Introgressive hybridization between two species of waterstriders (Hemiptera: Gerridae: *Limnoporus*): geographical structure and temporal change of a hybrid zone

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principal component analysis;
speciation.

Abstract

Where the distribution ranges of the waterstriders *Limnoporus notabilis* and *L. dissortis* meet in western Canada, extensive hybridization and introgression occurs. Multivariate ordination analyses of genetic and morphometric data by principal component analysis revealed that a single axis separating the two parent species could account for nearly all the variation in both data sets. Maps of principal component scores for both data sets revealed geographical patterns of variation reflecting specific topographic features in the region. Comparisons of morphometric data from some of the samples collected in the 1980s and from the same sites revisited in the 1990s revealed substantial changes. An 'island' of *dissortis*-like populations inside the range of *L. notabilis* in interior British Columbia expanded, and a marked local protrusion of *notabilis*-like phenotypes into the range of *L. dissortis* on the east slope of the Rocky Mountains diminished during the decade between collections. We conclude that introgressive hybridization between these two species of waterstriders is a spatially complex and highly dynamic process.

Introduction

Hybridization and introgression are increasingly recognized as important factors in the diversification of both plants and animals, and provide an excellent opportunity to study evolutionary processes like selection, gene flow and speciation (Harrison, 1993; Arnold, 1997; Dowling & Secor, 1997). Because they require direct contact between different populations, hybridization and introgression are inherently localized. Therefore, the geographical setting is a crucial determinant of these processes and, in turn, the conditions under which they have occurred can be inferred from the details of geographical structure of a hybrid zone. Moreover, the dynamics of hybridization and introgression are in many cases sufficiently fast so that the resulting evolutionary changes, for instance in the location of a hybrid zone, can be followed directly over time (e.g. Kohlmann & Shaw, 1991; Hafner *et al.*, 1998).

The waterstrider genus *Limnoporus* illustrates the potential for interspecific hybridization. In spite of the ancient origin of this group (Andersen *et al.*, 1993), laboratory studies have shown that, unless size differences prevent mating, all species combinations are partially interfertile (Sperling *et al.*, 1997). Therefore, some degree of hybridization is possible in principle wherever the distribution ranges of two species overlap. The two North American species *L. dissortis* (Drake & Harris) and *L. notabilis* (Drake & Hottes) form an extensive and spatially complex zone of introgressive hybridization in western Canada, where their distribution ranges come into contact (Spence, 1990). The dynamics of hybridization is substantially influenced by the details of mate choice and the genetic system. Crosses between *L. dissortis* and *L. notabilis* produce surviving offspring that are almost exclusively male

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(Spence, 1990; Sperling *et al.*, 1997). Moreover, because females prefer to mate with smaller males, hybridization and introgression occur primarily through matings of the relatively large *L. notabilis* females with the smaller *L. dissortis* or hybrid males (Spence, 1990). Together, these conditions result in introgression that is directed primarily from *L. dissortis* into populations of *L. notabilis*, and this asymmetry has been confirmed by genetic analyses (Sperling & Spence, 1991).

This asymmetry of introgressive hybridization has direct consequences for genetic and morphological variation. Here we investigate in detail the geographical structure of the hybrid zone, and relate morphometric and allozyme variation. If hybridization in this region is a continuing process, morphometric and genetic variation should be congruent and both express primarily the overall degree of introgression; in contrast, if local adaptation is the dominant force, one would expect different patterns of variation depending on the particular genetic or morphometric traits under study. We also examine to what extent the geographical patterns in both data sets reflect topographic features in the study area that may influence the process of hybridization. Finally, we compare morphometric data from a transect of sites sampled during the 1980s and a decade later, to test directly the hypothesis of continuing expansion of *L. dissortis* and hybrid populations into the range of *L. notabilis*. This analysis of actual change during a decade sheds new light on the dynamics of introgressive hybridization in this region (Spence, 1990; Sperling & Spence, 1991).

Materials and methods

Data collection

The waterstriders used in our study were collected in an extensive region of contact between *L. dissortis* and *L. notabilis* in British Columbia and Alberta (Fig. 1; sites numbered as in Sperling & Spence, 1991). Sampling was carried out in two episodes a decade apart. The first set of samples, designated '1980s' in the rest of this paper, was collected from 1983 to 1986, with most samples collected in 1984, and was the basis of a previous study of allozyme variation and body length (Sperling & Spence, 1991). We revisited a selection of these sites in 1996 and 1997 to collect new samples, designated '1990s' (Fig. 1: sites with numbers indicated in bold italics). These locations are along a transect running south-west to north-east through the entire region from the west coast of British Columbia to central Alberta, and two additional locations (nos. 22 and 23) from the Fraser Plateau in interior British Columbia, where the previous studies have found a core area of hybridization (Spence, 1990; Sperling & Spence, 1991).

