J. evol. Biol. 7: 697–712 (1994)

1010-061X/94/060697-16 $ 1.50 + 0.20/0

Morphometric variation in a hybrid zone of two subspecies of *Gerris costae* (Heteroptera: Gerridae) in the Maritime Alps

Carlo Rodolfo Largiadèr,[1]* Christian Peter Klingenberg[2] and
Manfred Zimmermann[1]

[1] *Department of Population Biology, Institute of Zoology, University of Berne, Baltzerstrasse 3, CH-3012 Berne, Switzerland*
[2] *Department of Entomology, University of Alberta, Edmonton, Alta. T6G 2E3, Canada*

Key words: Canonical trend surface analysis; Hemiptera; geographic variation; clines; rearing experiments; waterstrider; pondskater.

Abstract

Two morphologically distinct subspecies of *Gerris costae* form a contact zone extending from the Maritime to the Western Alps. Within this area canonical trend surface analysis revealed a geographic pattern of morphometric variation consistent with topography. From North to South, and from high to low elevation there was a transition from *G. c. costae*-like phenotypes to phenotypes resembling pure *G. c. fieberi*. The same pattern was confirmed with canonical variate analysis not taking geographic location into account; it is therefore not an artifact of trend surface analysis.

Comparisons of the pattern of morphometric variation of laboratory-reared offspring with the pattern of their parents sampled from natural populations show that geographic variation is mostly determined genetically. Intermediate individuals from field populations probably are natural hybrids between the two subspecies, because laboratory-reared hybrids were intermediate between the offspring of pure strains.

We did not find increased morphometric variation within the contact zone. This suggests unimpeded introgression and is in contrast with an increase in size

---

* Author for correspondence.

variability that is predicted to be associated with a transition between uni- and bivoltine forms.

Introduction

Many animal and plant species are subdivided into genetically and morphologically distinct groups, which can be recognized as races or subspecies. Many such groups are thought to have adapted to different ecological conditions through different selection regimes acting on geographically separated populations. In zones of secondary contact between these groups, hybridization and introgression provide excellent opportunities to study evolutionary processes (Barton and Hewitt, 1985 and 1989; Hewitt 1988; Harrison, 1990).

Two subspecies of the palaeartic waterstrider *Gerris costae* HERRICH-SCHAEFFER, 1853, *G. c. costae* and *G. c. fieberi*, occur in close geographic proximity in the Western Alps and the Apennines (Wagner and Zimmermann, 1955; Servadei, 1967; Nieser, 1978; Tamanini, 1979; Klingenberg, 1992). They differ in their ecological characteristics: *G. c. costae* is confined to high altitudes (above 800 m) in the Alps, whereas *G. c. fieberi* is also found in the lowlands of Italy, south-eastern Europe and Asia Minor. According to our observations, *G. c. costae* is found exclusively in lentic habitats whereas the habitats of *G. c. fieberi* are very diverse. In the Italian lowlands we found *G. c. fieberi* on stagnant or slowly flowing water in marshes and river basins in accordance with reports from other areas (Hoberlandt, 1948; Zimmermann, 1982; Klingenberg, 1992). At high elevations (above 800 m), however, *G. c. fieberi* reproduces even on very small puddles, e.g., in truck or cattle tracks.

In this study, we investigated geographic variation in north-western Italy and south-eastern France to locate the contact zone, and tried to find populations with morphometrically intermediate individuals of possible hybrid origin. We assessed the genetic and environmental basis of this geographic variation by comparing laboratory-reared F1-hybrids, laboratory-reared offspring from pure *G. c. costae* and *G. c. fieberi* strains, and their parental samples from field collections.

Materials and measurements

Field samples, including live bugs for initiating mass rearings, were collected from various parts of Italy, France and Switzerland in the summer of 1988 and spring of 1989 (Appendix). Additional samples from Austria, Greece, Italy and Switzerland, previously analysed by Klingenberg (1992), were also included in our analyses (Appendix). All laboratory mass rearings were carried out under standardised conditions (20° C; 18 L:6 D) as specified by Grossen and Hauser (1982). The animals were killed by deep-freezing and stored in 70% Ethanol prior to measurement.

A set of 14 linear distance measurements (Tab. 1) were taken on fully sclerotized adults of both sexes using a video image analysis system (Java Image analysis

Table 1. List of measured variables. Morphological terms according to Andersen (1982). The numbers for the variables described by Klingengberg (1992) are shown in parentheses.

1. Total length	(1)
2. Pronotum length	(2)
3. Eye length	(6)
4. Distance between outermost points of metaacetabula	(18)
5. Head width	(19)
6. Smallest distance between eyes	(20)
7. Distance between anterior margin of prosternum and posterior margin of middle coxae	( )
8. Length of second abdominal sternite	(37)
9. Length of fourth abdominal sternite	(39)
10. Length of seventh abdominal sternite	(42)
11. Length of third antennal segment	(45)
12. Length of first segment of middle tarsus	(51)
13. Length of second segment of middle tarsus	(52)
14. Length of hind femur	(53)

software; PCVISION*plus*™ framegrabber board). This set of variables differs from the one recommended by Klingenberg (1992) by exclusion of many variables important only to separate *G. c. costae* and *G. c. poissoni*. In terms of standard distances (D_p), the set used in this study (Largiadèr, 1991) clearly improves the separation of *G. c. costae* and *G. c. fieberi* from (D_{12}=) 4.52 for males and 4.40 for females to (D_{14}=) 5.97 for males and 6.27 for females.

