doi:10.1111/j.1558-5646.2007.00146.x

CONTRASTING PATTERNS OF HYBRIDIZATION IN LARGE HOUSE SPIDERS (*TEGENARIA ATRICA* GROUP, AGELENIDAE)

Peter J. P. Croucher,[1,2] Ross M. Jones,[1] Jeremy B. Searle,[1] and Geoff S. Oxford[1,3]

[1]*Department of Biology, University of York, PO Box 373, York YO10 5YW, United Kingdom*

Received November 7, 2006
Accepted March 13, 2007

The integrity of species is not fixed and may vary geographically. Here we investigate the geographic distributions and interactions of species in the *Tegenaria atrica* group (Araneae: Agelenidae). Detailed mapping of *T. saeva* and *T. gigantea* in England and Wales shows them to be broadly allopatric in southern England with a tightly defined, and possibly long-standing, narrow zone of parapatry in central southern England. In the north of England (Yorkshire), by contrast, the species are broadly sympatric as a result of recent range expansions. GIS techniques are used to map the species distributions and to quantify, we believe for the first time, the intimacy of interspecific interactions. The extent and nature of hybridization in these two areas is examined through regression and multivariate analyses of morphology. We show that the relative incidence of hybridization is much greater in Yorkshire than within the parapatric zone in the south. Clear patterns of asymmetric introgression are observed in both northern and southern England, with a greater impact of *T. gigantea* on *T. saeva* than vice versa. We find no sign of morphological reproductive character displacement at the zone of parapatry that might indicate reinforcement, although we cannot exclude more subtle effects, for example via cuticular pheromones. The integrity of these two species seems to be breaking down in northern England, a process that might gain momentum as the gene pools become more similar.

KEY WORDS: **Asymmetric introgression, GIS, hybrid zone, morphometrics, range expansion, reinforcement, speciation.**

There are now many well-studied examples of natural secondary contacts between taxa that hybridize to varying extents in areas of parapatry (e.g., crickets, *Gryllus* [Harrison et al. 1987]; house mice, *Mus* [Boursot et al. 1993]; fire-bellied toads, *Bombina* [Szymura 1993]; grasshoppers, *Chorthippus* [Butlin 1998]; warblers, *Hippolais* [Secondi et al. 2006]). Many of these cases involve ancient contacts between species or subspecies, often established as a result of population expansion at the end of the last Ice Age (Taberlet et al. 1998; Hewitt 1999) and have been used to investigate factors influencing hybridization rates and the possible roles played by adaptive processes in reducing gene flow between taxa (Butlin 1998; Noor 1999). Hybridization and gene flow between allopatric species or subspecies more recently brought into contact, often through human transportation or habitat modification, has also received much attention (Cox 2004; Ellstrand 2005). These cases may involve, among other factors, invasive organisms (e.g., Albert et al. 1997), the escape of farmed species (e.g., Rubidge et al. 2001), disturbance or alterations to a species' habitat (e.g., Lamont et al. 2003), hybridization between domesticated and wild species (e.g., Beaumont et al. 2001), and natural expansion of species ranges (e.g., Nolte et al. 2006).

More recently, studies have been made of the ongoing spatial dynamics of species interactions and their implications for the introgression of genes from one taxon to another (Secondi et al. 2006, and references therein). Of particular interest are cases in which species usually meet in a zone of long-standing parapatry but have also recently expanded their geographical distributions

[2]Present address: Department of Environmental Science, Policy and Management, University of California Berkeley, 137 Mulford Hall, Berkeley, CA 94720–3114.
[3]Corresponding author: E-mail: gso1@york.ac.uk

to areas in which they now find themselves in sympatry. Such situations potentially offer a replay of an earlier evolutionary phase in the species interactions so that comparisons between levels of introgression and gene flow in ancient parapatry and modern sympatry may illuminate the processes that originally led to reduced gene exchange and speciation. There are, as yet, few investigations of this kind although cases in which species integrity in sympatric populations varies geographically offer similar opportunities (e.g., Bettles et al. 2005, and references therein).

One well-studied example, allowing such a comparison, is provided by pied and collared flycatchers (*Ficedula hypoleuca* and *F. albicollis*). The ranges of these two species are predominantly allopatric with the exception of Central and Eastern Europe where they make contact with little hybridization or introgression (Sætre et al. 1997; Borge et al. 2005). In allopatry, the males of the two species are similarly colored but where their ranges coincide there has been divergence in male coloration. These data, assortative female mate preferences and the lower fitness of hybrids, have been interpreted as strong evidence for reinforcement of prezygotic isolation in the area of parapatry. Within the past 150 years, populations of these same species have become sympatric on the Swedish islands of Gotland and Öland (Alatalo et al. 1990). Here the degree of introgression is considerably higher and the hybrids are at less of a selective disadvantage than further south. Thus the strength of the barrier to gene exchange between the two species varies geographically. This may be a result of a breakdown of existing species recognition systems (Borge et al. 2005), an indication that there has been insufficient time for such a system to have evolved between formerly allopatric populations that now interact in sympatry or simply a consequence of differences in the dynamics of species interactions in space and time. In this paper, we present a study of hybridization of spiders in the *Tegenaria atrica* group (Agelenidae) in Britain, which also exhibit both a long-standing parapatric boundary and a region of recent sympatry.

The *T. atrica* group (Merrett 1980; Maurer 1992; see also Croucher et al. 2004) comprises three species; *T. atrica* C.L. Koch, 1843, *T. gigantea* Chamberlain and Ivie, 1935, and *T. saeva* Blackwall, 1844. As for many other closely related spiders, taxonomic identification to the species level within the *T. atrica* group is based exclusively on the relative size and shape of the various sclerites comprising the external copulatory organs-–the male pedipalps and the female epigyne. These species have long been suspected of hybridizing because males have occasionally been reported with apparently intermediate pedipalp morphologies (e.g., Locket 1975; Merrett 1980).

Tegenaria atrica is found across the whole of continental Europe except for the more arid southeastern Mediterranean region and southern Italy (Nikolić and Polenec 1981; Maurer 1992; Pesarini 1994). *Tegenaria saeva* and *T. gigantea*, by contrast, have a predominantly Atlantic distribution (Portugal, Spain, and France) and are widespread in Britain, where *T. atrica* is a rare introduction (Merrett 1980; Oxford and Chesney 1994). Preliminary surveys have suggested that whereas they occupy largely allopatric distributions across much of England and Wales (Merrett 1980; Oxford and Chesney 1994) in northern England the species' ranges overlap much more. Finer-scale mapping (Oxford and Smith 1987) showed that in the northeast English county of Yorkshire the two species are broadly sympatric and that individuals with intermediate morphologies are relatively frequent and widespread (Oxford and Smith 1987; Oxford and Plowman 1991; Croucher 1998). The greater overlap in the north of England is almost certainly the result of recent colonization by the two species, presumably from further south. Despite the north of England having a long history of arachnological recording (Bristowe 1951; Parker 1984), these two *Tegenaria* species were first reported in Yorkshire in the 1970s (Smith 1985) and in the northwest English county of Cumbria at about the same time (Parker 1984). Along the south coast of England preliminary surveys showed the species to be largely allopatric, with *T. saeva* to the west and *T. gigantea* to the east (Merrett 1980). The species make contact in the county of Dorset.

Here we use fine-scale mapping to establish the detailed distributions of *T. saeva* and *T. gigantea* in England and Wales and to examine the geographical incidence of specimens of intermediate morphology (putative hybrids). We use a GIS approach to position the species boundary and, we believe for the first time, to quantify the intimacy of interspecific interactions. Although current and recent gene flow between taxa may be examined using specific genetic markers, considerable insights into levels of genetic exchange can also be obtained from the examination of morphological characters controlled by a large number of quantitative trait loci (QTLs). These characters may include traits that contribute to prezygotic incompatibility. Particular attention is paid to the species' distributions along the south coast of England and in Yorkshire and the extent of intermediacy observed in specimens from these regions is compared. Morphological variation is assessed using developments of previously described methodologies for distinguishing these taxa. A plotting-based approach for males, using measurements of prosoma length and combined tegulum and conductor length (Merrett 1980), is extended through linear regression and analysis of covariance. A discriminant function (DF) approach (Oxford and Plowman 1991) is extended using new landmark measurements and multiple-group principle component analysis. These analyses allow not only an assessment of the extent of hybridization but also of asymmetries in introgression manifest at the morphological level.

The results of the analyses are used to address three questions. (1) What is the incidence of phenotypic intermediacy along the long-standing zone of parapatry on the south coast compared

with that found in recent sympatry in Yorkshire? (2) Is there evidence for any asymmetry in hybridization and, if so, what are the causes? (3) If the species barrier is stronger in the south, is there any evidence for reinforcement, for example, reproductive character displacement (Howard 1993, but see Lemmon et al. 2004)?

Materials and Methods

MAPPING SPECIES DISTRIBUTIONS

Within England and Wales, most of our effort has concentrated on mapping the distributions of *Tegenaria* species in Yorkshire and along the south coast of England. Specimens from these areas were obtained from surveys in 1994 and 1995 in which the general public was encouraged to collect large house spiders alive and deposit them at a number of local centers. These individuals were returned to the University of York where they were frozen, with diagnostic parts subsequently preserved in 70% ethanol. Immature specimens were reared to maturity before preservation. Most of these spiders were male and came from within houses.

In these same geographic areas we have intensively sampled specimens living in a variety of habitats outside buildings using the "fishing" technique, whereby a maggot is placed in the web and the spider lured from its usually inaccessible retreat to be caught by hand (Oxford and Croucher 1997). To provide a fuller picture of the broad-scale distributions of species in England and Wales we sampled a number of other geographically widespread locations (between 1994 and 2004) using the same technique, and re-examined an extensive collection of preserved material made by J. E. Dalingwater and J. A. B. Kennet. A small number of additional records were obtained from county organizers of the British Arachnological Society's Spider Recording Scheme (SRS) and from an unpublished house-spider survey (P. Smithers, pers. comm.).

Preserved specimens from these surveys were categorized by either PJPC or GSO as *T. saeva*, *T. gigantea*, or "intermediate" on the basis of the morphology of the male palps or the female epigyne (Merrett 1980; Roberts 1985, 1995). The term intermediate is used to refer to a specimen with diagnostic features falling between those of "good" *T. saeva* and "good" *T. gigantea*; these may represent first- or subsequent-generation hybrids. The identification of specimens from the Smithers survey and from SRS organizers was not confirmed by us and it is possible that intermediates in this material may have been overlooked or misclassified. Excluding these data from the analyses makes no difference to our conclusions. In our material, intermediates are classified as *Ts*?, *Tg*?, and *Ts/Tg*? according to how closely they resemble *T. saeva* and *T. gigantea*.

Broad-scale mapping of the distributions of *T. saeva* and *T. gigantea* was based on the 10 × 10 km squares of the British Ordnance Survey grid. Results from the more intensive surveys of the south coast and of Yorkshire were unique-location based. To examine more quantitatively the distributions of species and intermediates, we used Gaussian kriging interpolation (Burrough and McDonnell 1998) and plotted species isoclines. To preserve information, multiple species from a single location were moved in position by 1 m. The cell sizes of the interpolation grids were 1000 × 1000 m for the England and Wales map, and 100 × 100 m for the Yorkshire and south coast maps. A *T. saeva* individual was assigned a value of 1, a *T. gigantea* individual a value of 0 and an intermediate (*Ts/Tg*?) individual a value of 0.5. Isoclines were drawn for interpolated trait values of 0.22, 0.5, and 0.78. The 0.5 isocline represents the center of the parapatric zone whereas the distance between the trait-frequency isoclines of 0.22 and 0.78 approximates the inverse of the maximum slope of the tanh curve often used in the analysis of widths of hybrid zones (specifically tension zones: Szymura and Barton 1991; Barton and Gale 1993). The width of the parapatric/hybrid zone was estimated by taking every node (inflection) on the 0.22 isocline and measuring the minimum Euclidean distance between it and the 0.78 isocline (Burrough and McDonnell 1998; Jones 2005). This will result in more nodes being identified on longer, more convoluted lines than on shorter, more linear lines. However, any constraints put on the number of nodes used would be arbitrary and so we have used here the total number identified by GIS. Estimates of parapatric zone widths have to be treated cautiously because of the considerable deviations of the species boundaries from a simple linear pattern, especially in Yorkshire but also elsewhere. We considered that the current distributions of data sampling points, and the mosaic nature of parapatry, preclude a more detailed analysis of the parapatric/hybrid zone structure. All calculations were carried out in the ArcInfo module of ArcGis 9.1 (Environmental Systems Research Institute).