Enzyme electrophoretic data are all from the 1980s samples, and have been analysed previously by Sperling & Spence (1990, 1991), who also provide detailed descriptions of the methods used. Here, we reanalyse allele frequencies of three loci that provide diagnostic information between *L. dissortis* and *L. notabilis*: malic enzyme (*ME*), glucose-6-phosphate dehydrogenase (*G6PD*) and adenylate kinase (*AK*). Across the 48 sites

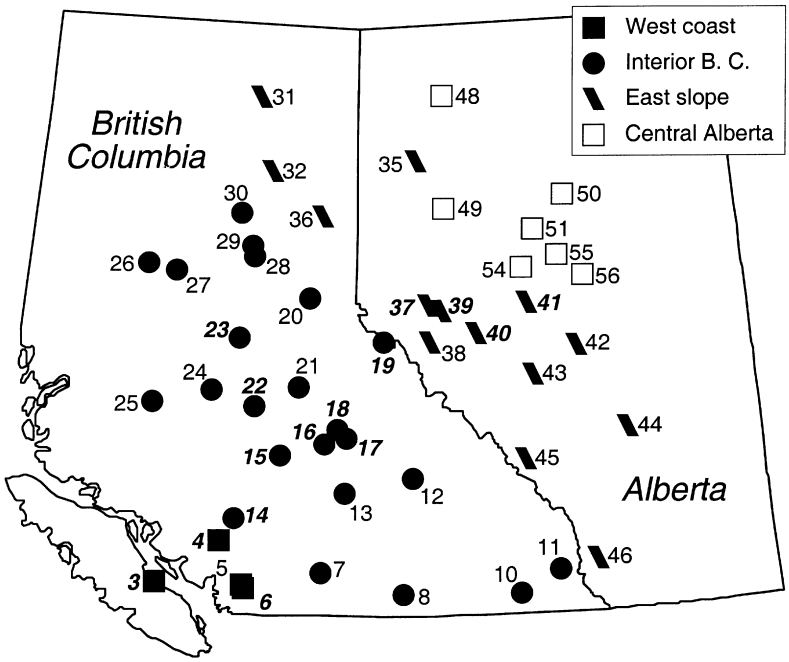

Fig. 1 Map of the locations sampled. All sites were sampled during the 1980s (numbers correspond to those of Sperling & Spence, 1991). Sites sampled again in the 1990s are indicated by bold italicized numbers. The northern and western limits of the map do not correspond to the borders of Alberta and British Columbia.

included in the allozyme analysis, sample sizes were 54.6 on average (range 8–134).

A set of nine morphometric measurements was selected from a larger set of variables in a preliminary stepwise discriminant analysis (Klingenberg, 1992) between laboratory-reared bugs of both species, and augmented with diagnostic characters described by Andersen & Spence (1992, table 3). The final list of variables is the following (numbers correspond to those in figs 2 and 3 of Klingenberg, 1992): length of the mid-femur (49), length of the mid-tibia (50), length of the fourth antennal segment (46), width of the head across the eyes (19), length of the thorax measured along the ventral midline (combined 31–33), width of the thorax (17, but measured in ventral view), length of the second to seventh abdominal segments measured along the ventral midline (combined 37–42), length of the seventh abdominal segment measured along the ventral midline (42) and width of the seventh abdominal segment at its anterior margin (27, but measured in ventral view). All morphometric data were taken using a dissecting microscope fitted with a video camera and digital measurement system.

Only males were included in the morphometric study because surviving offspring from crosses of the two parental species are almost exclusively male, and F_1 hybrids can thus only be discovered among males (Spence, 1990). For the samples from the 1980s, morphometric data were measured in bugs not included in the electrophoretic survey, but which had been collected as part of the same samples. Sample sizes averaged 12.6 (range 3–15) for all the samples from the 1980s, and 12.6 (range 1–29; 10 of the 15 samples had 10–17 specimens) for the samples from the 1990s.

Statistical analyses

Because this study specifically addresses the possible congruence between genetic and morphometric data, the analyses of both data sets need to be compatible. Therefore, although this approach is rarely used for genetic analyses, we analyse both data sets with multivariate ordination techniques. Unlike some previous studies, our ordinations are not based on genetic distances like, for instance, studies of genetic variation using multidimensional scaling (Lessa, 1990). Instead, our analyses represent the different local populations as points in a multidimensional space whose coordinates are defined by allele frequencies directly, and then assess the distribution of populations (Menozzi *et al*., 1978; Rendine *et al*., 1986). The same manoeuvre is conventionally used in morphometric studies, where populations are represented as points in a multidimensional space defined by the measurements. Accordingly, the relationship between the two data sets can be studied directly via the arrangements of points in the two different multivariate spaces. For instance, in a data set with two parent species and various hybrid and backcross intermediaries, one would expect that variation for both morphometric and genetic data is mostly along a single axis that separates the two parent species and orders intermediate populations according to the degree of introgression. More complex patterns, if present, should also be easy to diagnose.

We use principal component analysis (PCA) as the ordination technique for the analyses of both genetic and morphometric data (e.g. Jolliffe, 1986). PCA can be interpreted as a rigid rotation of the coordinate system so that the new axes (principal components, PCs) are mutually uncorrelated and aligned with those directions of multidimensional space that contain maximal variation. Thus, PCA provides an optimal approximation of the total multidimensional variation in fewer dimensions.