Statistical analysis

Statistical analyses were carried out using the SAS statistical software package (SAS Institute Inc., 1990). Correspondence in the results of separate analyses for males and females was taken as an indication of the reliability for observed patterns.

Canonical variate analysis

We used canonical variate analysis (CVA) to characterize the morphometric variation in the study area. CVA maximizes the differences between predefined groups relative to the variation within groups (Albrecht, 1980; Campbell and Atchley, 1981; Reyment et al., 1984). This results in a set of uncorrelated linear functions (canonical variates: CV's) that separate these predefined groups maximally. We carried out CVAs using two different *a priori* grouping criteria: 1) sampling sites and 2) predefined geographic regions (Appendix). The second classification criterion was chosen to facilitate the interpretation of morphometric variation by reducing the number of *a priori* groups. These analyses do not use information about the spatial relations between sampling locations or regions.

CVA, therefore, does not impose any geographic structure and its results can be interpreted as purely morphological variation.

We also used CVA to compare morphometric variation of laboratory-reared offspring with their parents from field populations and laboratory-reared hybrid individuals. According to their geographic origin we assigned parents and offspring of each culture separately to different *a priori* groups. We interpreted correspondence in the patterns of variations between parental groups and those between offspring groups as genetically determined variation. Accordingly, we interpreted differences in variation between parental groups and their offspring as nongenetic variation (effects of laboratory conditions). Therefore, if the observed variation has a strong genetic basis, the position of laboratory-reared hybrids relative to the offspring of pure strains should be similar to the position of natural hybrids relative to field samples of pure *G. c. costae* and *G. c. fieberi*.

Because of limited space, we were not able to rear the hybrid offspring of reciprocal crosses separately. Thus, we were not able to detect morphometric variation due to maternal effects or effects linked to sex chromosomes. Comparison of offspring from reciprocal crossings of *G. c. costae* and *G. c. poissoni* suggest that such effects have at most a modest influence on morphometric variation (Largiadèr, 1991). Therefore, we do not expect such influences to seriously affect the results of this study.

Canonical trend surface analysis

Canonical trend surface analysis (CTS) was used to detect two-dimensional geographic trends in morphometric variation (Lee 1969; Gittins 1979; Wartenberg, 1985). CTS fits smooth surfaces to spatial data by canonical correlation analysis (CCA; Pimentel, 1979). CCA is used to find pairs of maximally correlated variables (pairs of components or canonical variates) derived from the morphometric data and from geographic coordinates. Thus, it represents the multivariate extension of bivariate correlation analysis, i.e., the investigation of relationships between two sets of variables. In this study, one set of variables consisted of the morphometric characters and the second set of geographic coordinates and their squares and cross products. The linear functions of the coordinates define a trend surface that can be laid over the area of interest. Higher order polynomials for the geographic coordinates are calculated as for trend surface analysis (TSA; Gittins, 1968) to fit a more and more structured trend surface on the morphometric data. For example, surfaces of the first order can only define a plane with the function $Geo = \beta_1 X + \beta_2 Y$; where Geo is the canonical variate of the geographic variables, X and Y are the geographic coordinates and β_i is the linear coefficient for the i-th variable. Accordingly, second order surfaces are defined by the equation $Geo = \beta_1 X + \beta_2 Y + \beta_3 X^2 + \beta_4 XY + \beta_5 Y^2$. These surfaces can be dome- or saddle-shaped.

For a given dataset, the order of trend surfaces obtained with CTS is limited by the sum of the numbers of variables in each set compared to the number of sampling sites. The number of variables should not exceed the number of sampling sites, thus the highest order fits calculated for our data are of the fourth order. However, we decided to use only surfaces of low orders (first and second) which showed significant correlation with morphometry. We chose this restriction to reduce problems with overfitting and edge effects (Wartenberg, 1985). We based our interpretation of trend surfaces mainly on two statistical measures, the canonical correlation coefficient *C* and the redundancy coefficient *Rd* (Wartenberg 1985). *C* quantifies the correlation between two corresponding components of the variable sets. It ranges from zero for independent variation to unity for complete matching between character sets. *Rd* gives the actual overlap between the components, i.e. the amount of the total variation of one dataset explained by the component derived from the other dataset.

The trend surfaces were calculated for an area extending 150 kilometres East and 300 kilometres North from the point 43° 30′ N and 6′ E. This area is almost identical to the one shown in Fig. 3. We included 34 samples (518 females and 502 males) in CTS analyses. Sample sizes ranged from 1 to 43 individuals. In two samples either females or males were missing (see Appendix Table 1 for details). We analysed the data (1) using individual values of the morphometric variables or (2) the means of the variables for each sample.

Results

Using geographic regions instead of sampling sites as *a priori* grouping criterion did not alter the overall pattern of variation. This is reflected by high correlations between corresponding CV1s and CV2s (CV1s: 0.993 for males and 0.995 for females; CV2s: 0.899 and 0.950 respectively). Therefore we only report the results of CVA based on this reduced number of *a priori* groups.