SPECIMENS FOR MORPHOMETRIC ASSESSMENT

Material for a detailed morphometric assessment originated from the public surveys and hand-collections carried out between 1994 and 1996. A small number of F_1 hybrids were also generated in the laboratory. The strategy was to compare morphological variation in an area of recent sympatry (the area around the city of York in northern England) and an area of, presumably much older, parapatry (a zone on the south coast of England in the county of Dorset) against "type" specimens from areas containing relatively pure "allopatric" examples of *T. saeva* and *T. gigantea* (from the extreme southwest and southeast of England, respectively). These areas are labeled "York," "Para *Ts* Para *Tg*," "Allo *Ts*," and "Allo *Tg*" in Figure 1. No specimens were used from areas of the south coast between the allopatric and parapatric regions ("Mid *Ts*" and "Mid *Tg*" in Fig. 1).

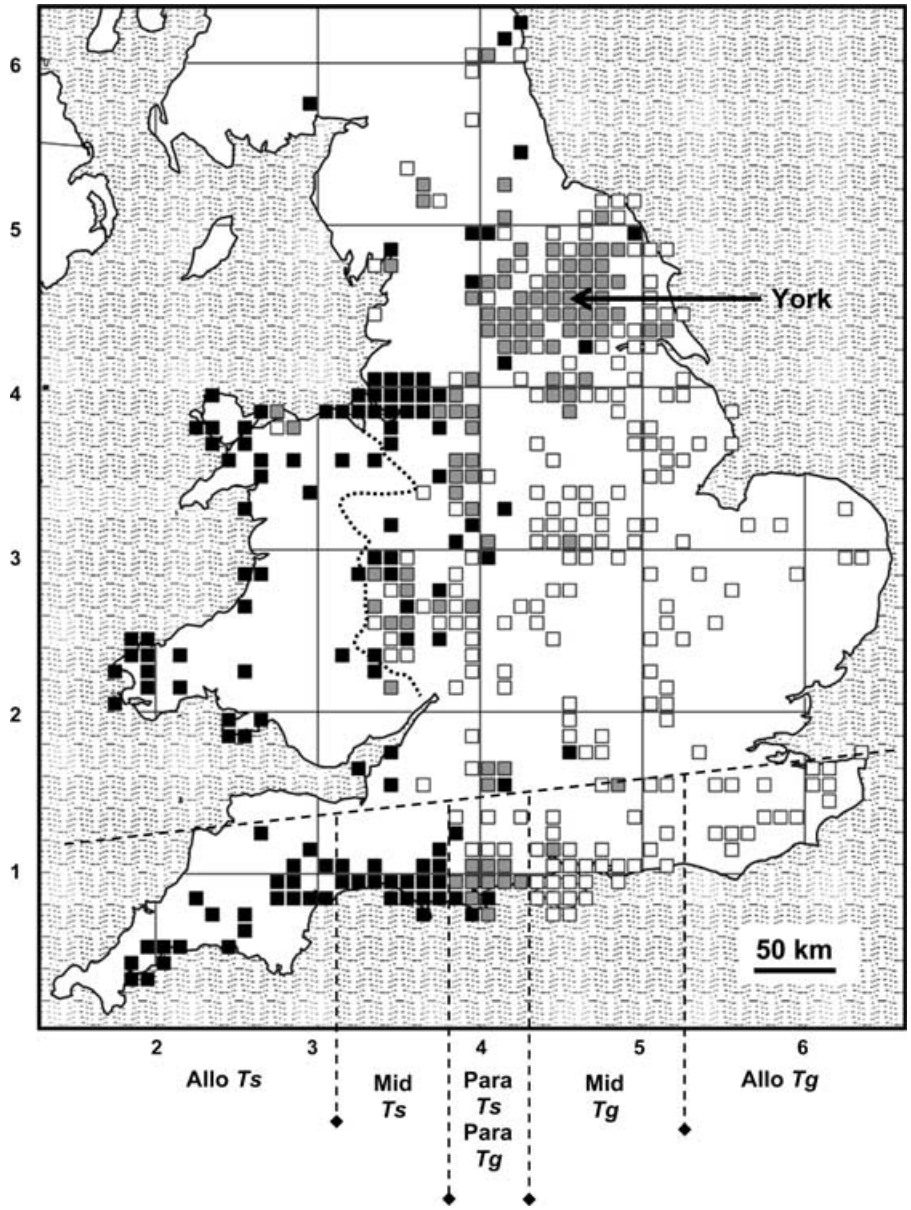

Figure 1. **Distribution in England and Wales of *Tegenaria saeva* and *T. gigantea* based on 10 × 10 km grid squares of standard maps (Ordnance Survey, the national mapping agency of Great Britain). The 100 × 100 km grid squares of the Ordnance Survey system are indicated as marginal numbers. Black squares, *T. saeva*; white squares, *T. gigantea*; gray squares, those containing both species. Spiders with intermediate morphologies are not included. The sampling zones along the south coast, from which specimens for morphological analyses were selected, are indicated; the sampling area around the city of York is shown in Figure 5. The English–Welsh border is denoted by a dotted line.**

All specimens from southern England were easily identified as *T. saeva* or *T. gigantea* with the exception of four intermediate females (*Ts*/*Tg*?). Overall, specimens were placed into the following ten categories: Allo *Ts*, Para *Ts* (allopatric and parapatric *T. saeva* from southern England); Allo *Tg*, Para *Tg* (allopatric and parapatric *T. gigantea* from southern England); Para *Ts*/*Tg*? (the females with intermediate morphology from southern England); Sym *Ts*, Sym *Ts*?, Sym *Ts*/*Tg*?, Sym *Tg*?, and Sym *Tg* (visually defined sympatric categories for *T. saeva*, *T. gigantea* and individuals of intermediate morphology from the York area). The sample sizes in each of these categories, as used in each analysis, are given in the Results section.

Twenty characters were measured on male spiders and 15 on females using a binocular microscope fitted with an eyepiece graticule. All measurements were made by one person (PJPC). The characters used are shown in Figure 2. The majority of characters were taken from the pedipalps of males (18) and the epigyne of females (11). These secondary reproductive characters were

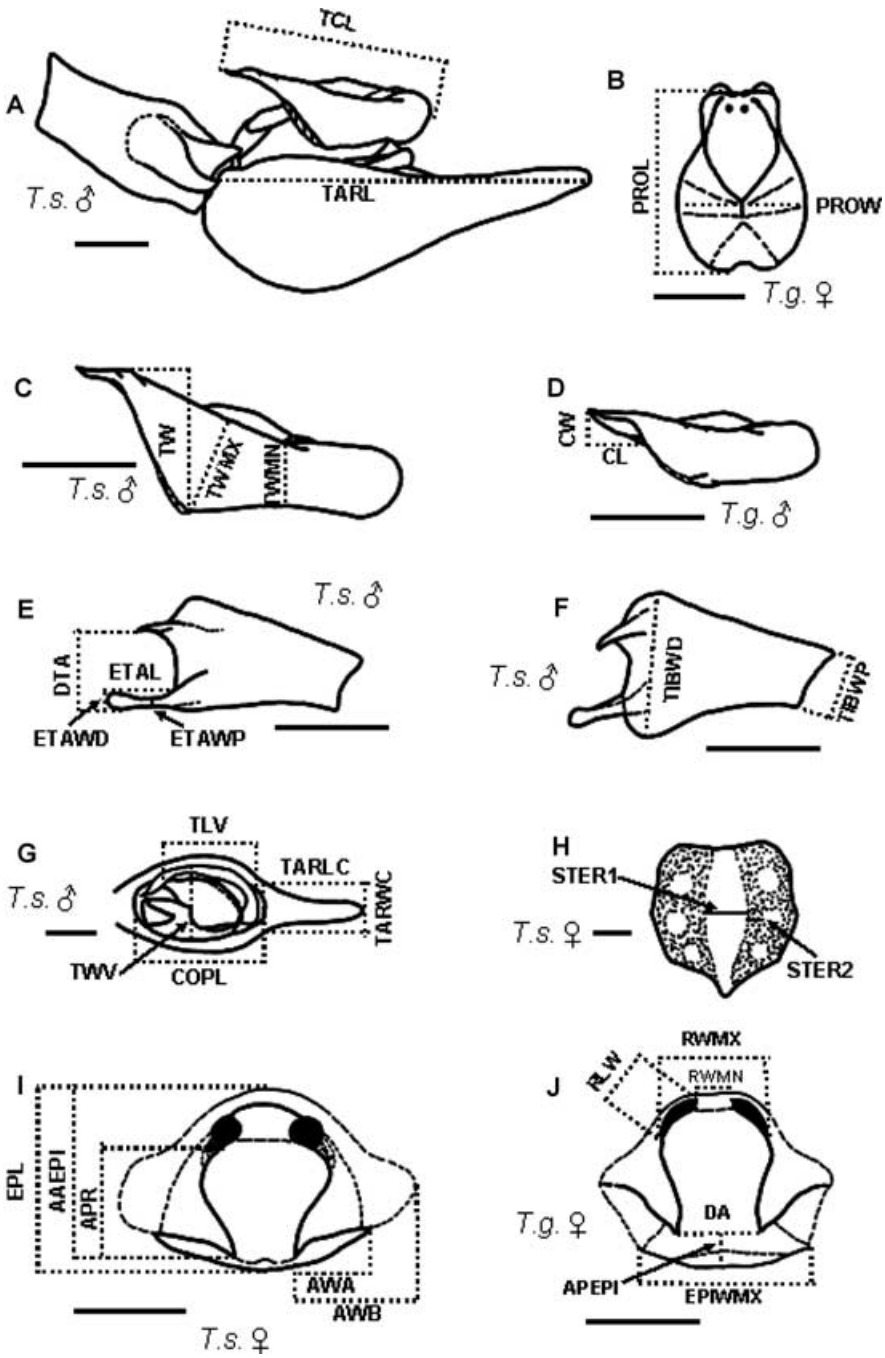

Figure 2. Measurements used in the morphological analyses. Scale bar = 0.5 mm (except B = 3.0 mm). Diagrams A, C, and D are ectal views (from the outside) of the left pedipalp with ventral side uppermost, E is a ventroectal view and F is also ventroectal but slightly more ventral. Diagram B is a dorsal view of the prosoma. Diagram H is a ventral view of the sternum, I and J are ventral views of the epigyne. AAEPI: tip of apophysis to anterior limit of epigyne, APEPI: tip of apophysis to posterior limit of epigyne, APR: tip of apophysis to posterior edge of receptacle, AWA: apophysis width "A", AWB: apophysis width "B", CL: conductor length, COPL: maximum cymbial operculum length, CW: maximum conductor width, DA: distance between tips of apophyses, DTA: distance between tips of tibial apophyses, EPIWMX: maximum width of epigyne at posterior limit, EPL: maximum epigyne length, ETAL: ectal tibial apophysis length, ETAWD: ectal tibial apophysis *maximum* width (distal), ETAWP: ectal tibial apophysis *minimum* width (proximal), PROL: prosoma (carapace) length (left side measurement), PROW: prosoma width (PROL and PROW were also measured in males), RLW: receptacle long width, RWMN: minimum width between receptacles, RWMX: maximum width between receptacles, STER1: sternum pattern width 1, STER2: sternum pattern width 2, TARL: tarsus length, TARLC: tarsus length from (distal) end of cymbium, TCL: maximum tegulum + conductor length, TIBWD: maximum distal tibia width, TIBWP: maximum proximal tibia width, TLV: tegulum length (ventral), TW: tegulum width, TWMN: minimum tegulum width, TWMX: maximum tegulum width, TWV: tegulum width (ventral).

emphasized because they would be the most likely to distinguish efficiently the species and because they would also be the most likely to be involved in any mechanical species isolation barrier. Characters were selected from an initially larger set (including those recommended by Oxford and Plowman [1991]) after an initial assessment of measurement reproducibility. All paired characters were measured on both the left and right sides and averaged. Care was taken to prevent recorder bias during measurement by selecting specimens blindly from a pool to be measured, thus avoiding runs of individuals from the same identification category.

DATA ANALYSIS

Comparing scatter-plots of combined tegulum + conductor length (TCL) against prosoma length (PROL) represents an established method of separating graphically males of the *T. atrica* group (Merrett 1980; Oxford and Smith 1987). This approach was therefore used, as an initial analysis, to compare the degree of separation and variation among the nine sample groups from southern England (139 males) and the York area (274 males). Measurements of TCL were regressed against PROL and the residual mean squares (RMS) compared among the groups. Analysis of covariance (ANCOVA) tested the homogeneity of the group means (the regression intercepts) for TCL (adjusted for the covariate PROL), after ensuring homogeneity of the regression slopes. Significant differences among the adjusted TCL means were determined using the GT-2 method for unplanned multiple comparisons and the Gabriel approximation to generate 95% comparison intervals (Sokal and Rohlf 1995).