PCA is a standard technique in morphometrics (e.g. Klingenberg, 1996), but not for genetic studies (but see, e.g. Menozzi *et al*., 1978; Rendine *et al*., 1986). A potential problem with PCA, as explained by Lessa (1990), is that it assumes that variation is linear, or at least mostly so. Deviations from linearity are possible in genetic studies. For example, if specific alleles are abundant only in the centre of a cline, the simultaneous absence of those alleles at both ends of the cline makes them similar to each other. This apparent similarity of extremes causes populations along the cline to be arranged in a U-shape in multidimensional space. In the case of the *Limnoporus* hybrid zone, however, this is unlikely to apply because all the alleles occur predominantly in one of the two parent species (Sperling & Spence, 1991). Moreover, if nonlinearity is present, it is easy to detect in scatter plots of PC scores (see Results).

Transformation of data values is a further measure that can be taken to render the scale of variation more linear. We used arcsine-square-root transformation for the allele frequencies (Sokal & Rohlf, 1995). To render morphometric variation more linear and to eliminate differences of scale between variables, the morphometric data were transformed to natural logarithms. The PCAs used the among-sample covariance matrices, weighted by sample size, of the data transformed in these ways.

To relate the electrophoretic and morphometric data, we used the partial least squares (PLS) method, a technique for analysing the covariation between two separate sets of variables (e.g. Rohlf & Corti, 2000). Just as PCA gives a lower-dimensional approximation of the covariance matrix for a single set of variables, PLS provides an analogous approximation for the matrix of covariances between two sets of variables (Klingenberg & Zaklan, 2000, Appendix). PLS analysis provides pairs of axes (PLS axes) that have maximal covariances between sets of variables. The PLS method has been used in dose–response studies (Bookstein *et al*., 1990), in ecomorphological studies (Corti *et al*., 1996; Klingenberg & Ekau, 1996; Adams & Rohlf, 2000) and to analyse covariation

between two parts of a morphological structure (Klingenberg & Zaklan, 2000). Our PLS analysis was based on the matrix of among-sample covariances between allele frequencies and morphometric measurements, transformed and weighted by sample size in the same way as for the respective PCAs.

To investigate the geographical structure of the hybrid zone, we produced contour maps for the PC1s of the genetic and morphometric data and for the total variance within each sample (sum of the variances of all variables) for the data from the 1980s. Preliminary analyses showed that the spatial structure of this hybrid zone was too fine-grained for methods like canonical trend surface analysis (Wartenberg, 1985; Largiadèr *et al*., 1994), as that method only identified a linear trend from south-west to north-east that was not informative regarding the more detailed spatial patterns that are the focus of our study. Therefore, we present the geographical patterns as contour maps (e.g. Menozzi *et al*., 1978), based on a nonparametric spline interpolation.

For the 15 sites sampled both in the 1980s and the 1990s, we compared averages and within-site variability of the morphometric data between the two periods. Because the among-sample PC1 was a good summary of differences between the two parental species and hybrids (i.e. an effective hybrid index; see Results), we used these PC1 coefficients to compute scores for each individual within samples. As a measure of variability, we used the total variance within each local sample. We tested for change using a permutation test against the null hypothesis that the samples from the two periods were drawn from the same distribution (Edgington, 1995). For each location, we randomly redistributed individuals 10 000 times between the samples from the 1980s and the 1990s, and each time calculated the difference in average PC1 scores and total variance. The significance level of the test was determined as the proportion of reshuffled samples in which the absolute differences were greater than in the original sample. To account for the 15 within-location tests, we applied a sequential Bonferroni correction (e.g. Sokal & Rohlf, 1995) separately for the comparisons of PC1 and total variance.

Table 1 Principal component analyses of variation in allozyme and morphometric data, and PLS analysis of covariation between the two data sets. Alleles are designated by the enzyme abbreviation and the relative mobility, and if an allele is diagnostic for one parent species, this is indicated in parentheses (Sperling & Spence, 1991).

Variable	PC1	PLS1
Allozymes		
ME-100 (*notabilis*)	0.431	0.427
ME-109 (*dissortis*)	−0.197	−0.196
ME-118 (*dissortis*)	−0.254	−0.246
G6PD-60 (*notabilis*)	0.112	0.116
G6PD-90 (*dissortis*)	−0.572	−0.570
G6PD-100 (*notabilis*)	0.559	0.568
AK-81 (*dissortis*)	−0.171	−0.171
AK-100	0.172	0.170
AK-119	−0.009	−0.006
Morphometrics		
Mid femur	0.446	0.447
Mid tibia	0.500	0.503
Antennal segment 4	−0.067	−0.076
Head width	0.159	0.159
Thorax length	0.293	0.230
Thorax width	0.246	0.237
Abdomen length	0.488	0.493
Abd. segment 7 length	0.361	0.360
Abd. segment 7 width	0.070	0.055