For both sexes canonical variate analyses of field samples gave similar patterns (Fig. 1). Along CV1, which explains 75% of between-group variance for males and 71% for females, the regions are arranged in a continuum. Two well defined groups are clustered around both the lower and upper extremes. The first group consists of samples of *G. c costae* from the Central, Eastern, Southern Alps and samples from the Haute Savoie. The second group with high values for CV1 is formed by samples of *G. c fieberi* from Greece and samples from Emilia-Romagna and Liguria. The few specimens from Southern Italy (regions Campagna and Puglia) are well within the range of the second group. The Maritime and Western Alps (Aosta Valley, Dauphiné, Provence, Northern Piemonte and Southern Piemonte) connecting the two extreme groups geographically, show intermediate values, thus connecting the extreme groups along the CV1 axis (Fig. 1). A slight geographic trend is visible, if the scores for these intermediate regions and their geographic positions are compared. From North to South the means of these regions score closer to the *G. c. fieberi* group. The means on the French side of the mountain ridges of the Maritime and Western Alps (Dauphiné and Provence) lie closer to the *G. c. costae* group than

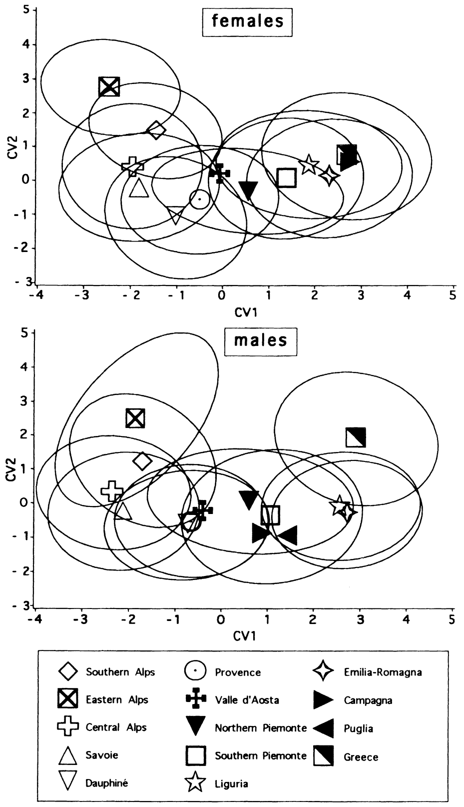

Fig. 1. Plot of the second versus the first canonical variate of CVA for females and males from field samples using geographic regions as grouping criterion. Confidence ellipses (75%) for the scores and the means (symbols) for each region are shown. Ellipses for regions with less than 13 individuals are not shown.

the regions on the Italian side (Aosta Valley, Northern Piemonte and Southern Piemonte) which spread across the entire intermediate space.

The second canonical variate (CV2) accounts for 12% (females) and 10% (males) of the between group variance. It mainly separates the regions Central, Eastern and Southern Alps, Thus, CV1 explains the variance relevant for the separation of *G. c. costae* and *G. c. fieberi*, whereas CV2 reflects variation within the subspecies *G. c. costae*. The following CV's each accounted for less than 7% of the between-group variance in both sexes and are therefore excluded from further interpretation.

Canonical trend surface analyses using sample means or individual scores of the morphometric measures show qualitatively similar results. Sample means yield higher canonical correlation and redundancy coefficients (Tab. 2) than individual scores, since morphometric variance within each sample site is excluded from the

Table 2. First and second order canonical correlations for morphometric data.

Canonical variates	Individual morphometric scores						Sample means of the morphometric scores					
	Females			Males			Females			Males		
	C^2	Rd_{std}	Rd_{raw}	C^2	Rd_{std}	Rd_{raw}	C^2	Rd_{std}	Rd_{raw}	C^2	Rd_{std}	Rd_{raw}
1st-order fit												
1	0.436	0.145	0.234	0.471	0.150	0.224	0.789	0.197	0.263	0.803	0.132	0.168
2	0.242	0.032	0.021	0.221	0.018	0.015	0.557	0.019	0.010	0.690	0.077	0.057
2nd-order fit												
1	0.483	0.142	0.234	0.586	0.151	0.228	0.909	0.085	0.115	0.933	0.141	0.176
2	0.303	0.064	0.059	0.230	0.026	0.023	0.792	0.141	0.141	0.748	0.058	0.030
3	0.092	0.011	0.004	0.145	0.025	0.022	0.516	0.009	0.005	0.646	0.016	0.006

Abbreviations: C^2 is the squared canonical correlation; Rd_{std} is the standardized and Rd_{raw} the raw morphometric redundancy.

analysis. There is, however, a larger quantitative difference between sexes in trend surfaces based on means than on individual values. This difference seems to be due to small samples (number 21 with 3 females and number 25 with one male; Fig. 3). These are not shared by the sexes and lay at opposite corners of the trend surfaces. By using means these small samples are weighed equal to larger samples, and therefore effects of random errors increase, especially at the edge of the surfaces. For this reason, our interpretations are based on trend surfaces for the individual scores.