Separate multivariate analyses of morphology were undertaken for male and female spiders. The allopatric *T. saeva* and allopatric *T. gigantea* samples were employed as a training set in a canonical discriminant analysis and the resulting DF was then used to ordinate the remaining individuals relative to these reference samples. Multiple group principal component analysis (MGPCA) (Thorpe 1988) was carried out on the reference samples and used to generate variables for input to the discriminant analyses. All raw measurements were log-transformed before analysis. The principal component (PC) coefficients from the MGPCA were applied to the data for each individual prior to ordination with the DF. The first PC from MGPCA is usually considered to be a vector describing "size" and thus, using this approach, the effects of size can be removed from the data (e.g., Thorpe 1988; Lynch and Haden 1995; Prenter et al. 1995; Overton et al. 1997). "Size in" and "size out" analyses were compared. The principal aim of these analyses was to produce a single score encapsulating the morphology of each individual and enabling it to be mapped along a *saeva–gigantea* axis.

Where multiple statistical tests are made original probability levels are reported but they are interpreted on the basis of adjusted significance levels using the Dunn–Šidák method (Sokal and Rohlf 1995: 241) with $\alpha = 0.05$.

Results

BROAD-SCALE DISTRIBUTIONS IN ENGLAND AND WALES

The distributions of *T. saeva* and *T. gigantea* over England and Wales on a scale of 10 km grid squares are shown in Figure 1, and the isocline map based on individual specimens comprises Figure 3. These maps confirm the patterns suggested by earlier, coarser-scale surveys (Merrett 1980; Oxford and Chesney 1994). *Tegenaria gigantea* is the only species present over much of central and eastern England. *Tegenaria saeva*, on the other hand, is the sole representative in southwest England and west Wales. In the central and southern English–Welsh border region the parapatric boundary seems to extend further west than it does on the south coast or across north Wales (Fig. 3). The distance between the 0.22 and 0.78 isoclines for the whole area shown in Figure 3 is 27.06 ± 0.21 km (mean ± SE, number of Euclidean measurements, $N = 7074$). For most of southern England and Wales the boundary between the species is relatively linear, but with a few notable "outliers" in which one species has established in an area principally occupied by the other. For example, *T. saeva* forms an enclave in what is otherwise an allopatric *T. gigantea* region in central southern England, and *T. gigantea* has a similar outpost in north Wales (Figs. 1, 3). It is possible that the apparent discreetness of the zone of parapatry along the Welsh border is an artifact resulting from insufficient sampling.

Harvey et al. (2002) published similar distribution maps for these species based on the British Arachnological Society's Spider Recording Scheme (SRS). These maps contain a higher numbers of cases of one species apparently in the allopatric area of the other. For example, there is a knot of *T. gigantea* grid squares in the southwest county of Cornwall where our records show only *T. saeva*. Many people contributed to the SRS and it is likely that some of these outliers are a result of misidentification or of occasional hybrids forced into one species or the other for recording purposes. Misidentification certainly seems to be the case for the records of *T. gigantea* in Cornwall mentioned above (S. P. Hopkins, pers. comm.). In contrast, the vast majority of specimens contributing to the present analysis were scored by just two people with extensive cross-referencing between them.

FINE-SCALE DISTRIBUTIONS AND PATTERNS OF HYBRIDIZATION

The two detailed surveys of *T. saeva* and *T. gigantea*, along the south coast of England and in Yorkshire, reveal very different geographical patterns of hybridization, which are confirmed by molecular markers (Croucher 1998). Close to the south coast

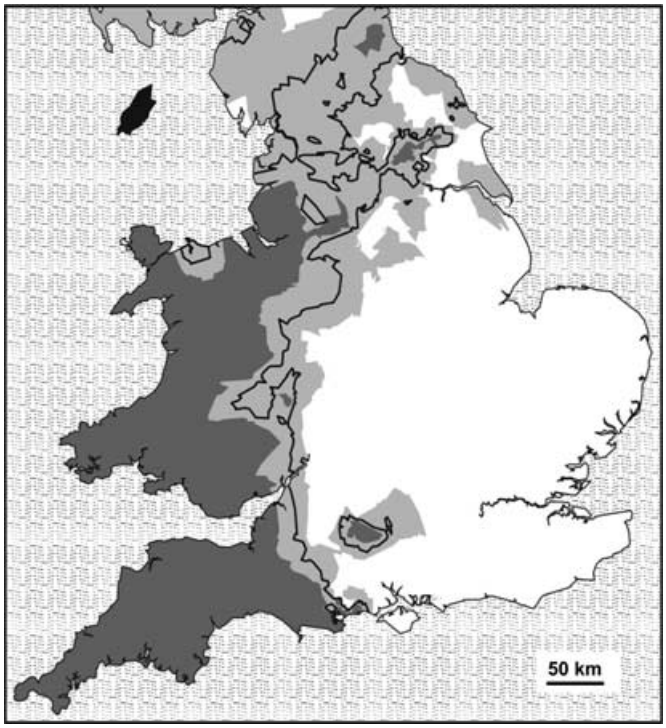

Figure 3. GIS-derived distributions of *Tegenaria saeva* and *T. gigantea* in England and Wales (see Methods for more details). Dark gray indicates areas with *T. saeva* at a predicted frequency of 0.78 or more, whereas white areas are those in which the predicted frequency of *T. saeva* is 0.22 or less. Light gray represents the parapatric zone (predicted *T. saeva* frequencies between 0.22 and 0.78) and, within it, the solid line shows the predicted 0.5 frequency isocline. The Isle of Man, where we have no spatial information, is shown in black. More detailed GIS-based maps of the south coast and Yorkshire study areas are shown in Figures 4B and 5B.

(Fig. 4), the species meet at a fairly well-defined boundary with *T. saeva* extending to the west and *T. gigantea* to the east. There are also sporadic individuals, and sometimes more extensive groupings, found in areas predominantly occupied by the other species. Only four intermediate spiders (all female) have been identified along the south coast (Fig. 4A); all have been ordinated in the multivariate analyses (see below). Isoclines derived from individual specimens are shown in Fig. 4B and give a mean parapatric zone width of 6.51 ± 0.05 km ($N = 6341$).

In Yorkshire, as mentioned above, both species are widespread. However there is a tendency for *T. gigantea* to be found alone in more rural areas (Fig. 5), giving rise to the higher number of single-species 10 km grid squares for this species in Figure 1. The stronghold for *T. saeva* in the region is the city of York (Fig. 5A) and its surrounding villages (see also Oxford and Smith 1987). In contrast to the largely parapatric distributions along the south coast, here the two species are broadly sympatric across much of the region although, on a finer level, particular villages may contain only one (Oxford and Smith 1987). In parallel with this, intermediate individuals in Yorkshire are relatively common (Fig. 5A: note that *Ts*? and *Tg*? are, for clarity, not plotted). Intermediate individuals are not always found with both putative parent species, but this could be the result of the loss of one parental species, incomplete sampling, or the translocation of intermediates from other locations. The mean width of the "parapatric zone" in Yorkshire is 7.41 ± 0.03 km ($N = 14543$) (Fig. 5B).

It is not straightforward to compare quantitatively the relative propensity for hybridization within the two study areas because of differences in species distributions and hence the opportunity for interspecific matings. One approach is to consider the spiders sampled within a 30 km wide, north–south strip centered on the grid line labeled 4 in Figure 4A, and a similar strip centered on York in Figure 5A (the dotted line). These strips are roughly comparable in both numbers and spatial distributions of species. In Figure 4A, 141 *T. saeva*, 86 *T. gigantea*, and 1 intermediate (*Ts*/*Tg*?) individuals were recorded in this strip (unique locations on the maps may represent multiple specimens), whereas in Figure 5A the corresponding numbers are 167, 115, and 49. The proportions of *T. saeva* and *T. gigantea* are almost the same in the two areas (frequencies of *T. saeva* of the total of *T. saeva* and *T. gigantea* are 0.62 and 0.59, respectively), but numbers of intermediates versus good species are highly significantly different ($\chi^2_{(1)} = 34.21$, $P \ll 0.001$). Thus the proportion of intermediate spiders out of the total sample size for the defined strip of the south coast is 0.4%, compared with 14.8% for central Yorkshire.

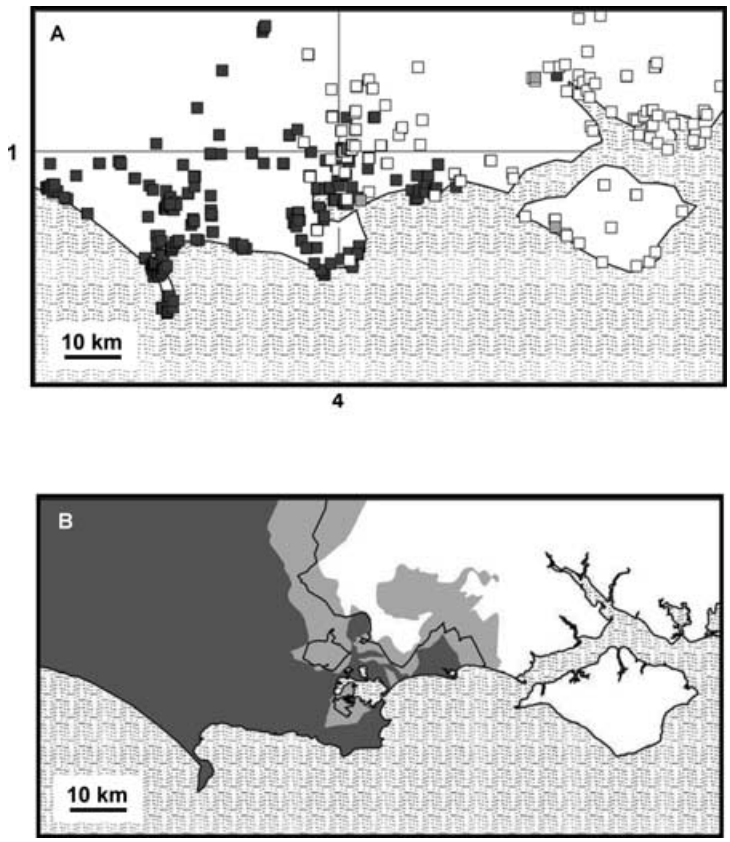

Figure 4. **Detailed distributions for *Tegenaria saeva* and *T. gigantea* in the area of parapatry in southern England. (A) Plot of unique spider locations (but those containing mixed taxa are shown as separate symbols that are slightly displaced). Dark gray squares, *T. saeva*; white squares, *T. gigantea*; light gray squares, putative hybrids (i.e., *Ts*/*Tg*?). Grid lines of the Ordnance Survey 100 × 100 km squares are indicated and numbered. (B) GIS-derived distributions of *Tegenaria saeva* and *T. gigantea* on the south coast (for explanation see legend to Fig. 3 and the Methods section).**

A second way of estimating the expected numbers of intermediate spiders in Yorkshire and on the south coast is based on the assumption that the propensity to hybridize is the same in both areas, and that the length of the 0.5 isocline can be used as a measure of the intimacy of species contact. The more convoluted (i.e., the longer) the line within a specified area the greater the opportunity for interspecific matings. Expected numbers will also increase with sample size. The expected number of intermediate spiders in Yorkshire (E_Y) in the sample from the area shown in Figure 5B was therefore calculated as

$$E_Y = T_I \frac{(N_Y \,.\, L_Y)}{(N_Y \,.\, L_Y + N_S \,.\, L_S)}, \qquad (1)$$

where T_I is the total number of intermediates collected across both study areas, N_Y is the total sample size for the Yorkshire area, L_Y is the length of the 0.5 isocline in Yorkshire, and N_S and L_S are the equivalent values for the south coast area shown in Figure 4B. The expected numbers of intermediate spiders in the sample from the south coast is $E_S = T_I - E_Y$. The estimate assumes that doubling the sample size, for example, will generate the same increase in expected numbers of intermediate spiders as would doubling the length of the contact zone. Values for the variables in equation 1 are $T_I = 56$, $N_Y = 457$, $L_Y = 601.9$ km, $N_S = 549$, $L_S = 203.5$ km, yielding expected values $E_Y = 39.82$ and $E_S = 16.18$. These were compared with the observed values of intermediates in the two areas (52 and 4, respectively) in a goodness-of-fit chi-squared test ($\chi^2_{(1)} = 12.89$, $P < 0.001$). The isocline lengths will be influenced by the numbers and distributions of intermediate spiders, which are weighted 0.5 during interpolation. The lengths of the 0.5 isocline were therefore recalculated ignoring intermediates (i.e., a purely parapatric line) to yield $L_Y = 556.5$ km and $L_S = 203.6$ km, and expected values of $E_Y = 38.90$ and $E_S = 17.10$ ($\chi^2_{(1)} = 14.44$, $P < 0.001$).