Results

Variation in allozyme and morphometric data

The analysis of variation in allele frequencies is dominated by a single dimension, as the PC1 alone accounts for 93.3% of the total variance, and the PC2 for a further 3.9%, whereas all of the other PCs take up less than 1%. Because the variation is so tightly concentrated along a single dimension, nonlinearity is not a serious problem in this data set, and PCA is an adequate method for analysis (cf. Lessa, 1990). The PC1 is a contrast between alleles predominantly occurring in the two parent species (Table 1). Although this contrast allocates different weights to the loci, the sum of coefficients for *notabilis*-typical alleles (with positive sign) at each locus has almost exactly the same magnitude as the sum of coefficients for *dissortis*-typical alleles (with negative sign). The PC1 is therefore similar to the hybrid index defined by Sperling & Spence (1991), except for the weighting of loci. *ME* and *G6PD* have similar weights, whereas that of *AK* is substantially lower. This probably reflects the fact that both species, outside the hybrid zone, have high frequencies exclusively for diagnostic alleles of *ME* and *G6PD*, whereas for *AK*, pure *L. dissortis* populations contain the typical allele *AK*-81 at intermediate frequencies together with *AK*-100, which is the predominant allele in *L. notabilis* populations (Sperling & Spence, 1990).

The scatter plot of the first two PCs also reflects that the PC1 accounts for most of the variation in allele frequencies, as it defines two widely separate clusters of populations (Fig. 2). There is a compact group of *dissortis*-like populations in central Alberta and on the east slope of the Rocky Mountains, which all have negative PC1 scores. This is opposed by a cluster of extreme *notabilis*-like populations on the west coast (highest positive PC1 scores) that blends into a gradation of populations from interior British Columbia and the east slope of the Rockies. Except for the low scores of coastal *L. notabilis* populations for the PC2, the small amount of variation for which the PC2 (and subsequent PCs) accounted was not related to any intelligible geographical pattern, and

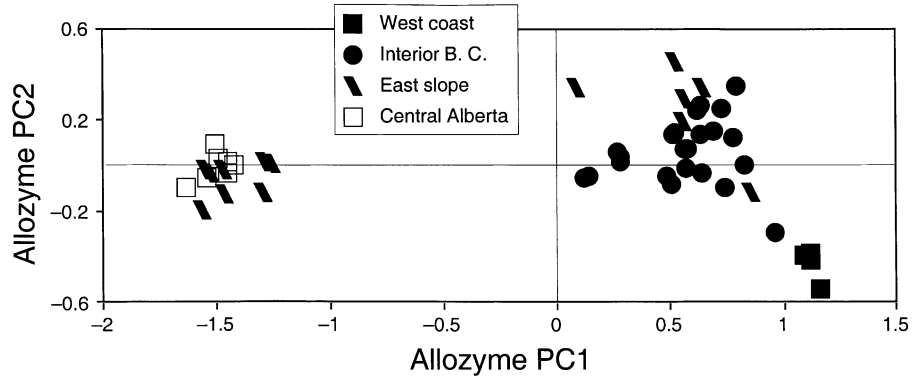

Fig. 2 Ordination of the electrophoretic data collected in the 1980s by principal component analysis (covariance matrix of arcsine-square root transformed allele frequencies for three loci).

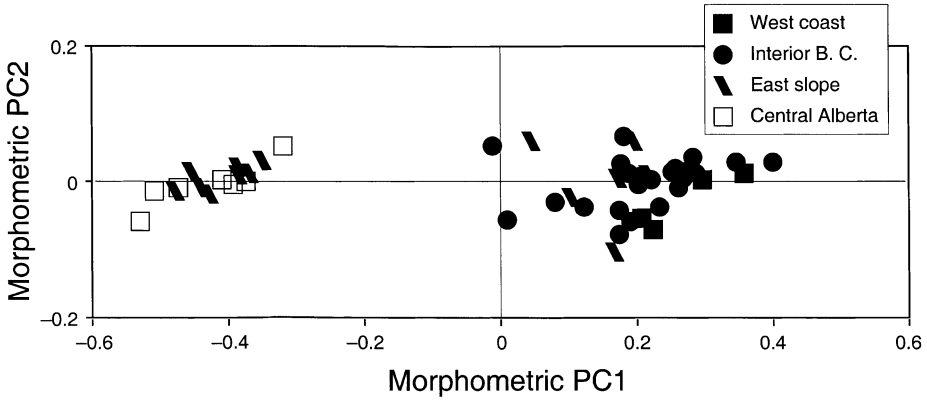

Fig. 3 Ordination of morphometric data from the collections in the 1980s by principal component analysis of the covariance matrix of log-transformed measurements.

we suspect that they primarily express random variation or local factors that are not interpretable on the scale of our study.

The PC1 for the morphometric variables takes up 97.6% of the total variance in that data set, and the PC2 accounts for 1.4%. The PC1 is primarily a size axis, as most PC coefficients are positive, although of variable magnitude (Table 1). Interestingly, some of the variables selected because they define diagnostic characters (antennal segment 4, head width, width of abdominal segment 7; Andersen & Spence, 1992) have smaller coefficients than the remaining variables. The PC1 of the morphometric variables defines two widely separated clusters of *dissortis*-like and *notabilis*-like populations (Fig. 3) similar to those of the analysis of allozyme data. The PC2 and subsequent PCs do not show any discernible geographical patterns.