The planes defined by the first order fits show almost identical geographic trends for both sexes (Fig. 2A–D) for CTS with individual scores for the morphometric variables. The first geographic component declines gently from North to South and

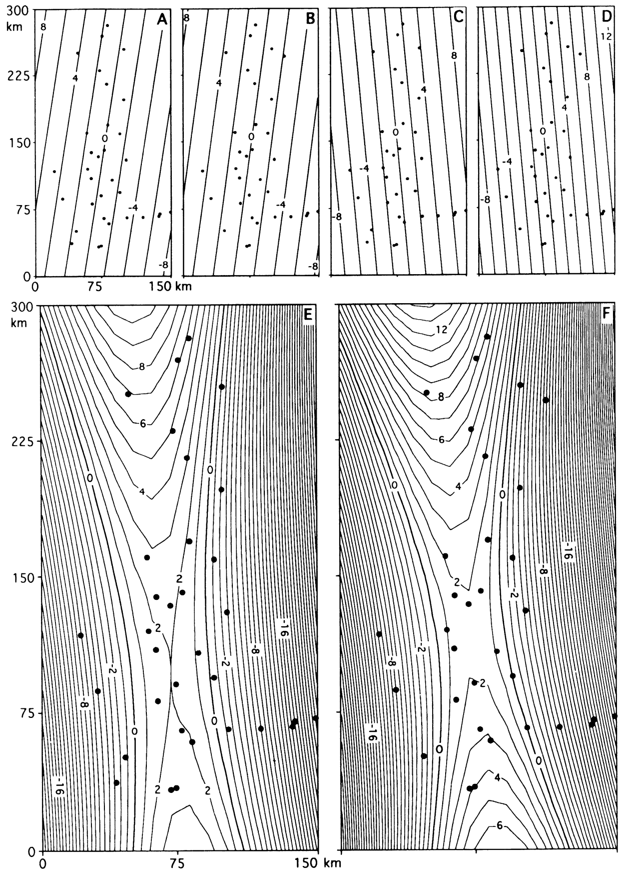

Fig. 2. First and second order trend surfaces of CTS of individual scores for females and males. The dots represent the sample sites (see Fig. 3) and the surfaces are drawn with contour lines. (A), (C) and (E): females ; (B), (D) and (F): males; (A) and (B): first components of the first order fits; (C) and (D): second components of the first order fits; (E) and (F): first components of the second order fits.

more strongly from West to East. The surface of the second component has a similar North-South slant, but the West-East slope is tilted in the opposite way. Both canonical correlation and morphometric redundancy (Tab. 2) are distinctly higher for the first component. Thus, the first component describes the predominant linear trend in the area.

The first components of the second fits are again similar for both sexes (Fig. 2E, F). They show a saddle-shaped surface reflecting the major topographic units of the study area, the Maritime and Western Alps and apparently incorporate the trends of the two planes of the first order components.

The canonical trend surfaces show geographic tendencies (Fig. 2) similar to the sample means of the CV1 (Fig. 3). Accordingly, we find high correlation between the canonical variate scores of CTS and CVA (Fig. 4). This confirms that both methods show the same geographic trends. The samples from low altitudes from east and west of the Maritime and Western Alps show CV1 scores closer to *G. c. fieberi*, whereas samples from high altitudes near the mountain ridges are closer to *G. c. costae*. Yet, there is only a weak correlation between altitude and CV1 scores in our dataset. This is not surprising since, according to CTS, we found a general North-South trend from *costae* to *fieberi*-like variation.

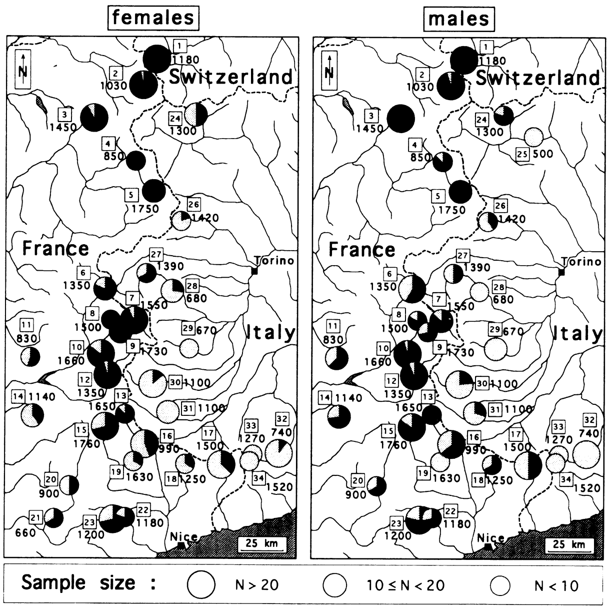

Fig. 3. Frequencies of *G. c. costae*-like and *G. c. fieberi*-like individuals in the field samples. Only the sample sites selected for CTS are shown. Black portions of the circles indicate specimens with first canonical variate scores < 0 (presumably *G. c. costae*, see Fig. 1), and shaded portions for specimens with scores > 0 (presumably *G. c. fieberi*). The altitude of the sampling locations is given in metres above sea level.

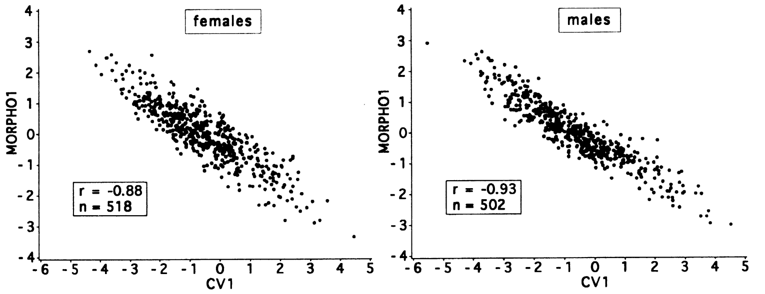

Fig. 4. Comparison of CVA and CTS. Plotted values are the individual scores for the first canonical variates of the CVA (CV1) and CTS (MORPHO1: first component for the morphometric character set) for females and males.

