	w	F^a
pronotum length			NC[b]	
pronotum ratio	6.54 ± 1.88	6.36*	6.22 ± 2.36	3.49
hindwing length	2.50 ± 2.30	—	NC	
wing ratio	2.74 ± 1.38	—		
femur ratio	6.84 ± 2.90	—		
peg row length	16.62 ± 3.98	—		
peg density	4.06 ± 1.00	—		
lateral sclerite			5.86 ± 1.26	51.7***
apical sclerite			7.58 ± 2.46	40.1***
ovipositor length			7.59 ± 1.84	54.2***
CV1	7.04 ± 0.82	2.12	5.84 ± 1.48	52.6***
CV2			NC	

[a] F, Variance ratio test for the comparison of this model with a model in which $d0$ was allowed to vary;—indicates that the more complete model did not reduce the residual variance; $*p < 0.05$, $***p < 0.001$.

[b] NC, The optimization process failed to find a fit to the model.

the north in both Cols but have narrow clines at Quillane and very much wider clines at Pourtalet. Male wing ratio and peg density, and female pronotum ratio are displaced to the south at Pourtalet but show no significant displacement at Quillane.

In both Cols CV1 in males is heavily influenced by peg number and the estimated cline is very similar for CV1 and peg number. In females CV1 is most strongly influenced by the two ovipositor measurements, which are the most divergent characters between subspecies. The cline for CV1 in females is displaced north of that for CV1 in males in both Cols, but by very different amounts (by 1.2 km at Quillane and 9.8 km at Pourtalet), and the clines have very different widths (less than 1 km at Quillane and 22.6 km at Pourtalet). CV2 in both Cols and for both sexes appears to be broadly size associated.

(*c*) ***Supplementary collections***

Finer scale sampling in the Col du Pourtalet (figure 1*e*) shows a pattern of change in stridulatory peg number that is consistent with the main transect samples. The centre of the cline, at a peg number of about 125, lies approximately on the watershed for the 1.5 km width of suitable habitat between the Pic d'Aneou and Pic d'Estremere. A similar transition occurs to the east in the Col de Peyrelue (between Pic d'Estremere and Pic de Peyrelue). This col represents a more direct path for gene flow between the subspecies but has a narrower band of suitable habitat. The transect is, in fact, only about 1 km shorter than that through the Col du Pourtalet and so, whereas narrower cline widths would probably have been estimated for a transect through this col (at least for clines more than 4 km wide), this alone cannot explain the differences in cline width observed between Pourtalet and the Col de la Quillane.

At the Col de la Quillane the topography is more complex and the areas of habitat suitable for *Chorthippus parallelus* are dissected by areas of woodland (figure 1*b*, *c*). The supplementary samples identify several interesting features. A second contact between the subspecies exists in the Tet valley where a change in peg number of 44 occurs over just 0.5 km, compared to more than 4 km in the main transect. The Tet valley has steep sides mostly covered with dry scrub vegetation unsuitable for *Chorthippus parallelus*, except for pockets of moist grass around streams and where the valley side has been terraced to create small hay meadows. Thus grasshopper populations are much more patchy than in the main transect and this may underlie the abrupt transition in peg number.

Populations in the valleys between the Tet valley and Capcir, which runs down to meet the Tet valley in the extreme northeast of figure 1*b*, have high peg numbers despite their apparent isolation from the main body of the *C. p. erythropus* populations. Populations in clearings in the wooded area between Cerdagne and Capcir also have high peg numbers, indicating colonization from the *C. p. erythropus* side. *Chorthippus parallelus* appears to be capable of colonizing suitable habitat despite intervening areas of woodland much wider than the average dispersal distance of about 30 m per generation (Virdee 1991). The peg numbers indicate more gene flow into these areas from *C. p. erythropus* than from *C. p. parallelus* populations as the clearings between Cerdagne and Capcir all have peg numbers typical of *C. p. erythropus*. Also the populations between Capcir and the Tet show evidence of gene flow from *C. p. erythropus*, apparently across a barrier of some 2 km of woodland, despite their potential connection to the main body of *C. p. parallelus* populations in the Tet valley. Historical changes in land use may also have contributed to the present pattern of variation in peg number, for example if the area of forest has spread southward into Cerdagne.