The calculations above assume that the fine-scale intimacy of contact between the two species is similar in both areas. This can be tested by estimating the expected numbers of mixed species sample sites (mostly households–-identified here as spider locations with the same 6-figure map reference). The majority of sample sites yielded just one individual: Yorkshire, 70.5% (total

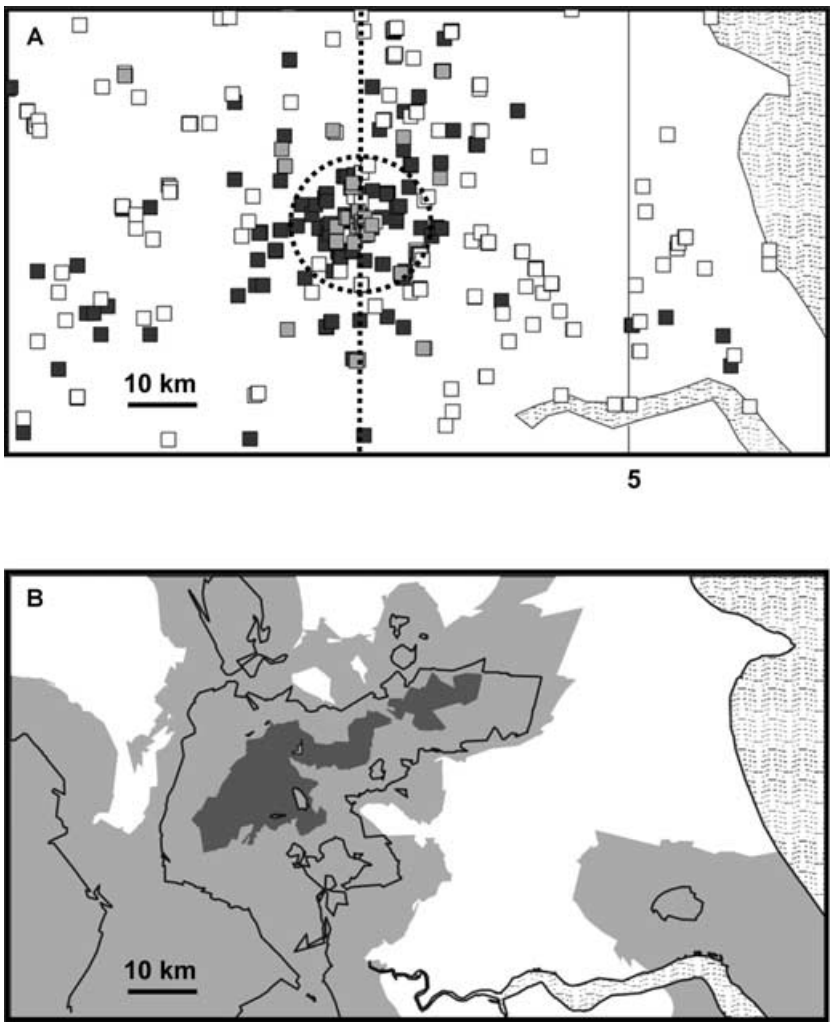

Figure 5. Detailed distributions for *Tegenaria saeva* and *T. gigantea* in the area of sympatry in Yorkshire. (A) Plot of unique spider locations (but those containing mixed taxa are shown as separate symbols that are slightly displaced). Dark gray squares, *T. saeva*; white squares, *T. gigantea*; light gray squares, putative hybrids (i.e., *Ts*/*Tg*?). Individuals classified as *Ts*? and *Tg*? are not included. Grid lines of the Ordnance Survey 100 × 100 km squares are indicated and numbered. The dotted circle locates the city of York and immediate environs; the source of specimens for morphometric analysis. The vertical dotted line indicates the axis around which the 30 km wide band, used for assessing the propensity for hybridization, was centered (see text). (B) GIS-derived distributions of *Tegenaria saeva* and *T. gigantea* in Yorkshire (for explanation see legend to Fig. 3 and the Methods section).

sample sites, $N = 254$); south coast, 70.4% ($N = 341$). In those sites with more than one spider (multiple sites) the frequency distributions of numbers of individuals per site are not significantly different in the two areas ($\chi^2_{(5)} = 3.19$, n.s.). The expected number of mixed species sites in the sample from Yorkshire (E_{MY}) was calculated as:

$$E_{MY} = T_M \frac{(M_Y \,.\, L_Y)}{(M_Y \,.\, L_Y + M_S \,.\, L_S)}, \qquad (2)$$

where T_M is the total number of mixed species sites sampled across both study areas, and M_Y and M_S are the number of multiple sites observed in Yorkshire and on the south coast, respectively. As before, it is assumed that the number of mixed species sites sampled will increase as both the sample size of multiple sites and the length of the 0.5 isocline increase. The expected numbers of mixed sites among those sampled on the south coast are $E_{MS} = T_M - E_{MY}$. In this case, $T_M = 23$, $M_Y = 75$, and $M_S = 101$, giving expected values of $E_{MY} = 15.41$ and $E_{MS} = 7.59$, when the more conservative estimates of $L_Y = 556.5$ km and $L_S = 203.6$ km are used. Comparing observed (18 and 5) and expected values yields $\chi^2_{(1)} = 1.32$ (n.s.). Thus, given the assumptions made, there is no evidence from our data that mixed species houses are more likely in Yorkshire than on the south coast when sample sizes and the length of the 0.5 isoclines are taken into consideration.

These analyses strongly suggest that there are relatively fewer hybrids found on the south coast, and more in Yorkshire, than would be expected on the assumption that the propensity to

hybridize, given the opportunity, is the same in the two areas. Individuals classified as *Ts*? and *Tg*? in Yorkshire were not considered above and are not plotted on the maps; their inclusion would obviously increase even more the proportion of individuals in Yorkshire influenced by hybridization (see below).

MORPHOMETRICS

Species and intraspecific category identification in males was based on the shape of the conductor (Fig. 2D, compare with Fig. 2C) and the presence/absence of a strong angle on the ventral margin of the tegulum + conductor (where the TW and TWMX lines meet in Fig. 2C, compare with Fig. 2D). These are therefore independent of the combined TCL and PROL used in the plots described below.

Plots of the combined TCL against PROL indicated that male *T. saeva* and *T. gigantea* from southern England could easily be separated based on these criteria, and both allopatric and parapatric datasets appeared similar. Sympatric specimens from the York area showed a much poorer separation although the categories Sym *Ts* and Sym *Tg* could still be distinguished clearly (data not shown). These data were formally analyzed using linear regression and the RMS between the nine categories compared using *F*-tests. These comparisons are given in Table 1 and indicate that, for southern England, the data for Para *Ts* were more variable than for Allo *Tg*, Allo *Ts*, and Para *Tg*, although no contrast within these groups was formally significant after correction for multiple testing. Sample categories from the York area were generally more variable than those from southern England, with the notable exception of Para *Ts*. The Sym *Tg* category appeared somewhat less variable than the other sympatric sample categories, the possible significance of which is discussed below.

The ANCOVA performed on the nine species and intraspecific category datasets indicated that the regression lines did not differ significantly in slope ($F_{(8,385)} = 1.86$; n.s.). However, the elevations of the regression lines (mean TCL adjusted for covariation with PROL) were highly significantly different ($F_{(8,403)} = 153.72$; $P \ll 0.0001$). These results fully support those from previous analyses of independently gathered specimens from Yorkshire (Oxford and Smith 1987). The adjusted means for TCL together with their 95% comparison intervals and bars highlighting the homogeneous groupings are given in Figure 6. This figure dramatically demonstrates the pattern of variation among the datasets with respect to this important character. The adjusted means exactly reflect the visually defined morphological groupings, with the Allo *Ts* and Allo *Tg* exhibiting the greatest differences and the Sym *Ts*/*Tg?* group falling approximately half-way between these values. It is interesting to note that whereas the Sym *Tg* group forms an homogeneous group with the Allo *Tg* and Para *Tg* groups, the Sym *Ts* group exhibits significantly shorter adjusted mean TCL than the Allo *Ts* and Para *Ts* groups. In other words sympatric *T. saeva* are, on average, displaced to a greater extent toward *T. gigantea* with respect to this character.

The Allo *Ts* and Allo *Tg* groups were used as the training set in the multivariate analyses as the basis for the MGPCA and subsequent canonical DF. Initial univariate one-way ANOVA on each of the landmark measurements indicated that, after correction for multiple testing within the sexes, 10 of 20 of the measurements taken on males and 9 of 15 of the measurements taken on females contained significant levels of between-group variation (see Appendix). Therefore a high degree of reliance could be placed on these variables to demonstrate real differences between *T. saeva* and *T. gigantea*. As indicated above, the species and intraspecific category identification was based on a visual inspection of shape (in males, of the conductor and the extent of the angle on the ventral margin of the tegulum and conductor, and in females, primarily the openness of the receptacle opening and the shape of the epigynal apophyses; see Fig. 2). Although the MGPCA and discriminant analyses may be interpreted as separating the taxa on the basis of a multivariate "shape," this is derived from linear landmark measurements that do not directly correspond to the overall

Table 1. Comparison of residual mean squares from linear regression of TCL on PROL (see Fig. 2). Values in the matrix are *P*-values from *F*-tests of the residual mean squares (RMS) from the linear regression. Shaded blocks denote within-area comparisons (left, south coast; right, Yorkshire). Probabilities still significant after correction for multiple testing are in bold.

	Allo Ts	Allo Tg	Para Ts	Para Tg	Sym Ts	Sym Ts?	Sym Ts/Tg?	Sym Tg?	Sym Tg
N	30	30	40	39	145	43	31	15	40
RMS (10^{-3})	0.699	0.766	1.283	0.580	1.659	1.692	1.752	2.155	1.163
Allo Tg	0.361								
Para Ts	0.038	0.079							
Para Tg	0.338	0.212	0.009						
Sym Ts	0.003	0.010	0.179	**0.000**					
Sym Ts?	0.006	0.015	0.196	**0.001**	0.450				
Sym Ts/Tg?	0.006	0.016	0.182	**0.001**	0.400	0.452			
Sym Tg?	0.005	0.011	0.106	**0.001**	0.220	0.267	0.309		
Sym Tg	0.066	0.127	0.382	0.018	0.101	0.123	0.117	0.070	

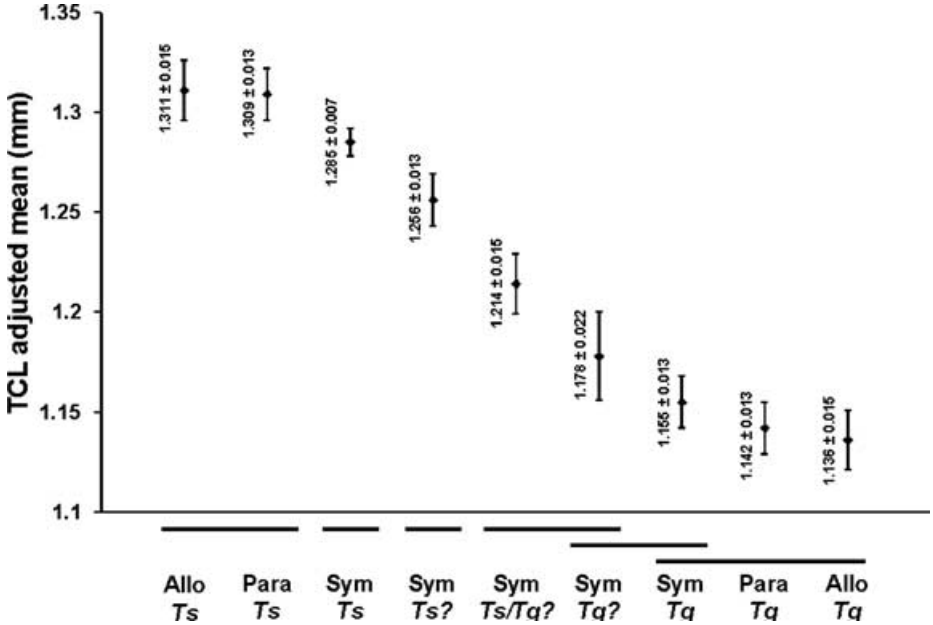

Figure 6. Analysis of covariance: tegulum + conductor length (TCL) adjusted for variation in prosoma length (PROL). Morphological sample groups (for explanation see Methods) are ordered by decreasing adjusted mean TCL. Adjusted mean TCL lengths are given with their 95% comparison intervals. Homogeneous groupings are indicated by horizontal bars below the chart.

structures interpreted during visual categorization. Furthermore, even those characters that appear either to distinguish the species well, or fail to distinguish the species, on the basis of univariate ANOVA, do not necessarily receive corresponding weightings in the PCs or DF (see Appendix). For example, in females EPIWMX exhibits little apparent between-group variation when examined independently yet is weighted heavily in PC11; the PC with the greatest correlation with the DF. Similarly, in males ETAL exhibits little between-group variation yet is weighted heavily in PC10 and PC7; the second and third PCs most strongly correlated with the DF. Conversely TCL, as employed in the ANCOVA analyses on males, contributes relatively little to the PCs correlating most strongly with the DF. Overall then, the three approaches to identification and categorization employed here, plots of combined TCL against PROL in males, visual classification of the genitalia, and discriminate function analyses in both sexes, are substantially different.