To assess the influence of genetic and environmental factors, samples from various wild populations and their laboratory-reared offspring were compared by CVA. Patterns of variation among offspring groups correspond to the variation among parental samples (Fig. 5). CV1 explains 43.8% (females) and 49% (males) of the between-group variance and CV2 22.3% (females) and 16.7% (males). CV1 separates mainly the subspecies, demonstrating the strong genetics basis of morphometric variation. CV2 discriminates mainly parents from their offspring, indicating a laboratory effect on morphometric characters. The laboratory effect is probably due to the increased food availability in the laboratory. Laboratory-reared F1 hybrids have intermediate scores compared to the offspring of pure parental strains (Fig. 5). Offspring of intermediate parents also score intermediately and can thus be interpreted as descendants of natural hybrid populations.

Discussion

Our morphometric data demonstrate an extensive transition zone between the two morphologically and ecologically distinct subspecies *G. c. costae* and *G. c. fieberi*, which is associated with the major topographical features of the area, the Maritime and Western Alps. Samples from the northern and south-eastern parts of the study area are similar to samples of the pure subspecies collected far from the contact zone. The occurrence of intermediate specimens in the transition zone, and our laboratory breeding experiment suggest that hybridization and introgression occur in this area.

Klingenberg (1992) speculated that the present geographic distribution of the two subspecies and their differences in ecological characteristics are the result of divergence during the last Ice Age due to isolation in separate refuges. The refuge of *G. c. costae* presumably was between the Alpine and Scandinavian ice shields, whereas *G. c. fieberi* probably had its refuge in the Adriatic area, in south-eastern Europe and Italy. In this scenario, the transition zone described here is due to

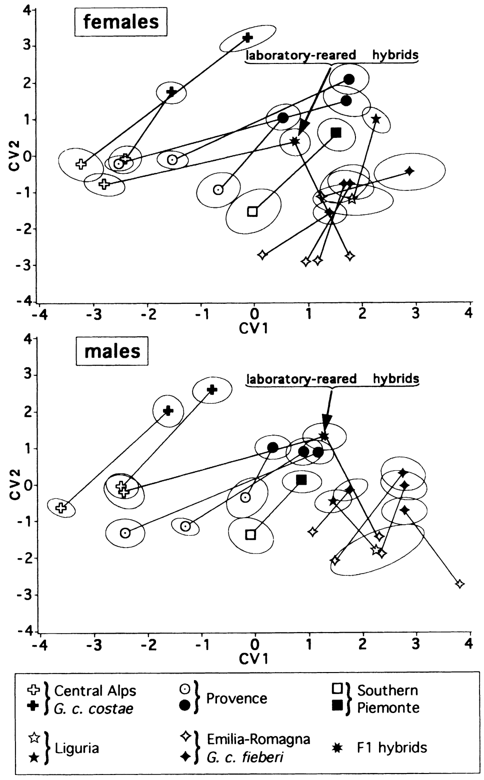

Fig. 5. Comparisons between laboratory-reared offspring and their parents from natural populations. The plot shows the second versus the first canonical variate of the CVA for females and males. The open symbols represent the parents and the solid symbols their offspring. Confidence ellipses (75%) for the group means with more than ten individuals.

secondary contact of the two subspecies. As is consistent with the hypothesis of range expansions from the North and Southeast, the geographic trends are expressed at different scales on the eastern (Italy) and western (France) slopes (Fig. 3). On the eastern side we find pure *G. c. fieberi* populations at the entrance of large West-East directed valleys, whereas at higher elevations we find intermediate populations. On the western slope this trend is much weaker, and we were not able to find pure *G. c. fieberi* populations at low altitudes. High elevation of the Maritime Alps by itself is probably not a sufficient barrier to dispersal of *G. c. fieberi* into France, because *G. c. fieberi* can be found at high altitudes in regions where *G. c. costae* does not occur (e.g. in Emilia-Romagna at 1650 m). Based on its present geographic distribution, we suspect that *G. c. costae* is better adapted to alpine habitats than *G. c. fieberi*. Hence, competitive exclusion of *G. c. fieberi* by *G. c. costae* in alpine areas of the contact zone could explain the absence of pure *G. c.*

fieberi populations from France. Common garden experiments using field exclosures could be used to test this hypothesis.