4. DISCUSSION

The two parapatric subspecies of *Chorthippus parallelus* differ strongly in features of the male stridulatory peg

Table 3. *Fitted parameters for male and female characters at the Col du Pourtalet*

(Conventions as in Table 1.)

Character	parameter value ± standard error					variance ratio
	a	*b*	*m*	*d*0/km	*w*/km	
Males						
a pronotum length/mm	2.95 ± 0.032	−0.223 ± 0.022	0.076 ± 0.014 (0.56)	22.9(±0.085)	*x*	8.23**
b pronotum ratio	1.06 ± 0.0022	—	−0.056 ± 0.0043 (0.86)	25.9 ± 0.130	0.374	22.47**
c forewing length/mm	9.11 ± 0.148	−0.452 ± 0.095	—	—	—	6.80*
d hindwing length/mm	5.43 ± 0.190	−0.954	1.36 ± 0.244 (2.42)	17.7 ± 1.93	20.2 ± 5.78	14.94**
e wing ratio	1.81 ± 0.018	—	−0.239 ± 0.062 (1.71)	27.1 ± 1.61	11.28 ± 4.98	15.23**
f femur length/mm	8.99 ± 0.068	−0.318 ± 0.044	−0.261 ± 0.031 (0.73)	25.8(±0.17)	*x*	5.67**
g femur width/mm	1.83 ± 0.0052	—	0.069 ± 0.0074 (0.77)	22.8(±0.045)	*x*	14.33**
h femur ratio	4.84 ± 0.057	—	−0.532 ± 0.092 (2.63)	20.8 ± 1.38	21.2 ± 6.30	18.49**
i row length/mm	3.47 ± 0.035	—	0.483 ± 0.054 (1.80)	20.7 ± 0.814	11.74 ± 3.30	15.70**
j peg number	99.2 ± 2.04	—	56.9 ± 4.97 (4.95)	23.3 ± 0.606	17.94 ± 2.84	106.53**
k peg density/pegs per mm	25.9	3.60 ± 0.300	7.53 ± 1.09 (2.73)	27.2 ± 0.712	8.58 ± 2.84	50.07**
l CV1	2.97 ± 0.220	—	−6.63 ± 0.562	23.7 ± 0.612	19.42 ± 2.76	143.07**
m CV2	−2.96 ± 0.272	2.22 ± 0.180	−0.788 ± 0.112	22.9(±0.075)	*x*	10.09**
Females						
n pronotum length/mm	3.69 ± 0.060	−0.272 ± 0.044	0.162 ± 0.019 (0.84)	23.6 ± 0.463	1.12 ± 0.882	7.29**
o pronotum ratio	1.05 ± 0.0057	—	−0.114 ± 0.039 (1.72)	29.9 ± 2.66	12.84 ± 2.56	21.93**
p forewing length/mm	6.25 ± 0.028	—	—	—	—	—
q hindwing length/mm	4.34 ± 0.022	—	—	—	—	—
r wing ratio	1.46 ± 0.0043	—	—	—	—	—
s femur length/mm	10.5 ± 0.090	−0.368 ± 0.058	—	—	—	6.68*
t femur width/mm	2.11 ± 0.010	—	0.103 ± 0.015 (0.74)	24.4 ± 0.677	3.86 ± 1.93	3.92*
u femur ratio	5.03 ± 0.154	—	−0.738 ± 0.275 (3.06)	21.9 ± 3.45	36.4 ± 18.1	9.23**
v lateral sclerite mm	0.290 ± 0.0173	0.117 ± 0.012	0.0923 ± 0.0082 (1.56)	25.2 ± 0.516	2.92 ± 1.44	27.73**
w apical sclerite mm	0.608 ± 0.0072	—	0.0680 ± 0.0082 (1.67)	18.0 ± 1.37	11.22 ± 2.70	13.53**
x ovipositor length/mm	0.925 ± 0.0197	—	0.283 ± 0.0231 (4.19)	15.6 ± 0.949	17.56 ± 2.40	39.77**
y CV1	4.90 ± 0.456	—	−6.40 ± 0.559	13.9 ± 1.05	22.6 ± 3.10	47.27**
z CV2	−1.81 ± 0.317	1.56 ± 0.22	−1.39 ± 0.140	24.1 ± 0.415	2.00 ± 0.784	9.09**

row and the ovipositor of females and less markedly, but still significantly, in several other morphological characters. These differences are qualitatively and quantitatively similar in the two transects separated by about 200 km, suggesting that each subspecies is broadly homogeneous in morphology. However, the present data have revealed variation in cline width and position among characters within transects, and for some of the characters between cols.