The PLS analysis showed that almost all the covariation between the two data sets was accounted for by a single pair of PLS axes (taking up 98.5% of the sum of singular values, an analogue of total variance in PCA). Both PLS axes showed a similar clustering of *dissortis*-like and

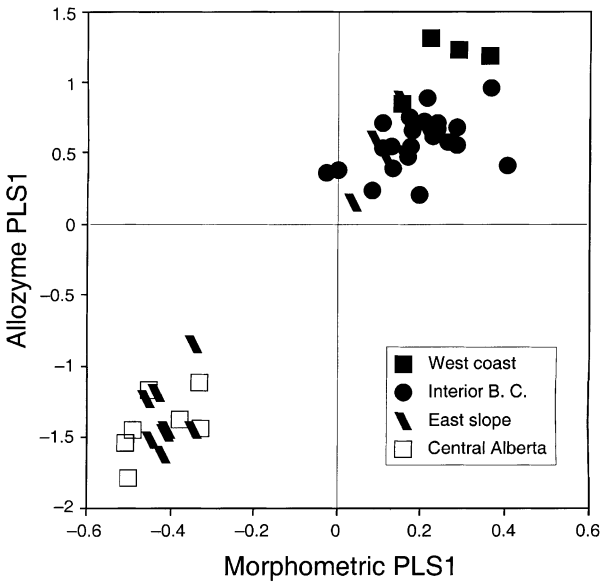

Fig. 4 Partial least squares analysis relating the morphometric and allozyme variation. The PLS axes are linear combinations of the two sets of variables that have maximal covariance.

notabilis-like populations (Fig. 4), indicating that one data set can therefore be used to infer the variation in the other data set to a considerable degree. Moreover, this pair of PLS axes was nearly identical to the PC1s from the separate PCAs of the two data sets (Table 1). Altogether, these results suggest that the PC1s not only provide a good summary of the variation in the respective data set, but also that both convey very similar information, and therefore are nearly interchangeable for the assessment of morphological and genetic variation in this hybrid zone.

Geographical structure of the hybrid zone

The contour map of the PC1 scores for the allozyme data (Fig. 5) shows a region with higher values in the south-west of the study area and a region with lower values in the north-east, corresponding with the distribution of populations dominated by *L. notabilis* and *L. dissortis*, respectively. Between them, there is a zone of steeper transition, shown by a band of concentrated isolines, which coincides approximately with the east slope of the Rocky Mountains. Some features of this map deserve special mention. First, there is a depression in the surface, indicating the presence of *dissortis*-typical alleles, in the Fraser Plateau of central British Columbia (locations 15 and 21–24, surrounded by the 0.3 isoline). Second, the zone of rapid transition has a small, but distinctive ridge projecting out toward the north and north-east from site 38 and especially site 39 in the Athabasca River valley,

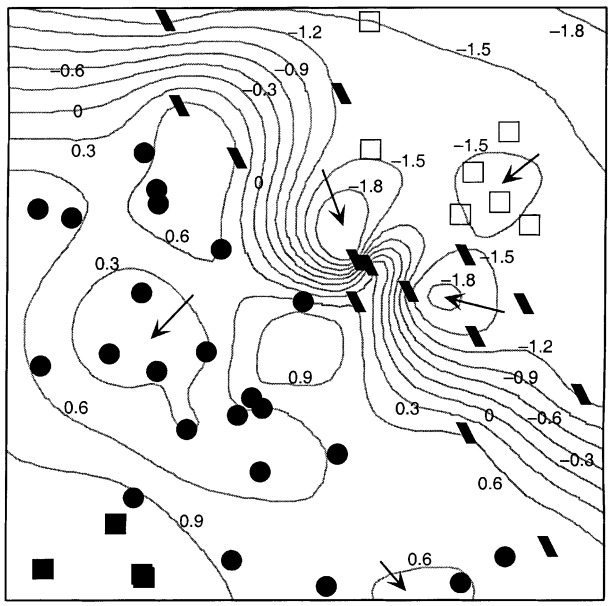

Fig. 5 Contour map for the PC1 scores of the allozyme data. Arrows point toward depressions in the surface. The symbols used to designate sampling locations in different geographical regions and the map projection are the same as in Fig. 1.