Geographic variation of morphometric characters of *G. costae* is largely determined genetically, as shown by using CVA comparisons of laboratory-reared offspring with their parents from natural populations (Fig. 5). These findings are consistent with the results of Klingenberg (1992). Laboratory-reared F1 hybrids between the two subspecies are morphologically intermediate between offspring of pure parental strains. This suggests that intermediate samples from the field also are the result of hybridization. We found no apparent increase in morphometric variation within samples from the contact zone as compared to pure populations (Fig. 1). This is in contrast to the results from a study on a hybrid zone between closely related waterstrider species (Spence, 1990). The increased variability in the hybrid zone between two species of *Limnoporus* may result from reduction of gene flow due to chromosomal incompatibility (Spence, 1990) generating linkage disequilibria, as demonstrated by enzyme electrophoretic studies (Sperling and Spence, 1991). Even under a polygenic model of inheritance for morphometric traits, the resulting linkage groups would behave like genes of major effect, with backcrosses and segregation in the F2 leading to increased phenotypic variability in the hybrid zone. Without such barriers to recombination, the increase in variability is expected to be much smaller, and is unlikely to be statistically detectable. The absence of increased variation in our intermediate populations therefore suggests unimpeded introgression. This is consistent with intraspecific studies of allozyme variation, which did not find linkage disequilibria (Preziosi and Fairbarin, 1992; Gallant et al. 1993).

The two subspecies generally occur at different altitudes, and their phenologies differ accordingly. *G. c. costae* starts to reproduce after snowmelt, which may be as late as June in the Alps, and seems to be strictly univoltine (Köpfli et al., 1987). In its low-altitude habitats, *G. c. fieberi* has a much longer reproductive season. In our study area, overwintered adults can be observed already in March, larvae in April, and adult offspring in early June (Largiadèr, 1991), but copulating adults and larvae are still seen in October (Largiadèr, pers. obs.), which suggests that this subspecies may be bi- or perhaps even multivoltine. In a model of a cline in season length, Roff (1980) considered the consequences of a transition from a univoltine to a bivoltine life cycle on adult size. He assumed that size positively correlates with development time, and therefore predicted a general increase in size with increasing season length. Where a bivoltine life cycle becomes feasible, however, development time must be shorter, and bivoltine adults are expected to be smaller than univoltine adults in the same location. The resulting " saw-tooth" pattern was confirmed, e.g. for a latitudinal cline in a cricket species (Mousseau and Roff, 1989). Nevertheless, we did not find such a pattern or the increase in size variability predicted to be associated with a transition between uni- and bivoltine forms. This again supports the hypothesis of secondary contact as the divergence in their glacial refuges might have involved both morphology and voltinism.

We have no evidence for inviability of F1 hybrids (Largiadèr, 1991; C. P. Klingenberg. pers. obs.). Nevertheless, we were not able to rear F2 hybrids in the laboratory. This is almost certainly due to an artifact and not F1 hybrid sterility, because other mass rearings of pure strains broke down at the same time, and because we observed a few first instar larvae for F2 hybrids and backcrosses. Two factors may have caused this breakdown: our cold treatment for diapausing adults may have been to short (six months instead of about eight months in nature), or disease in the laboratory culture.

Whereas Klingenberg (1992) described geographic variation of the subspecies of *G. costae* at the continental scale, here we analyse the structure of a contact zone with a resolution of several kilometres. The mechanisms generating the small-scale structure of this zone remain unclear. The width of a hybrid zone depends largely on dispersal rates, on the degree of pre- and postzygotic reproductive isolation and on population densities of the taxa on either side of the contact zone (Endler, 1977). The structure of the hybrid zone is further determined by spatial and temporal variability of waterstrider habitats and populations. For *G. costae*, there are no reliable estimates for relevant parameters. There is, however, circumstantial evidence for long distance migration in *G. c. costae* (Bächler, 1985), which can cross watersheds by flight. Furthermore, a single female of *G. costae* can lay more than 500 eggs (R. Hauser, pers. comm.) and 8–12 weeks are sufficient to produce a large number of adult offspring. Given this high potential for dispersal and reproduction, we expect that the hybrid zone is highly complex and dynamic.

Three isolating mechanisms might be important for the persistence of the cline. (1) There may be partial prezygotic reproductive isolation. In mass rearings with *G. c. fieberi* females and *G. c. costae* males, the small *G. c. costae* males seemed to have difficulties to grab the large *G. c. fieberi* females for copulations, whereas this was not true in mass rearings with *G. c. fieberi* males and *G. c. costae* females, which are about the same size (C. R. Largiadèr, pers. obs.). (2) If habitat use reflects habitat selection in these two taxa, continued colonization of temporary habitats by *G. c. fieberi* will tend to preserve the cline. (3) Disruption of locally adapted life cycle syndromes might constitute a postzygotic isolation mechanism, i.e. the increased variability of season length in the contact zone will select in one year for univoltine life cycles and in another for bivoltine life cycles.

A detailed understanding of the processes structuring this hybrid zone must include combined studies of morphological variation and phenological adaptions on a local geographic scale, pre- and post-zygotic isolation mechanisms, and metapopulation structure.

Acknowledgements

We thank R. Hauser for support and encouragement for this project. We are grateful to J. R. Spence for a constructive review of an earlier version of this paper. We are also grateful to L. Frauchiger and R. Köpfli for assisting in the laboratory work, and to R. Hauser and A. Scholl for providing additional field samples. C. R. L. thanks the Keller family from Castino, Italy, for their hospitality. Financial support was provided by a University of Alberta Ph.D. Scholarship of C. P. K.

References

Albrecht, G. H. 1980. Multivariate analysis and the study of form, with special reference to canonical variate analysis. Amer. Zool. 20: 679 693.

Andersen, N. M. 1982. The Semiaquatic Bugs (Hemiptera, Gerromorpha). Entomonograph 3, Scandinavian Science Press Ltd., Klampenborg (Denmark).