The resulting DFs for both the males and females provided very good separation for these samples. For the males the eigenvalue (ratio of between groups variation to within groups variation) was high (60.63) with a corresponding canonical correlation between the discriminant score and the Allo *Ts* and Allo *Tg* groups of 0.99 ($r^2 = 0.98$, indicating that 98% of the variation was explained by group differences). Wilk's λ was small (Wilk's $\lambda = 0.02$, $\chi^2_{(20)} = 218.42$, $P < 0.001$) showing that the group means were highly significantly different. The DF for females was less-efficient but still good (eigenvalue = 11.56; canonical correlation = 0.96 [92% of variation due to group differences]; Wilk's $\lambda = 0.08$, $\chi^2_{(15)} = 163.20$, $P < 0.001$). For both males and females 100% of the training sets were correctly reclassified by the DFs. In both sexes, the first component of the MGPCA could be interpreted as an indicator of "size." Excluding this component did not improve the discrimination between the two groups and therefore only "size-in" results are reported.

Figure 7 shows the ordination for each specimen from each group as a histogram of the discriminant scores and group means are given in Table 2. Figure 7A illustrates the clear separation between *T. saeva* and *T. gigantea* males in southern England. The distribution of the Para *Tg* group closely mirrors that of the Allo *Tg* group. However, compared with Allo *Ts*the Para *Ts* group is slightly, but significantly, displaced toward the origin (i.e., towards *T. gigantea*) (Mann–Whitney *U*-test: $P < 0.001$). The Para *Ts* group also exhibits a significantly higher variance than Allo *Ts* (Levene's test: $F_{(1,71)} = 5.285$, $P = 0.025$) and is left-skewed with a tail of individuals exhibiting intermediate scores (Shapiro–Wilks *W*-test: $W = 0.863$, $P < 0.001$). Three laboratory-generated F_1 hybrids are also ordinated and these, as expected, fall between the *T. saeva* and *T. gigantea* distributions. Figure 7B shows the discriminant scores for males from the York area. There are highly significant differences between the five recognized categories (ANOVA: $F_{(4,102)} = 84.36$, $P \ll 0.001$) and posthoc tests show that all pairwise comparisons except for *Tg* and *Tg*? are also significant. The Sym *Ts* and Sym *Tg* groups, although clearly differentiated, are significantly displaced toward the origin when compared to the Allo *Ts* and Allo *Tg* groups in Figure 7A ($t_{(49)} = 4.37$, $P < 0.001$, and $t_{(51)} = 8.35$, $P < 0.001$, respectively). In agreement with the ANCOVA results for TCL versus PROL (Fig. 6), the Sym *Ts*/*Tg*? group falls between the Sym *Ts* and Sym *Tg* groups and is flanked

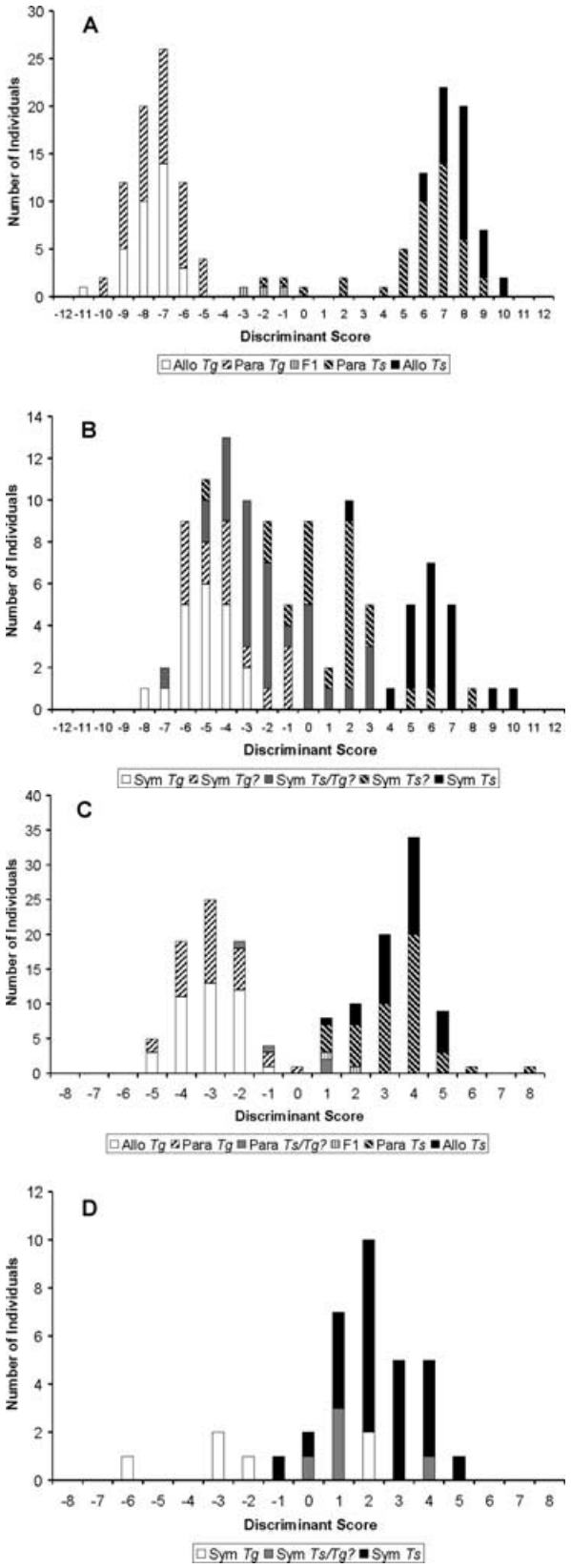

Figure 7. Discriminant scores for each of the morphological groupings (for explanation see Methods). (A) males, southern England; (B) males, York area; (C) females, southern England; (D) females, York area. F_1: laboratory-generated F_1 hybrids.

Table 2. Descriptive statistics for the discriminant scores of each dataset

	Males			Females		
Dataset	*N*	Mean	SD	*N*	Mean	SD
Allo *Ts*	32	7.78	1.04	34	3.63	1.00
Para *Ts*	41	6.10	2.06	45	3.41	1.29
Para *Tg*	44	−7.28	1.26	31	−2.94	1.09
Allo *Tg*	33	−7.55	0.96	40	−3.09	1.00
Sym *Ts*	19	6.09	1.73	24	2.24	1.40
Sym *Ts?*	22	1.42	2.70	–	–	–
Sym *Ts/Tg?*	31	−1.86	2.43	5	1.37	1.56
Sym *Tg?*	15	−3.72	1.76	–	–	–
Sym *Tg*	20	−4.97	1.28	6	−1.61	3.13

by the Sym *Ts*? and Sym *Tg*? groups. Thus the subjective visual categories really do appear to reflect real morphologically intermediate groupings.

Although the DF for females generated a smaller separation (Fig. 7C, Table 2), the differences between the four categories are very highly significant (ANOVA: $F_{(3,146)} = 429.29$, $P \ll 0.001$). In post hoc tests, Para *Ts* and Para *Tg* exhibit distributions that did not differ significantly from their respective allopatric groups, but were highly significantly different from each other ($t_{(74)} = 22.43$, $P < 0.001$). The four putative hybrid individuals (Para *Ts*/*Tg*?) and two laboratory-generated F_1 hybrids fell between the *T. saeva* and *T. gigantea* distributions but the poorer discrimination between females means that they are not distinct from the main species groups. Unfortunately, relatively few female specimens were included from the York area (Fig. 7D, Table 2). However, the results do show that there is significant discrimination between the three categories (ANOVA: $F_{(2,32)} = 10.97$, $P < 0001$) and, as for the York males, the mean discriminant scores for the two species are displaced toward each other when compared to southern England (*T. saeva* – $t_{(56)} = 4.42$, $P < 0.001$; *T. gigantea* – $t_{(44)} = 2.39$, $P = 0.02$). In post hoc tests the Sym *Ts* and Sym *Tg* groups are significantly different from one another, although the putative "hybrid" group Sym *Ts*/*Tg*?, which falls between the two, does not differ significantly from either.

Discussion

Previous work by Croucher et al. (2004) has suggested that *T. saeva* and *T. gigantea* originated in the Iberian peninsula and expanded their ranges northwards after the last glaciation. Given this, the distributions of *T. saeva* and *T. gigantea* in England and Wales (Fig. 3) are intriguing. There is currently no indication of any ecological differences between the species that would explain the sharp geographical divide observed in southern England, as underlined by their broadly sympatric distributions across northern England. Furthermore, sampling from inside and outside houses suggests no obvious differences in habitat utilization between the

species in Yorkshire (Oxford and Smith 1997) or along the south coast (Croucher 1998). The species' present-day distributions in southern Britain are likely, therefore, simply to reflect post-glacial colonization history, whether natural and/or human-mediated.

The persistence of remarkably discrete allopatric distributions in the south, one of the most densely populated regions of England, is surprising given the propensity for human-mediated dispersal of large house spiders, and increased human mobility, over the last 200–300 years. That some exchange of individuals does occur between allopatric areas is suggested by the finding of occasional, morphologically intermediate individuals in deep allopatry, and the detection of mtDNA sequences characteristic of *T. gigantea* in *T. saeva* sampled from the far south-west of England (Croucher et al. 2004). Equally surprising is the recent, and rapid, expansion of *T. saeva* and *T. gigantea* into broad sympatry in northern England. Whether this shift in distribution is climate related is currently unknown.

The use of spatially explicit modeling in population genetics and evolutionary research is growing (e.g., Avise 2000; Epperson 2003; Manel et al. 2003; Storfer et al. 2007). Here we use a GIS approach to determine the location and width of the parapatric zone. GIS techniques have been little used in analyses of hybrid zones, and are primarily employed to map the positions of the zones with respect to environmental or other factors (Kidd and Ritchie 2000; Ritchie et al. 2001; Jones and Searle 2003; Swenson and Howard 2004, 2005).

Our isocline analyses suggest that the width of the parapatric zone over the whole of England and Wales is ~27 km compared with ~7 km in the more intensively sampled areas of Yorkshire and the south coast. This contrast is unlikely to be a result of the different grid sizes used for interpolation across the two spatial scales, and may simply reflect the more dispersed distribution of sampling sites outside of the main study areas. Given the broadly sympatric distribution of species in Yorkshire, the similarities of the estimated widths of the parapatric boundaries here and for the south coast are unexpected. However, the evaluation of the 0.22 to 0.78 isocline distances might not be reliable where boundaries are very convoluted and interdigitating, and the estimates presented should be treated with caution.

The fitting of the 0.5 isoclines, however, provided a novel application of GIS to quantify the potential intimacy of species interactions in the two study areas. We show that the species microdistributions (the chance of both cohabiting the same house) are the same in Yorkshire and on the south coast, when the lengths of the parapatric boundaries and sample sizes are taken into account. This allows an estimate to be made of the expected numbers of spiders with intermediate morphologies in each area, based on the assumption that hybridization probability is constant per unit length of the 0.5 isocline. The results demonstrate that there are significantly more intermediate spiders in Yorkshire, and fewer on the south coast, than expected. This agrees with the result of the comparisons between numbers of the two "good" species and those with intermediate morphologies in similar-sized strips defined within the two areas. The strong implication is that hybridization is a relatively more frequent event in Yorkshire, a conclusion that can only be strengthened further by the inclusion of the *Ts*? and *Tg*? categories. In other words, species barriers are considerably less leaky in the south than in the north where a high proportion of individuals are influenced to some extent by introgression.