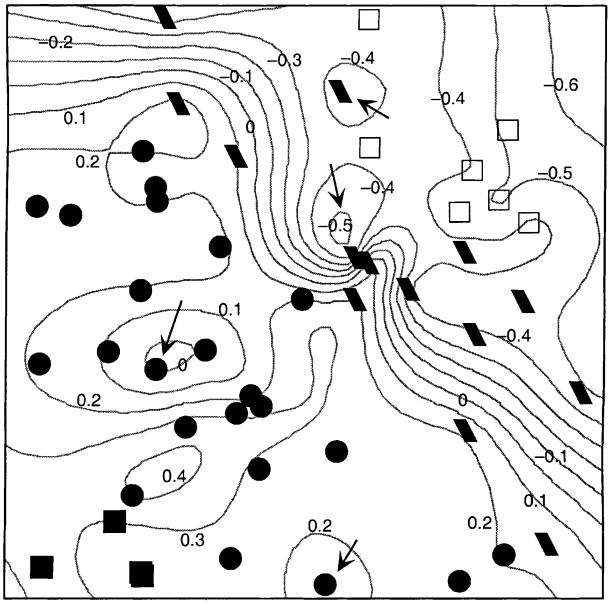

Fig. 6 Contour map for the PC1 scores of the morphometric data from the 1980s. Arrows point toward depressions in the surface. The symbols used to designate sampling locations in different geographical regions and the map projection are the same as in Fig. 1.

suggesting that *notabilis*-typical alleles are locally more frequent than at neighbouring locations. This ridge is surrounded by three depressions with extremely low PC1 scores, indicating that the populations in that area harbour almost exclusively *dissortis*-typical alleles.

The contour map for the average PC1 scores of the morphometric data (Fig. 6) is similar in that it shows the same general structure, with regions of high and low scores in the south-west and north-east of the area, respectively, and a zone of transition corresponding to the east slope of the Rockies. The correspondence to the geographical patterns in the allozyme data goes further, as the depression in the Fraser Plateau is also present (locations 21, 22, 24, surrounded by the 0.1 isoline), as well as the northward-directed ridge along the Athabasca River at locations 38 and 39, and the depressions to extremely low PC1 averages that surround this ridge from the north-east.

The contour map of total morphometric variance (Fig. 7) is quite different. Little overall trend is discernible, except perhaps a weak increase toward the west, but a series of pronounced peaks are present throughout the study area, of which two coincide with local features recognized in the maps of the average PC1 scores. First, the highest peak of total variance, at sites 22 and 23 (surrounded by the 0.105 isoline) coincides with the depression of the average PC1 scores in the Fraser Plateau. Second, a smaller peak of elevated variability coincides with the ridge along the Athabasca River at site 39. There are also discrepancies, however, such as the

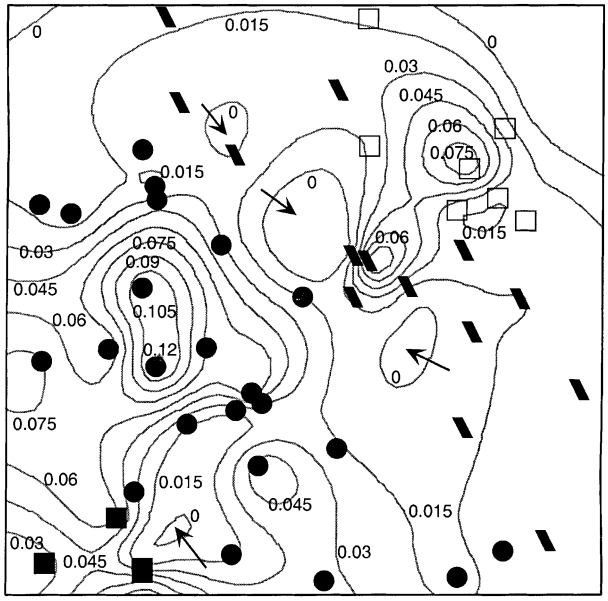

Fig. 7 Contour map for the total amount of total morphometric variance in the samples from the 1980s. Arrows point toward depressions in the surface. The symbols used to designate sampling locations in different geographical regions and the map projection are the same as in Fig. 1.

local increase of variability at site 51 relative to its neighbouring sites 50 and 54–56 in central Alberta, which has no match in the maps for average PC1 scores. Finally, it is also noteworthy that for most of the east slope of the Rockies (except near site 39), where the greatest change in average PC1 scores takes place, variance of morphometric traits is consistently low. This suggests that the *Limnoporus* populations in this region are fairly homogeneous, rather than mixed populations in which recognizably *dissortis*-like and *notabilis*-like forms coexist.

Changes between 1980s and 1990s samples

The comparison of samples collected in the 1980s and the 1990s shows that most sites along the transect running from the Pacific coast to central Alberta saw only small changes in the morphometric PC1 scores, of which most were not statistically significant (Fig. 8). This makes the few large changes all the more remarkable. One of these is a large drop in the average PC1 score for location 16, from a value typical of most sites in interior British Columbia to a value much closer to the *dissortis*-like values on the east slope of the Rockies. This change may be part of a regional trend, as there is also a smaller, but statistically nonsignificant, decrease in the PC1 score for the nearby site 22. Therefore, the depression of the PC1 surface in the Fraser Plateau (Fig. 6) appears to have deepened and expanded toward the south-east. Other decreases of the PC1 scores are found in location 39, reducing the local peak of more *notabilis*-like PC1 scores in a region of mostly *dissortis*-like populations, and in site 40 where the average shifted toward more extreme *dissortis*-like values. There was only one statistically significant increase of PC1 scores toward more *notabilis*-like values, in location 3 on the coast.