Bächler, M. 1985. Geographische Isolation und genetische Differenzierung bei Populationen des alpinen Wasserläufers *G. costae* (Hemiptera: Gerridae). Lizentiatsarbeit am Zoologischen Institut der Universität Bern.

Barton, N. H. and G. M. Hewitt. 1985. Analysis of hybrid zones. Ann. Rev. Ecol. Syst. 16: 113–148.

Barton, N. H. and G. M. Hewitt. 1989. Adaption, speciation and hybrid zones. Nature 341: 497 503.

Campbell, N. A. and W. R. Atchley. 1981. The geometry of canonical variate analysis. Syst. Zool. 30: 268 280.

Endler, J. A. 1977. Geographic Variation, Speciation, and Clines. Monographs in Population Biology 10. Princeton University Press, Princeton, New Jersey.

Gallant, S. L., R. F. Preziosi and D. J. Fairbairn. 1993. Clinal variation in eastern populations of the waterstrider *Aquarius remigis*: gradual intergradation or discontinuity? Evolution, 47(3): 957 964.

Gittins, R. 1968. Trend-surface analysis of ecological data. J. Ecology 56: 845 869.

Gittins, R. 1979. Ecological applications of canonical analysis, pp. 309 535. *In* L. Orloci, C. R. Orloci and W. M. Stiteler (eds.), Multivariate Methods in Ecological Work. International Co-operative Publishing House, Fairland, Maryland.

Grossen, B. and R. Hauser. 1982. Über den Umgang mit Wasserläufern (Hemiptera, Gerridae). Mitt. schweiz. entomol. Ges. 55: 394 395.

Harrison, R. G. 1990. Hybrid zones: windows on evolutionary process, pp. 69 128. *In* D. Futuyma and J. Antonovics (eds.), Oxford Surveys in Evolutionary Biology, Volume 7. Oxford University Press.

Hewitt, G. M. 1988. Hybrid zones – Natural laboratories for evolutionary studies. TREE, 3(7): 158–167.

Hoberlandt, L. 1948. Results of the zoological scientific expedition of the National Museum in Praha to Turkey. Acta Entomol. Mus. Nat. Pragae 26: 1 74.

Klingenberg, C. P. 1992. Multivariate morphometrics of geographic variation of *Gerris costae* (Heteroptera: Gerridae) in Europe. Revue suisse de Zoologie 99(1): 11 30.

Köpfli, R., R. Hauser and M. Zimmermann. 1987. Diapausedetermination bei Wasserläufern (Hemiptera, Gerridae). Revue suisse Zool. 94: 533–543.

Largiadèr, C. R. 1991. Multivariate morphometrische Untersuchungen zur Erfassung der geographischen Variabilität zweier Unterarten von *Gerris costae* (Heteroptera: Gerridae) in den Alpen und in den Apenninen. Lizentiatsarbeit am Zoologischen Institut der Universität Bern.

Lee, P. J. 1969. The theory and application of canonical trend surfaces. J. Geology 77: 303 318.

Mousseau, T. A. and D. A. Roff. 1989. Adaptation to seasonality in a cricket: patterns of phenotypic and genotypic expression along a cline in season length. Evolution 43(7): 1483 1496.

Nieser, N. 1978. Heteroptera, pp. 280 285. *In* J. Illies (ed.), Limnofauna Europaea. Gustav Fischer, Stuttgart.

Pimentel, R. A. 1979. Morphometrics. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Preziosi, R. F. and D. J. Fairbairn. 1992. Genetic population structure and levels of gene flow in the stream dwelling waterstrider, *Aquarius (= Gerris) remigis* (Hemiptera: Gerridae). Evolution 46(2): 430 444.

Reyment, R. A., R. E. Blackith and N. A. Campbell. 1984. Multivariate Morphometrics. Academic Press, London.

Roff, D. A. 1980. Optimizing development time in a seasonal environment: the 'ups and downs' of clinal variation. Oecologia 45: 202 208.

SAS Institute Inc., 1990. SAS/STAT User's Guide, Version 6, Fourth Edition Volume 1 and 2. SAS Institute Inc., Cary, North Carolina.

Servadei, A. 1967. Rhynchota. Heteroptera Homoptera Auchenorrhyncha. Catalogo topographico e sinonimico. Fauna d'Italia. IX. Edizioni Calderini, Bologna.

Spence, J. R. 1990. Introgressive hybridization in Heteroptera: the example of *Limnoporus* Stål (Gerridae) species in western Canada. Can. J. Zool. 68: 1770–1782.

Sperling, F. A. and J. R. Spence. 1991. Structure of an asymmetric hybrid zone between two water strider species (Hemiptera: Gerridae: *Limnoporus*). Evolution 45(6): 1370–1383.

Tamanini, L. 1979. Eterotteri acquatici (Heteroptera: Gerromorpha, Nepomorpha). Consiglio nazionale delle ricerche.

Wagner, E. and S. Zimmermann. 1955. Beitrag zur Systematik der Gattung *Gerris* F. (Hemiptera-Heteroptera, Gerridae). Zool. Anz. 155: 177–190.

Wartenberg, D. 1985. Canonical trend surface analysis: a method for describing geographic patterns. Syst. Zool., 34(3): 259–279.