As a method for the analysis of hybrid zones, the GIS approach we have used may be particularly valuable for interactions between species or races distributed in a mosaic pattern in that spatial structure is necessarily incorporated into the analyses. A problem arises if species or race distributions are mosaic on a very fine geographical scale, as is the case of the interaction of *T. saeva* and *T. gigantea* in Yorkshire, when isoclines almost cease to be meaningful. Although the width of the parapatric zone is of interest, of more importance here is our use of the 0.5 isocline as a surrogate for species intimacy and, thus, the opportunity for hybridization. The present data are derived from specimens gathered by the general public and by ourselves from accessible habitats. We therefore had little control, on a very local scale, over where specimens originated from and, more importantly, how many were caught at each location. Under these circumstances, obtaining a direct measure of the opportunity for the two species to hybridize is difficult. Choosing the 0.5 isocline presents a way round this problem. Of course, species can hybridize away from this line where relative proportions are not equal, and F_1 hybrid themselves contribute to the process. Nevertheless, the very similar widths of the parapatric zones in the south and in Yorkshire mean that the 0.5 isocline length will probably represent the opportunity for hybridization fairly well. The probability of hybridization is expected to vary with the proportions of the two species at any one locality, reflecting the chance that different species meet and mate. Although the absolute number of hybrids generated might be greater where both species are equally frequent, the probability of an individual being involved in an interspecific encounter is expected to increase as its species becomes relatively rarer. If this probability function is known, it can be incorporated into the spatial model to provide more realistic predictions of expected hybridization rates. The approach we have used for *Tegenaria* is both simple and general, and might be applicable to many other situations.

The finding of the GIS analysis that barriers to gene flow between the *Tegenaria* species are stronger in the south is fully endorsed by the detailed morphometric analyses. The effect of hybridization on morphology appears to be asymmetrical in both study areas. Samples of male *T. saeva* from the parapatric zone in southern England exhibit increased levels of variation and

distributions that are skewed toward *T. gigantea* both in the regression analyses of palpal TCL versus PROL, and in the multivariate analyses. Sympatric samples from the York area also show increased variances and shifted distributions in both analyses with, again, *T. saeva* (and *T. saeva*-like intermediates) being most affected (Tables 1 and 2 and Figs. 6, 7A, and 7B). The multivariate data for females show the same trends (Table 2 and Figs. 7C and 7D). The multivariate analyses employed MGPCA, which means that the variables entering the discriminant analysis are uncorrelated. Although not essential for DA, orthogonal variables generated in this way do allow an examination of the effects of size per se and ensure that the variables represent largely independent genetic traits. The analyses suggest that intermediate discriminant scores do represent the results of hybridization, which is consistent with the placement of known F_1 progeny.

The conclusion that hybridization is asymmetrical, with *T. saeva* experiencing greater introgression of *T. gigantea* genes than vice versa, is further supported by an analysis of allozyme markers (Croucher 1998). Croucher et al. (2004) also demonstrated the occurrence in southern England of mitochondrial gene sequences attributable to *T. gigantea* in *T. saeva* individuals, but not the reverse. Asymmetrical introgression has been reported in a number of other organisms, for example, house mice (Ferris et al. 1983), cave-dwelling crayfish (Cesaroni et al. 1992), salamanders (Mead et al. 2001), and carabid beetles (Sota et al. 2001), and the extent to which different markers show asymmetric gene flow can vary (e.g., Sætre et al. 2003; Bettles et al. 2005; Johannesen et al. 2005). In the pied and collared flycatchers, more *F. hypoleuca* genes flow to *F. albicollis* than vice versa, especially on the Swedish islands (Borge et al. 2005). Borge et al. (2005) speculate that this might be a result of enhanced gene flow from migrating allopatric *F. hypoleuca* individuals in which adaptations to avoid hybridization have not evolved, and/or the numerical dominance of this species. In *Tegenaria* this is not the case, and Croucher (1998) has proposed that asymmetric hybridization probably stems, at least in part, from stronger mechanical barriers to copulation between male *T. gigantea* and female *T. saeva* than in the reciprocal cross (for a parallel see Sota et al. 2001).

The results from the present study have shown that the degree of hybridization between the two *Tegenaria* species is far greater in the area of recent sympatry that at an older parapatric boundary. This parallels the case of the pied and collared flycatchers where hybridization, and introgression, is much higher in the more recently established populations on Gotland and Öland (Borge et al. 2005). That isolating mechanisms exist between *T. saeva* and *T. gigantea* is indicated by the low incidence of hybridization along the southern England zone of parapatry reported here. In light of the narrowness and apparent stability of this parapatric boundary we have suggested previously (Croucher 1998; Croucher et al. 2004) that this constitutes a "tension zone" (Barton and Hewitt 1985) in which hybrids experience reduced fitness. Support for the tension zone hypothesis comes from the observation that laboratory-generated hybrids between allopatric parents have markedly elevated mortality rates compared with progeny from intraspecific matings reared under identical conditions (Croucher 1998). As a result of this selection against hybrids, we might expect to find evidence of character displacement in reproductive structures within the parapatric zone. In fact, intraspecific sexual morphology was no different between spiders from deep allopatry and those from parapatry. This does not necessarily mean that reinforcement has not occurred-–we have not investigated more subtle reproductive cues such as web or cuticular pheromones, which may be very important in spider courtship (Suter et al. 1987; Trabalon et al. 1997), and Lukhtanov et al. (2005) found that genitalia morphology was not involved in the reinforcement process in *Agrodiaetus* butterflies. Indeed, the results from some simulation models suggest that reproductive character displacement may not be a necessary requirement for reinforcement to occur (Lemmon et al. 2004; see also Servedio and Noor 2003).

In summary, the recent range expansions of both *T. saeva* and *T. gigantea* across the north of England has produced broadly sympatric distributions within which hybridization is shown to be relatively, and absolutely, much more common than within a more ancient, narrow parapatric zone in the south of England. Spatial and temporal differences in species interactions in the two areas may be responsible for this contrast. In southern England, a relatively slow meeting of allopatric populations during a period of little or no human-mediated dispersal might have allowed time for species barriers to develop. The relatively linear nature of the parapatric zone, and selection against hybrids, may mean that here the rate of F_1 production, and subsequent backcrossing is too low to compromise species integrity. Despite evidence of selection against hybrids there is no indication of reinforcement involving morphological characters in the zone of parapatry. Although the origins of spiders in the recently colonized north of England are unknown, it is more likely the populations derived from more southerly allopatric areas than from the relatively narrow parapatric zone. The increased sympatry generated during colonization, and the vagaries of species dispersal into unoccupied areas (Ibrahim et al. 1996), may have enhanced the frequency of interspecific matings and allowed any viable and fertile F_1 hybrids to have a greater chance of meeting and generating segregating F_2 individuals. Some of these are expected to be morphologically (and/or pheromonally) similar to one or other parent and thus able to back-cross more easily with them. If the reproductive characters studied here are inherited in an additive way, the more gene flow there is between species the easier future gene flow becomes (Barton and Hewitt 1985).

In Yorkshire, therefore, we may be witnessing the beginnings of a hybrid swarm (e.g., Bettles et al. 2005; Taylor et al. 2006)

and possibly the ultimate fusion of hitherto separate species. If this prediction is true, it will create a geographical pattern of introgression reminiscent of a ring species (Irwin et al. 2001, 2005; Alexandrino et al. 2005) with a single "hybrid" species in the north and two effectively reproductively isolated species in the south. Differences from a classical ring species would be that (1) the geographically defined, species-free center of the ring does not exist, and (2), the evolutionary processes generating the observed spatial patterns of gene exchange are reversed, with reproductive barriers breaking down in areas of recent sympatry, rather than accentuated. The patterns seen within *Tegenaria* parallel, to a large extent, those demonstrated in flycatchers (Alatalo et al. 1990; Sætre et al. 1997; Borge et al. 2005).

Comparisons of interactions between taxa in ancient parapatry and in recent sympatry provide a novel angle on the study of speciation. Such opportunities are likely to become more common as species distributions alter as a result of global climate change (Rhymer and Simberloff 1996). Under these circumstances species interactions may change much faster than normal evolutionary time scales, and enable the processes to be followed, and analyzed, in real time. In other words, it may be easier to elucidate the elements underlying reproductive isolation of species by examining reasons for its breakdown in certain geographic areas, than through its original buildup.

ACKNOWLEDGMENTS

We are indebted to members of the British Arachnological Society, members of the general public, those who provided spider depositories, the BBC, and local newspapers in York, Dorset, and Hampshire, who helped with the publicity of surveys and the collecting of spiders across our two main study areas. We thank J. E. Dalingwater, P. Smithers, the late S. P. Hopkins, and Spider Recording Scheme County Organizers for further specimens, records, and correspondence. We are most grateful to P. T. Roberts and S. Sparrow for modifying Figures 3–5. Figures 1, 4A, and 5A were drawn using DMAP software (A. Morton). T. J. Crawford and M. H. Williamson provided valuable comments and information, and T. Lu, W. O. McMillan, and two anonymous referees made helpful suggestions, which have led to significant improvements. This paper contains maps based on copyright digital map data owned and supplied by HarperCollinsCartographic, and are used here with permission.

LITERATURE CITED

Alatalo, R. V., D. Eriksson, L. Gustafsson, and A. Lundberg. 1990. Hybridization between pied and collared flycatchers—sexual selection and speciation theory. J. Evol. Biol. 3:375–389.

Albert, M. E., C. M. D'Antonio, and K. A. Schierenbeck. 1997. Hybridization and introgression in *Carpobrotus* spp. (Aizoaceae) in California. 1. Morphological evidence. Am. J. Bot. 84:896–904.

Alexandrino, J., S. J. E. Baird, L. Lawson, J. R. Macey, C. Moritz, and D. B. Wake. 2005. Strong selection against hybrids at a hybrid zone in the *Ensatina* ring species complex and its evolutionary implications. Evolution 59:1334–1347.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard Univ. Press, Cambridge MA.

Barton, N. H., and K. S. Gale. 1993. Genetic analysis of hybrid zones. Pp. 13–45 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, NY.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. 16:133–164.

Beaumont, M., E. M. Barratt, D. Gottelli, A. C. Kitchener, M. J. Daniels, J. K. Pritchard, and M. W. Bruford. 2001. Genetic diversity and introgression in the Scottish wildcat. Mol. Ecol. 10:319–336.

Bettles, C. M., M. F. Docker, B. Dufour, and D. D. Heath. 2005. Hybridization dynamics between sympatric species of trout: loss of reproductive isolation. J. Evol. Biol. 18:1220–1233.

Borge, T., K. Lindroos, P. Nádvorník, A.-C. Syvänen, and G.-P. Sætre. 2005. Amount of introgression in flycatcher hybrid zones reflects regional differences in pre- and post-zygotic barriers to gene exchange. J. Evol. Biol. 18:1416–1424.

Boursot, P., J.-C. Auffray, J. Britton-Davidian, and F. Bonhomme. 1993. The evolution of house mice. Annu. Rev. Ecol. Syst. 24:119–152.

Bristowe, W. S. 1951. An introductory chapter on British arachnologists and their work. Pp. 1–14 *in* G. H. Locket, and A. F. Millidge, eds. British spiders Vol.I. Ray Society, London.

Burrough, P. A., and R. A McDonnell. 1998. Principles of geographic information systems. Oxford Univ. Press, Oxford, U.K.

Butlin, R. 1998. What do hybrid zones in general, and the *Chorthippus parallelus* zone in particular, tell us about speciation? Pp. 367–378 *in* D. J. Howard, and S. H. Berlocher, eds. Endless forms: species and speciation. Oxford Univ. Press, NY.

Cesaroni, D., G. Allegrucci, and V. Sbordoni. 1992. A narrow hybrid zone between two crayfish species from a Mexican cave. J. Evol. Biol. 5:643–659.

Cox, G. W. 2004. Alien species and evolution. Island Press, Washington, DC.

Croucher, P. J. P. 1998. Evolutionary interactions of two colonizing species of large house spider (Araneae: *Tegenaria* spp.)—testing the reinforcement hypothesis. DPhil thesis, Univ. of York, York, UK.

Croucher, P. J. P., G. S. Oxford, and J. B. Searle. 2004. Mitochondrial differentiation, introgression and phylogeny of species in the *Tegenaria atrica* group (Araneae, Agelenidae). Biol. J. Linn. Soc. 81:79–89.

Ellstrand, N. C. 2005. Dangerous liaisons? When cultivated plants mate with their wild relatives. The Johns Hopkins Univ. Press, Baltimore, MD.

Epperson, B. K. 2003. Geographical genetics. Princeton Univ. Press, Princeton, NJ.

Ferris, S. D., R. D. Sage, C-M. Huang, J. T. Nielsen, U. Ritte, and A. C. Wilson. 1983. Flow of mitochondrial DNA across a species boundary. Proc. Natl. Acad. Sci. USA 80:2290–2294.