The changes in total variance (Fig. 9) are more difficult to interpret because the sample sizes limit the statistical power for detecting genuine differences in variance. None of the changes is statistically significant after sequential Bonferroni correction for taking into account the number of simultaneous tests. However, there is a single change that is nominally significant: a large increase in the total variance at site 16, almost to the peak values in the core area of hybridization (locations

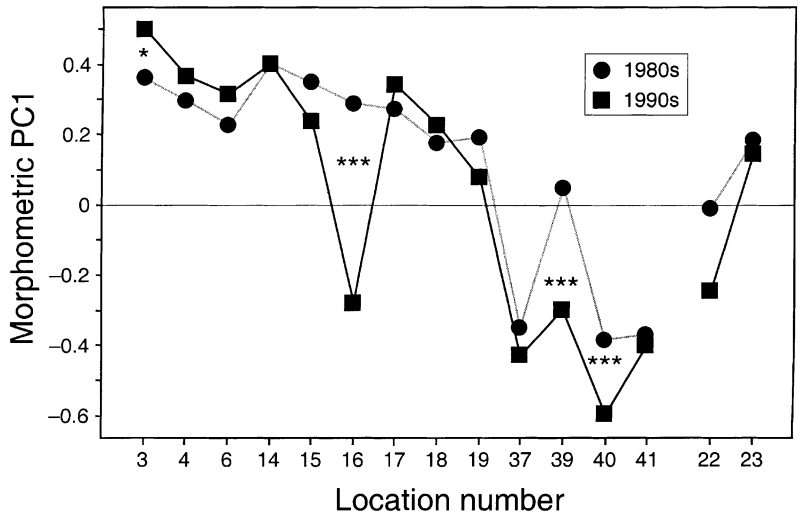

Fig. 8 Comparison of average morphometric PC1 scores between the samples from the 1980s and the 1990s. The 13 locations shown graphically to the left are part of a transect from coastal British Columbia to central Alberta, and the remaining two locations on the Fraser Plateau, to the north of the main transect (cf. Fig. 1). Asterisks indicate within-site comparisons significant after sequential Bonferroni adjustment (*$P < 0.05$; ***$P < 0.001$).

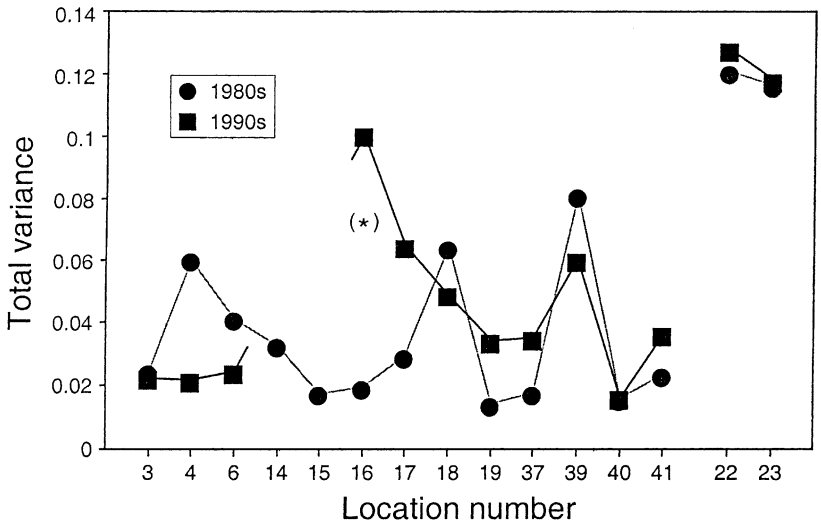

Fig. 9 Comparison of total variance in the samples from the 1980s and 1990s. The missing values in the 1990s transect for sites 14 and 15 are due to insufficient sample sizes. The asterisk in parentheses indicates a comparison that is nominally significant ($P < 0.05$) but not after sequential Bonferroni adjustment.

22 and 23; cf. Figs 7 and 9). This increase in variance coincides with a similarly drastic change in average PC1 scores at location 16 (cf. Fig. 8).

Discussion

Morphometric variation among populations coincides well with variation in enzyme allele frequencies, as both data sets are dominated almost exclusively by an axis summarizing the difference between *L. dissortis* and *L. notabilis*. We are unable to find any other significant patterns of variation among populations (e.g. coastal–inland or altidudinal gradients, etc.). This implies that variation in the overall proportion of genes from the two parent species exceeds by far any effects of selection or drift, which would act locally and affect different enzyme loci and the morphometric variation independently. Therefore, the congruence between data sets, implying a strong association between allozyme markers and the genes affecting morphometric differentiation, further emphasizes that introgressive hybridization is a recent and continuing process in this hybrid zone (Spence, 1990; Sperling & Spence, 1991). This congruence of genetic and morphometric data may be typical for hybrid zones between species (e.g. Shoemaker *et al.*, 1996; Shapiro, 1998). In contrast, marked incongruence has been found in a species pair of *Aquarius* waterstriders in eastern North America: electrophoretic data indicate a steep transition in a narrow zone between two large areas that appear genetically homogeneous (Gallant & Fairbairn, 1997), whereas the predominant pattern of morphometric variation is a cline that reverses its direction in the zone where hybrid genotypes occur (Brennan & Fairbairn, 1995).