Zimmermann, G. 1982: Beiträge zur Wasserwanzen-Fauna Griechenlands I (Heteroptera, Nepomorpha, Gerromorpha, Saldidae). Marburger Entomol. Publ. 1: 183–216.

Zimmermann, M. and A. Scholl. 1993. Specific status of *Aquarius cinereus* (Puton) and *Aquarius najas* (De Geer) (Hemiptera Gerridae) and the extent of hybridization in the Mediterranean region. Entomologica scandinavica 24: 197–210.

Received 14 September 1993;
accepted 12 August 1994.
Corresponding Editor: G. M. Hewitt

Appendix

Sizes of measured field samples

Region	**N Samples**	**Sampling sites**		**Sample size**		**Region total**	
		Site No.	Coordinates	Females	Males	Females	Males
Eastern Alps	3					32	50
Southern Alps	1					21	13
Central Alps	8	1	46° 4′15.7″ N; 6° 59′14.4″ E	27	38	167	185
Savoie	4	2	45° 57′ 9.8″ N; 6° 54′30.6″ E	37	21		
		3	45° 46′53.6″ N; 6° 32′46.1″ E	25	25		
		4	45° 35′54.0″ N; 6° 52′19.3″ E	2	8		
		5	45° 27′31.1″ N; 6° 58′17.8″ E	12	19	76	73
Dauphiné	6	6	44° 57′38.9″ N; 6° 40′50.2″ E	16	29		
		7	44° 47′ 8.5″ N; 6° 56′ 7.1″ E	30	15		
		8	44° 45′42.4″ N; 6° 44′42.2″ E	6	6		
		9	44° 43′ 9.0″ N; 6° 50′49.3″ E	10	7		
		10	44° 35′26.2″ N; 6° 41′27.5″ E	26	24		
		11	44° 34′ 7.0″ N; 6° 11′57.6″ E	9	16	97	97
Provence	12	12	44° 29′47.1″ N; 6° 44′33.6″ E	43	31		
		13	44° 19′27.5″ N; 6° 53′ 6.7″ E	8	8		
		14	44° 17′33.7″ N; 6° 19′ 2.9″ E	10	11		
		15	44° 14′22.0″ N; 6° 45′14.5″ E	41	32		
		16	44° 2′13.9″ N; 7° 0′ 0.0″ E	25	25		
		17	44° 6′15.1″ N; 7° 30′15.4″ E	27	24		
		18	44° 6′13.8″ N; 7° 16′ 2.5″ E	6	9		
		19	44° 5′34.8″ N; 6° 55′39.0″ E	3	3		
		20	43° 57′37.0″ N; 6° 30′51.7″ E	4	7		
		21	43° 48′59.5″ N; 6° 26′24.8″ E	3			
		22	43° 48′32.9″ N; 6° 52′51.3″ E	5	9		
		23	43° 48′ 3.6″ N; 6° 50′52.6″ E	21	28	196	186

Sizes of measured field samples (*continued*)

Region	**N Samples**	**Sampling sites**		**Sample size**		**Region total**	
		Site No.	Coordinates	Females	Males	Females	Males
Valle d'Aosta	2	24	45° 49′ 5.6″ N; 7° 13′43.1″ E	10	5		
		25	45° 44′37.7″ N; 7° 24′39.1″ E		1	10	6
Northern Piemonte	3	26	45° 18′ 7.3″ N; 7° 13′10.7″ E	5	5		
		27	45° 2′25.6″ N; 6° 59′22.6″ E	6	8		
		28	44° 56′57.9″ N; 7° 9′59.1″ E	11	6	22	19
Southern Piemonte	3	29	44° 41′ 2.9″ N; 7° 15′29.7″ E	7	14		
		30	44° 28′46.5″ N; 7° 3′ 3.1″ E	25	28		
		31	41° 21′32.3″ N; 7° 9′56.4″ E	11	10	43	52
Liguria	7	32	44° 9′12.8″ N; 7° 54′18.2″ E	28	23		
		33	44° 8′17.8″ N; 7° 45′ 1.8″ E	4	5		
		34	44° 6′44.8″ N; 7° 44′ 7.3″ E	2	3	48	49
Emilia-Romagna	15					205	184
Campagna	1					5	2
Puglia	1					–	1
Greece	7					41	64
TOTAL	73					966	979

Sample sizes of laboratory mass rearings

Rearing	**Site No.**	**Presumed subspecies status**	**Parents**		**Offspring**	
			Females	Males	Females	Males
Central Alps 1	1	*G. c. costae*	14	38	17	25
Central Alps 2		*G. c. costae*	27	28	25	25
Emilia-Romagna 1		*G. c. fieberi*	4	5	25	25
Emilia-Romagna 2		*G. c. fieberi*	5	2	14	15
Emilia-Romagna 3		*G. c. fieberi*	8	3	25	25
Emilia-Romagna 1 ×		*G. c. fieberi*	–	2	25	25
Emilia-Romagna 2		*G. c. fieberi*	7			
Liguria 1	32	*G. c. fieberi*	15	13	25	24
Southern Piemonte 1	30	?	15	15	24	24
Provence 1	17	?	14	14	25	25
Provence 2	15	?	41	32	21	25
Provence 3	12	?	43	31	11	21
Central Alps 2 ×		*G. c. costae*	16	11	25	25
Emilia-Romagna 1		*G. c. fieberi*	2	4		