Harrison, R. G., D. M. Rand, and W. C. Wheeler. 1987. Mitochondrial DNA variation in field crickets across a narrow hybrid zone. Mol. Biol. Evol. 4:144–158.

Harvey, P. R., D. R. Nellist, and M. G. Telfer. (eds). 2002. Provisional atlas of British spiders (Arachnida, Araneae). Vol. 2. Biological Records Centre, Huntingdon, UK.

Hewitt, G. M. 1999. Post-glacial re-colonization of European biota. Biol. J. Linn. Soc. 68:87–112.

Howard, D. J. 1993. Reinforcement: origins, dynamics, and fate of an evolutionary hypothesis. Pp. 46–69 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, NY.

Ibrahim, K.M., R. A. Nichols, and G. M. Hewitt. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Heredity 77:282–291.

Irwin, D. E., S. Bensch, J. H. Irwin, and T. D. Price. 2005. Speciation by distance in a ring species. Science 307:414–416.

Irwin, D. E., J. H. Irwin, and T. D. Price. 2001. Ring species as bridges between microevolution and speciation. Genetica 112–113:223–234.

Johannesen, J., B. Johannesen, E. M. Griebeler, I. Baran, M. R. Tunç, A. Kiefer, and M. Veith. 2005. Distortion of symmetrical introgression in a hybrid zone: evidence for locus-specific selection and uni-directional range expansion. J. Evol. Biol. 19:705–716.

Jones, R. M. 2005. The Oxford-Hermitage common shrew hybrid zone. Ph.D. thesis, Univ. of York, York, U.K.

Jones, R. M., and J. B. Searle. 2003. Mapping the course of the Oxford-Hermitage chromosomal hybrid zone in the common shrew *Sorex araneus*—a GIS approach. Mammalia 67:193–200.

Kidd, D. M., and M. G. Ritchie. 2000. Inferring the patterns and causes of geographic variation in *Ephippiger ephippiger* (Orthoptera, Tettigoniidae) using geographic information systems (GIS). Biol. J. Linn. Soc. 71:269–295.

Lamont, B. B., T. He, N. J. Enright, S. L. Krauss, and B. P. Miller. 2003. Anthropogenic disturbance promotes hybridization between *Banksia* species by altering their biology. J. Evol. Biol. 16:551–557.

Lemmon, A. R., C. Smadja, and M. Kirkpatrick. 2004. Reproductive character displacement is not the only possible outcome of reinforcement. J. Evol. Biol. 17:177–183.

Locket, G. H. 1975. The identity of Blackwall's *Tegenaria saeva* (Araneae, Agelenidae). Bull. Br. Arachnol. Soc. 3:85–90.

Lukhtanov, V. A., N. P. Kandul, J. B. Plotkin, A. V. Dantchenko, D. Haig, and N. E. Pierce. 2005. Reinforcement of pre-zygotic isolation and karyotype evolution in *Agrodiaetus* butterflies. Nature 436:385–389.

Lynch, J. M., and T. J. Hayden. 1995. Genetic influences on cranial form: variation among ranch and feral American mink *Mustela vison* (Mammalia: Mustelidae). Biol. J. Linn. Soc. 55:293–307.

Manel, S., M. K. Schwartz, G. Luikart, and P. Tarberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends. Ecol. Evol. 18:189–197.

Maurer, R. 1992. Checkliste der Europäischen Agelenidae nach der Roewerschen Systematik 1954 – unter Berücksichtigung Angrenzender Östlicher Gebiete 1: Listen. Privately printed.

Mead, L. S., S. G. Tilley, and L. A. Katz. 2001. Genetic structure of the Blue Ridge dusky salamander (*Desmognathus orestes*): inferences from allozymes, mitochondrial DNA, and behavior. Evolution 55:2287–2302.

Merrett, P. 1980. Notes on the variation, identification and distribution of British species of the *Tegenaria atrica* group (Araneae, Agelenidae). Bull. Br. Arachnol. Soc. 5:1–8.

Nikolić, F., and A. Polenec. 1981. Catalogus faunae Jugoslaviae. Ljubljana, Yugoslavia.

Nolte, A. W., J. Freyhof, and D. Tautz. 2006. When invaders meet locally adapted types: rapid moulding of hybrid zones between sculpins (*Cottus*, Pisces) in the Rhine system. Mol. Ecol. 15:1983–1993.

Noor, M. A. F. 1999. Reinforcement and other consequences of sympatry. Heredity 83:503–508.

Overton, J. L., D. J. Macintosh, and R. S. Thorpe. 1997. Multivariate analysis of the mud crab *Scylla serrata* (Brachyura: Portunidae) from four locations in Southeast Asia. Mar. Biol. 128:55–62.

Oxford, G. S., and H. C. G. Chesney. 1994. Large house spiders *Tegenaria* spp. in Northern Ireland: previously overlooked species or recent introductions? Ir. Nat. J. 24:354–357.

Oxford, G. S., and P. J. P. Croucher. 1997. Gone fishing . . . for *Tegenaria*. Newsl. Br. Arachnol. Soc. 78:9–10.

Oxford, G. S., and A. Plowman. 1991. Do large house spiders *Tegenaria gigantea* and *T. saeva* (Araneae, Agelenidae) hybridise in the wild?—A multivariate approach. Bull. Br. Arachnol. Soc. 8:293–296.

Oxford, G. S., and C. J. Smith. 1987. The distribution of *Tegenaria gigantea* Chamberlain and Ivie, 1935 and *T. saeva* Blackwall, 1844 (Araneae, Agelenidae) in Yorkshire. Bull. Br. Arachnol. Soc. 7:123–127.

Parker, J. R. 1984. Synanthropic spiders and other things—Part 2. Newsl. Br. Arachnol. Soc. 39:1–2.

Pesarini, C. 1994. Arachnida Araneae. Pp.1–42 *in* A. Minelli, S. Rufo, and S. LaPosta, eds. Checklist delle specie della fauna Italiana, 23. Calderini, Bologna, Italy.

Prenter, J., W. I. Montgomery, and R. W Elwood. 1995. Multivariate morphometrics and sexual dimorphism in the orb-web spider *Metellina segmentata* (Clerck, 1757) (Araneae, Metidae). Biol. J. Linn. Soc. 55:345–354.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. Annu. Rev. Ecol. Syst. 27:83–109.

Ritchie, M. G., D. M. Kidd, and J. M. Gleason. 2001. Mitochondrial DNA variation and GIS analysis confirm a secondary origin of geographical variation in the bushcricket *Ephippiger ephippiger* (Orthoptera: Tettigonioidea), and resurrect two species. Mol. Ecol. 10:603–611.

Roberts, M. J. 1985. The spiders of Great Britain and Ireland, Vol.1. Harley Books, Colchester, U.K.

Roberts, M. J. 1995. Spiders of Great Britain and Northern Europe. Harper-Collins, London.

Rubidge, E., P. Corbett, and E.B. Taylor. 2001. A molecular analysis of hybridization between native westslope cutthroat trout and introduced rainbow trout in southeastern British Columbia, Canada. J. Fish Biol. 59(Suppl. A):42–54.

Sætre, G.-P., T. Borge, K. Lindroos, J. Haavie, B. C. Sheldon, C. Primmer, and A.-C. Syvänen. 2003. Sex chromosome evolution and speciation in *Ficedula* flycatchers. Proc. R. Soc. Lond. B. 270:53–59.

Sætre, G.-P., M. Kral, S. Bures, and R. A. Ims. 1997. Dynamics of a clinal hybrid zone and a comparison with island hybrid zones of flycatchers (*Ficedula hypoleuca* and *F. albicollis*). Nature 387:589–592.

Secondi, J., B. Faivre, and S. Bensch. 2006. Spreading introgression in the wake of a moving contact zone. Mol. Ecol. 15:2463–2475.

Servedio, M. R., and M. A. F. Noor. 2003. The role of reinforcenment in speciation: theory and data. Annu. Rev. Ecol. Syst. 34:339–364.

Smith, C. J. 1985. The distribution of the larger *Tegenaria* spider species (Agelenidae) in the York area. Newsl. Br. Arachnol. Soc. 42:5–6.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research, 3rd ed. WH Freeman and Company, NY.

Sota, T., R. Ishikawa, M. Ujiie, F. Kusumoto, and A. P. Vogler. 2001. Extensive trans-species mitochondrial polymorphism in the carabid beetles *Carabus* subgenus *Ohomopterus* caused by repeated introgressive hybridization. Mol. Ecol. 10:2833–2847.

Storfer, A., M. A. Murphy, J. S. Evans, C. S. Goldberg, S. Robinson, S. F. Spear, R. Dezzani, E. Delmelle, L. Vierling, and L. P. Waits. 2007. Putting the 'landscape' in landscape genetics. Heredity 98:128–142.

Suter, R. B., C. M. Shane, and A. J. Hirscheimer. 1987. Communication by cuticular pheromones in a linyphiid spider. J. Arachnol. 15:157–162.

Swenson, N. G., and D. J. Howard. 2004. Do suture zones exist? Evolution 58:2391–2397.

———. 2005. Clustering of contact zones, hybrid zones, and phylogenetic breaks in North America. Am. Nat. 166:581–591.

Szymura, J. M. 1993. Analysis of hybrid zones with *Bombina*. Pp. 261–289 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, NY.

Szymura, J. M., and Barton, N. H. 1991. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. varigata*: comparisons between transects and between loci. Evolution 45:237–261.

Taberlet, P., L. Fumagalli, A. G. Wust-Saucy, and J. F. Cosson. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Mol. Ecol. 7:453–464.

Taylor, E. B., J. W. Boughman, M. Groenenboom, M. Sniatynski, D. Schulter, and J. L. Gow. 2006. Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. Mol. Ecol. 15:343–355.

Thorpe, R. S. 1988. Multiple group principal component analysis and population differentiation. J. Zool. Lond. 216:37–40.

Trabalon, M., A.-G. Bagnères, and C. Roland. 1997. Contact sex signals in two sympatric spider species, *Tegenaria domestica* and *Tegenaria pagana*. J. Chem. Ecol. 23:747–758.

Associate Editor: W. O. McMillan

Appendix

Table A1. Measurements, principal components, and DF coefficients: Males. Data given for the training samples from southern England: allopatric *T. saeva* (Allo *Ts*) (*N* = 32); allopatric *T. gigantea* (Allo *Tg*) (*N* = 33). Means and standard deviations (SD) are shown for each character listed in order of decreasing order of discriminatory power in univariate ANOVA (*WGV*, within-group variation; *BGV*, between-group variation). Principal components (PC) and their coefficients are shown in order of decreasing canonical correlation (*Corr.*) with the DF. The corresponding discriminate function coefficient (*DFC*) to be applied to each PC is given with the respective PC. DF constant = −73.74. Results from univariate ANOVA on each PC are provided at the base of the table. n.s., not significant; **P*<0.05; ***P*< 0.01; ****P*<0.001; *****P*<0.0001. Probabilities not significant after correction for multiple testing are in parentheses.