That only a single dimension accounts for nearly all geographical variation is not unique to this data set, but has been shown in earlier studies (e.g. Lessa, 1990, fig. 6). We also emphasize that this apparent preponderance of a unitary factor of population differentiation is not an artefact of the ordination methods used, because other genetic studies have used PCA to document multidimensional variation (Menozzi *et al.*, 1978; Lessa, 1990, fig. 5). Different PCs from empirical data on human allele frequencies across Europe and the Near East have been related to major waves of population movements (Menozzi *et al.*, 1978), and computer simulations confirmed that PCA of genetic data can recover as many as three subsequent waves of demic expansion from different points of origin (Rendine *et al.*, 1986).

Our ordination analyses for both genetic and morphometric data also reflect the directional nature of gene flow in this hybrid zone, as the clusters of *notabilis*-like populations have a substantially greater spread of PC1 scores from extreme values toward the overall average than the tight clusters of *dissortis*-like populations. This appears to be a consequence of asymmetric introgression, which is directed primarily from *L. dissortis* into the gene pool of *L. notabilis* populations due to sex-specific hybrid viability and the asymmetry of the mating system (Spence, 1990; Sperling & Spence, 1991).

In addition to the overall geographical pattern of *dissortis*-like populations in the north-east and *notabilis*-like populations in the south-west of the study area, the transition zone shows a more intricate structure, which can be related to regional topography and require more localized treatments than the larger-scale trends in a transition zone between two waterstrider subspecies (Largiadèr *et al.*, 1994). Two such localized features are particularly prominent. First, in the region of the Fraser Plateau in interior British Columbia (locations 21–24), there is an 'island' of more *dissortis*-like and more variable populations within the range otherwise occupied by *L. notabilis*. Second, where the Athabasca River emerges from the Rocky Mountains (locations 38 and 39), a

marked protrusion of more *notabilis*-like PC scores extends northward into the surrounding area that is dominated by typical *dissortis*-like values, and also coincides with a local peak of morphometric variance. In both regions Sperling & Spence (1991, fig. 2) found homozygote excess and linkage disequilibrium between allozyme loci within local samples, suggesting that both are areas of continuing hybridization and introgression.

Further evidence for the localized and dynamic nature of hybridization in these areas comes from the comparisons of samples collected in the 1980s and the 1990s, which document actual change in the hybrid zone. This direct evidence largely confirms the scenario inferred from historical collection records and genetic data from the 1980s (Spence, 1990; Sperling & Spence, 1991). Except for one small increase in a population of *L. notabilis* outside the hybrid zone (location 3), all significant changes of average PC1 scores were decreases toward more *dissortis*-like values (Fig. 8), as expected from the directional nature of introgressive hybridization in this system (Spence, 1990; Sperling & Spence, 1991).

Particularly, there was a marked expansion of the area of hybridization on the Fraser Plateau of British Columbia. This increase of *dissortis*-like phenotypes appears to be a continuation of the gradual decreases in average body lengths towards more *dissortis*-like values in this region from the mid-1970s (Spence, 1990, table 1). Similarly, historical records and genetic analysis for a zone of hybridization between the katydids *Orchelinum nigripes* and *O. pulchellum* near Washington, DC, suggest similar dynamics of an expanding 'island' bordered by a hybrid zone (Shapiro, 1998).

The second marked change between the 1980s and 1990s collections took place on the eastern slope of the Rockies. Whereas the data from the 1980s clearly indicate that populations in the Athabasca River valley (locations 39 and 40) were more *notabilis*-like than the neighbouring populations, this local focus of hybridization had vanished by the time our 1990s samples were collected, and the populations were entirely dominated by *L. dissortis*. This change includes both a significant drop in average morphometric PC1 scores in these locations and a decreasing trend in total variance, as one would expect as a consequence of decline in *notabilis*-like phenotypes.

Presumably, the *Limnoporus* hybrid zone is prone to rapid change due to the high mobility of these bugs (Spence, 1981, 2000) and the instability of populations that is associated with their temporary habitats and high susceptibility to egg parasitoids (Spence, 1986). Accordingly, it is plausible that sections of the hybrid zone can shift by several tens of kilometres over a decade, even though this estimate exceeds those in examples from less mobile insects. The well-documented shift of the hybrid zone between chromosomal taxa of the grasshopper *Caledia captiva* was of the order of 200 m over 3 years (Kohlmann & Shaw, 1991), and a shift of 700–900 m over 5 years was found in a contact zone between the chewing lice *Geomydoecus aurei* and *G. centralis* of the pocket gopher *Thomomys bottae* (Hafner *et al*., 1998). Further detailed follow-up studies will be needed to understand the rates and extent of expansion by *L. dissortis* in the regions pinpointed by our study. Our results clearly indicate, however, that introgressive hybridization between *Limnoporus* species is a spatially complex and highly dynamic process.

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