Variation in cline width among characters is expected on a variety of models for the maintenance of the hybrid zone (Barton & Hewitt 1985). It would not be expected if all clines were the result of neutral introgression, but it is consistent with either environmentally determined selection pressures, or selection against hybrids. Width variation may be explained either by selection operating directly on the characters involved, and more strongly on those with narrow

Table 4. *Estimated cline widths when the cline centre is assumed equal to the estimated centre for peg number (23.3 km) at Col du Pourtalet*

(Abbreviations as in Table 2. $^{**}p < 0.01$.)

character	males *w*	*F*	females *w*	*F*
pronotum length	0.74±0.42	—	0.72±0.40	—
pronotum ratio	4.34±2.08	19.7**	6.18±2.06	11.2*
hindwing length	25.60±13.64	—		
wing ratio	12.84±4.54	7.51*		
femur length	14.80±12.20	7.75*		
femur width	17.54±8.16	11.9*	3.40±2.38	—
femur ratio	26.32±8.84	4.37*	37.8±20.2	—
peg row length	21.36±5.34	—		
peg density	17.48±4.28	4.78*		
lateral sclerite			52.6±39.4	5.85*
apical sclerite			33.8±14.4	5.39*
ovipositor length			NC	
CV1	18.52±2.30	—	NC	
CV2	0.38±0.40	2.77	1.58±0.68	2.54

clines, or by variation in linkage between genes influencing the morphological characters and genes under selection. Width variation may also be explained by differences in the underlying genetics of the characters: for two characters under similar selection a wider cline is expected for the character determined by more loci. However, this relationship is weaker if the heritability of the character is high on either side of the cline (Slatkin 1978). For some of the characters studied here analysis of F1 and backcross hybrids indicates that the difference between subspecies is polygenic (Butlin & Hewitt 1988). Heritabilities have not been measured within the parental populations but a comparable set of morphological characters had estimated heritabilities ranging from 0.16 to 0.89 in the related species *Chorthippus brunneus* (Butlin & Hewitt 1986), with the highest value for stridulatory peg number.

Variation among characters within transects in the position of the cline centre may also be understood in a number of different ways. If clines are environmentally determined then the centres may vary in position because different environmental features do not change together. In a tension zone, determined by a balance between dispersal and selection against hybrids, clines for these selected characters are expected to be coincident (Barton & Hewitt 1985). However exceptions are possible. An advantageous allele at a locus that is loosely linked, or unlinked, to heterotically selected loci may be recombined away from them and spread into one of the taxa. Neutral or environmentally selected alleles may be left behind when the tension zone moves or is restructured (Hewitt 1989). Until recently there was virtually no evidence of such a lack of coincidence (Barton & Hewitt 1985; Hewitt 1989), but detailed investigations of mitochondrial DNA have revealed two clear examples (*Caledia*, Marchant 1988; *Mus*, Sage *et al.* 1986). Where the genetic determination of characters varies the underlying genotypic clines may be coincident but the phenotypic clines may be displaced, for example with directional dominance the cline centre would be shifted towards the recessive side. Alternatively, maintenance of a cline by a dispersal-selection balance does not preclude environmentally determined selection pressures operating on some characters and displacing clines in those characters.

Which of these possibilities might apply in *Chorthippus parallelus*? The genetics of hybrid sterility, the only known selection pressure against hybrids, has not yet been elucidated. If it is determined by a small number of loci there may be the possibility for morphological characters to vary independently of a tension zone for sterility loci. Those morphological characters that have been studied in crosses between *C. p. parallelus* and *C. p. erythropus*, characters of the hind femur and peg row in males, have polygenic inheritance without directional dominance (Butlin & Hewitt 1988). Dominance might possibly explain the displacement of the clines for ovipositor characters, especially since the displacement is in the same direction in the two cols. However, a further explanation would be necessary for the much greater displacement at the Col du Pourtalet. For a character like peg row length, which is displaced in opposite directions in the two transects, dominance is an unlikely explanation.