						Principal Component						
						PC5	PC10	PC7	PC16	PC8	PC2	PC6
					DFC→	+20.52	+4.13	−14.47	+9.38	+2.89	−6.13	−2.77
					Corr.→	+0.54	+0.37	−0.36	+0.31	+0.23	−0.18	−0.16
Character	*Allo Ts Mean(SD)*	*Allo Tg Mean(SD)*	*WGV*	*BGV*	*P*							
CW	0.04 (0.01)	0.11 (0.01)	0.06	0.94	****	−0.12	−0.25	+0.48	+0.02	−0.43	+0.60	+0.27
TW	0.59 (0.04)	0.33 (0.04)	0.07	0.93	****	+0.50	+0.23	−0.10	+0.14	−0.08	+0.16	−0.18
CL	0.17 (0.03)	0.27 (0.02)	0.19	0.81	****	−0.57	+0.14	−0.45	−0.03	+0.23	+0.54	−0.15
TWMX	0.39 (0.04)	0.28 (0.03)	0.30	0.70	****	+0.33	−0.05	−0.11	+0.03	+0.32	+0.26	+0.12
TCL	1.32 (0.07)	1.16 (0.05)	0.38	0.62	****	+0.15	−0.01	+0.01	−0.00	−0.01	−0.07	+0.01
ETAWD	0.14 (0.01)	0.10 (0.01)	0.38	0.62	****	+0.21	+0.39	−0.19	+0.04	+0.04	+0.13	+0.65
ETAWP	0.10 (0.02)	0.07 (0.01)	0.47	0.53	****	+0.38	−0.23	−0.07	−0.06	+0.13	+0.32	−0.46
TIBWD	0.85 (0.07)	0.75 (0.06)	0.65	0.35	****	−0.05	+0.04	−0.06	+0.39	−0.11	−0.08	+0.00
DTA	0.47 (0.03)	0.51 (0.03)	0.78	0.22	****	−0.02	+0.32	−0.02	−0.12	−0.12	−0.02	+0.12
TARLC	1.02 (0.17)	1.19 (0.16)	0.78	0.22	****	−0.12	−0.27	+0.18	+0.13	+0.53	−0.11	+0.25
TARL	2.45 (0.29)	2.62 (0.25)	0.91	0.09	(*)	−0.09	−0.02	+0.06	−0.44	+0.23	−0.08	+0.09
TLV	0.89 (0.08)	0.86 (0.05)	0.93	0.07	(*)	−0.01	−0.04	−0.03	+0.17	+0.07	+0.05	−0.09
PROW	4.24 (0.60)	4.50 (0.56)	0.95	0.05	n.s.	−0.14	+0.08	−0.10	−0.15	−0.26	−0.11	−0.16
ETAL	0.26 (0.03)	0.27 (0.02)	0.95	0.05	n.s.	−0.11	+0.50	+0.65	+0.06	+0.26	+0.02	−0.30
TWMN	0.27 (0.03)	0.28 (0.02)	0.96	0.04	n.s.	+0.07	−0.07	+0.08	+0.06	−0.13	+0.15	+0.01
TWV	1.02 (0.08)	0.99 (0.06)	0.96	0.04	n.s.	+0.10	−0.00	+0.03	−0.69	−0.11	−0.01	−0.01
PROL	6.11 (0.86)	6.32 (0.81)	0.98	0.02	n.s.	−0.10	+0.17	−0.16	+0.06	−0.29	−0.12	−0.09
TARWC	0.58 (0.08)	0.59 (0.06)	0.99	0.01	n.s.	−0.07	−0.39	−0.08	+0.07	−0.06	−0.22	+0.06
TIBWP	0.44 (0.06)	0.44 (0.05)	1.00	0.00	n.s.	−0.08	−0.08	−0.01	+0.17	−0.13	−0.07	−0.09
COPL	1.21 (0.09)	1.20 (0.08)	1.00	0.00	n.s.	−0.08	+0.17	−0.02	+0.16	−0.02	−0.06	−0.02
WGV						0.05	0.11	0.11	0.14	0.24	0.33	0.39
BGV						0.95	0.89	0.89	0.86	0.76	0.67	0.61
P						****	****	****	****	****	****	****

Continued

Table A1. Continued

	Principal Component												
DFC→	PC12	PC14	PC19	PC17	PC18	PC20	PC13	PC11	PC15	PC9	PC3	PC4	PC1
Corr.→	+3.84	+1.92	+12.67	−24.46	−14.77	+19.22	−9.22	−7.63	−14.44	+7.61	+48.47	−42.97	+3.05
Character	+0.15	−0.14	+0.14	−0.12	+0.10	+0.08	+0.06	−0.05	−0.04	+0.04	−0.03	+0.02	+0.01
CW	−0.16	+0.10	+0.02	+0.08	+0.01	−0.02	+0.02	−0.12	−0.04	−0.10	+0.03	−0.02	+0.10
TW	−0.52	−0.22	+0.05	+0.01	−0.13	+0.01	+0.14	−0.29	+0.01	+0.22	+0.15	+0.30	+0.10
CL	−0.04	−0.05	−0.02	−0.09	+0.01	+0.02	+0.03	−0.11	−0.00	+0.18	+0.14	−0.02	+0.04
TWMX	+0.36	+0.35	+0.01	+0.10	+0.07	−0.04	−0.23	−0.05	−0.13	−0.24	+0.31	+0.44	+0.08
TCL	+0.00	+0.01	−0.01	−0.01	−0.00	+0.01	−0.01	−0.01	+0.00	+0.01	+0.76	−0.62	+0.14
ETAWD	+0.22	−0.17	+0.00	−0.07	−0.07	−0.02	+0.10	−0.09	−0.11	+0.04	−0.29	−0.27	+0.22
ETAWP	+0.03	+0.09	−0.03	+0.02	+0.09	+0.00	−0.14	+0.10	+0.02	−0.00	−0.42	−0.40	+0.28
TIBWD	−0.07	+0.11	+0.49	−0.42	+0.54	+0.24	−0.04	+0.06	−0.05	−0.05	−0.01	+0.03	+0.20
DTA	−0.14	+0.48	+0.14	+0.23	−0.01	−0.08	−0.27	+0.24	+0.50	+0.36	−0.02	+0.01	+0.13
TARLC	−0.38	−0.03	−0.18	−0.28	−0.12	−0.23	−0.07	+0.07	+0.18	−0.02	−0.01	+0.06	+0.38
TARL	−0.23	−0.16	+0.32	+0.41	+0.02	+0.50	−0.02	+0.06	−0.20	−0.12	−0.00	+0.04	+0.27
TLV	+0.13	+0.14	+0.28	+0.20	−0.18	−0.16	+0.77	+0.30	+0.12	−0.11	+0.03	+0.04	+0.16
PROW	+0.07	−0.21	+0.35	−0.01	−0.20	−0.57	−0.29	−0.04	−0.16	−0.22	+0.02	+0.07	+0.36
ETAL	+0.23	+0.05	−0.01	−0.08	−0.03	+0.01	+0.03	−0.15	−0.17	+0.16	−0.01	−0.01	+0.13
TWMN	+0.23	−0.45	−0.12	−0.08	−0.02	+0.13	−0.13	+0.65	−0.04	+0.35	+0.15	+0.22	+0.15
TWV	+0.10	+0.03	−0.14	−0.41	+0.32	−0.08	+0.32	−0.08	+0.17	+0.05	+0.07	+0.15	+0.16
PROL	−0.13	+0.38	−0.46	−0.13	−0.25	+0.27	+0.05	+0.16	−0.31	−0.20	−0.02	+0.07	+0.36
TARWC	+0.18	+0.17	+0.01	+0.19	+0.05	−0.06	+0.09	−0.32	−0.30	+0.64	−0.03	+0.05	+0.25
TIBWP	+0.34	−0.22	−0.09	+0.07	−0.14	+0.34	−0.05	−0.37	+0.59	−0.15	+0.01	+0.08	+0.32
COPL	−0.09	−0.18	−0.39	+0.48	+0.63	−0.25	+0.07	+0.03	+0.00	−0.14	+0.01	+0.02	+0.17
WGV	0.44	0.44	0.47	0.52	0.62	0.72	0.84	0.85	0.90	0.93	0.95	0.98	1.00
BGV	0.56	0.56	0.53	0.48	0.38	0.28	0.16	0.15	0.10	0.07	0.05	0.02	0.00
P	****	****	****	****	****	****	(**)	(**)	(*)	(*)	n.s.	n.s.	n.s.

Table A2. Measurements, principal components, and discriminant function coefficients: Females. Data given for the training samples from southern England: allopatric *T. saeva* (Allo *Ts*) (*N* = 34); allopatric *T. gigantea* (Allo *Tg*) (*N* = 40). DF constant = −81.58. Otherwise, see legend to Table A1.

						Principal Component						
						PC11	PC2	PC5	PC8	PC7	PC3	PC13
					DFC→	+20.75	−3.43	−5.14	−6.83	+4.95	+2.76	+14.56
					Corr.→	+0.53	−0.37	−0.33	−0.29	+0.29	+0.29	+0.25
Character	*Allo Ts Mean(SD)*	*Allo Tg Mean(SD)*	*WGV*	*BGV*	*P*							
APR	0.59 (0.06)	0.42 (0.04)	0.29	0.71	****	+0.12	+0.08	−0.36	−0.23	+0.41	+0.17	+0.28
AAEPI	0.84 (0.10)	0.61 (0.05)	0.32	0.68	****	+0.31	+0.07	−0.12	−0.39	+0.36	+0.16	−0.29
RLW	0.14 (0.03)	0.21 (0.02)	0.34	0.66	****	−0.06	+0.37	+0.68	+0.22	+0.35	+0.10	−0.08
EPIL	0.90 (0.07)	0.73 (0.05)	0.36	0.64	****	+0.07	+0.05	−0.07	−0.21	+0.25	+0.17	−0.02
RWMN	0.34 (0.08)	0.19 (0.04)	0.36	0.64	****	+0.03	−0.74	+0.24	−0.08	−0.07	+0.52	+0.01
RWMX	0.60 (0.05)	0.50 (0.05)	0.53	0.47	****	+0.10	−0.10	+0.34	−0.05	+0.21	+0.19	+0.29
DA	0.30 (0.03)	0.35 (0.04)	0.64	0.36	****	−0.12	+0.12	+0.15	+0.02	+0.08	+0.19	−0.46
STER1	0.64 (0.13)	0.77 (0.11)	0.78	0.22	****	−0.01	+0.36	−0.00	−0.39	−0.47	+0.44	−0.08
AWA	0.22 (0.04)	0.19 (0.04)	0.81	0.19	(***)	+0.02	+0.01	−0.31	+0.33	+0.31	+0.21	−0.31
APEPI	0.08 (0.04)	0.13 (0.06)	0.81	0.19	(***)	+0.01	−0.08	−0.07	−0.00	+0.02	−0.15	+0.00
AWB	0.57 (0.07)	0.51 (0.08)	0.83	0.17	(***)	+0.06	+0.04	−0.26	+0.55	−0.19	+0.38	−0.19
PROW	4.30 (0.42)	4.47 (0.32)	0.95	0.05	(*)	+0.33	+0.17	−0.01	+0.25	−0.03	+0.17	+0.51
PROL	6.25 (0.60)	6.43 (0.47)	0.97	0.03	n.s.	+0.28	+0.18	−0.02	+0.20	−0.04	+0.20	+0.23
STER2	0.33 (0.05)	0.33 (0.07)	0.99	0.01	n.s.	−0.01	+0.27	+0.05	−0.19	−0.27	+0.14	+0.01
EPIWMX	0.83 (0.08)	0.83 (0.08)	1.00	0.00	n.s.	−0.82	+0.08	−0.16	−0.01	+0.18	+0.26	+0.30
WGV						0.23	0.38	0.44	0.50	0.50	0.51	0.59
BGV						0.77	0.62	0.56	0.50	0.50	0.49	0.41
P						****	****	****	****	****	****	****

Continued

Table A2. Continued.

	Principal Component							
DFC→	PC14	PC1	PC4	PC10	PC12	PC9	PC6	PC15
Corr.→	−16.03	−1.00	+1.56	−6.24	−6.56	−0.61	+0.92	+0.96
Character	−0.23	−0.18	+0.14	+0.14	+0.13	−0.04	+0.03	+0.01
APR	+0.10	−0.05	+0.00	+0.02	−0.70	+0.04	+0.09	−0.09
AAEPI	+0.40	−0.02	+0.08	−0.08	+0.46	−0.22	+0.25	−0.01
RLW	−0.01	+0.05	−0.02	−0.23	−0.22	−0.34	+0.01	−0.01
EPIL	−0.87	+0.08	+0.04	−0.05	+0.17	+0.03	+0.13	+0.19
RWMN	+0.03	+0.04	+0.08	−0.29	−0.11	−0.03	−0.04	−0.01
RWMX	+0.03	+0.03	−0.02	+0.81	+0.16	+0.08	−0.11	−0.06
DA	+0.11	+0.09	−0.05	+0.04	−0.10	+0.78	+0.20	+0.10
STER1	+0.01	+0.09	−0.46	+0.06	−0.08	−0.16	−0.21	+0.04
AWA	+0.00	+0.04	+0.02	+0.03	+0.06	−0.03	−0.74	+0.07
APEPI	+0.05	+0.98	−0.02	+0.02	−0.05	−0.08	+0.04	−0.03
AWB	−0.04	−0.00	+0.08	+0.28	−0.08	−0.29	+0.48	+0.01
PROW	+0.18	+0.07	−0.03	−0.23	+0.17	+0.19	+0.00	+0.60
PROL	−0.10	+0.06	−0.03	−0.23	+0.22	+0.25	−0.03	−0.76
STER2	+0.02	+0.08	+0.87	+0.04	−0.07	+0.02	−0.15	+0.01
EPIWMX	+0.11	+0.05	+0.02	−0.08	+0.27	−0.01	+0.09	−0.05
WGV	0.63	0.73	0.81	0.82	0.83	0.98	0.99	1.00
BGV	0.37	0.27	0.19	0.18	0.17	0.02	0.01	0.00
P	****	****	(***)	(***)	(***)	n.s.	n.s.	n.s.