Perhaps the most striking feature of the results is the difference between the two transects. Clines are both wider and more dispersed at the Col du Pourtalet than at the Col de la Quillane. Variation in width among transects is a common observation in hybrid zones with a number of possible explanations (Barton & Hewitt 1985). For environmentally determined clines variation in both width and relative position can clearly be accounted for by differences in environmental gradients. For neutral clines variation can be explained by differences in the time since contact was established together with subsequent patterns of population structure. However, it is unlikely that these morphological clines between *C. p. parallelus* and *C. p. erythropus* are wholly neutral given the narrowness of some clines, the variation in position and width within transects, the polygenic determination of the characters, and the observed hybrid sterility.

In the case of tension zones, three types of explanation are possible: genetic variation within taxa, modification, and population structure. Genetic variation within taxa also appears unlikely given the similarities in the total divergence at the two cols. Modification of tension zones is a contentious area (Barton & Hewitt 1985; Butlin 1987, 1989) whether it is by reinforcement or by reduction in selection against hybrids. It could explain the lack of sterility in field collected males of *Chorthippus parallelus* (Ritchie & Hewitt 1991) and the pattern of variation in female preference at the Col de la Quillane (Butlin & Ritchie 1991) but, as yet, there is no evidence to suggest that it could account for the observed differences between transects in morphological clines.

Population structure is the most probable explanation. Patchiness of population density in a contact area can broaden tension zones, especially at low overall population density and with shallow density gradients (Nichols & Hewitt 1986; Nichols 1989). However, this effect is dependent on the scale of patchiness: if there are large blocks of suitable habitat separated by more severe barriers to dispersal, differences between interacting taxa may be concentrated at these barriers. Despite the superficial habitat uniformity at the Col du Pourtalet, Virdee (1991) has shown marked differences in grasshopper density between habitat types that lead to a patchy overall distribution of grasshoppers. No comparable analysis has been conducted yet for the Col de la Quillane but it is clear that in the wooded areas grasshopper populations are very patchy with intervals of hundreds to thousands of metres between neighbouring populations. Thus it is possible that population structure has acted to concentrate clines at a barrier to dispersal in the Col de la Quillane whereas patchiness of populations, with extinction and recolonization, has acted to broaden clines at the Col du Pourtalet in the manner simulated by Nichols (1989). These models do not address the relative positions of clines in different characters but it seems likely that the same process would result in lack of coincidence between clines, especially for weakly selected characters. Colonists may originate from hybrid populations and carry a mixture of alleles from the two subspecies, or recombination and drift within patches may influence different characters in different ways. Thus population structure alone could explain many of the differences between cols. However, some specific problems remain, notably the group of characters that appear to change abruptly north of the Col de la Quillane within the area of suitable habitat in Capcir, and away from the wooded area that coincides with the transition in other morphological characters and in female preferences (Butlin *et al.* 1991).

The hybrid zone between *C. p. parallelus* and *C. p. erythropus* is complex. It is almost certainly secondary in origin, having formed when the grasshoppers' ranges expanded at the end of the last ice age. On a broad scale, many characters change close to the predicted point of secondary contact. The evidence presented here, plus the evidence for selection against hybrids (Hewitt *et al.* 1987), makes it very unlikely that the structure of the hybrid zone is simply determined by neutral diffusion. With selection against hybrids and narrow clines for many characters located at or near constrictions in the grasshoppers' distribution, the zone has many of the characteristics of a tension zone. The influence of population structure on a tension zone may explain much of the variation between transects, and variation in linkage relations, directional dominance or direct selection pressures may explain some of the differences among morphological clines within transects. It remains possible that environmental selection pressures influence some characters. For example, it is not clear whether population structure alone, or in combination with directional dominance is sufficient to explain the displacement of the ovipositor length clines at the two cols or the extreme difference in width between cols. However, environmental selection on some characters, even if clearly demonstrated, would not influence the conclusion that following secondary contact the position and characteristics of this zone are determined primarily by a balance between dispersal and selection against hybrids.

We are very grateful to Richard Nichols, Sonia Virdee and other members of the *Chorthippus parallelus* group for useful discussions during preparation of this paper. Hilary Ritchie and Ken Clark helped with the collection of specimens. The work was supported by SERC grants GR/D27426 and GR/E31390 to G.M.H., an SERC studentship to M.G.R., and a Royal Society University Research Fellowship to R.K.B. Insects were imported under MAFF licence PHF 275/49.

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Received 22 February 1991; accepted 10 May 1991