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Multivariate morphometrics of geographic variation of *Gerris costae* (Heteroptera: Gerridae) in Europe

by

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With 7 figures

ABSTRACT

The waterstrider species *Gerris costae* (Herrich-Schaeffer) has a large and disjunct distribution in the southern part of the palearctic region. Multivariate morphometric techniques were used to assess the geographic variation in the European part of its range, where three subspecies are currently recognized: *G. c. costae* (Herrich-Schaeffer), *G. c. fieberi* Stichel, and *G. c. poissoni* Wagner and Zimmermann. Twelve variables were selected from a larger set (56 or 57 variables for males and females, respectively) by stepwise discriminant analysis, and were found to represent the main patterns of morphometric variation in the full character set. Principal component analysis revealed that both 'size' and 'shape' contribute to geographic differentiation. Canonical variate analysis with two different criteria for the definition of *a priori* groups showed that the subspecies are coherent units separate from each other, and that the segregation into the three subspecies indeed reflects the main pattern of geographic variation in Europe. Comparisons of field samples with offspring reared in the laboratory under standardized conditions demonstrated that geographic differentiation is genetically determined for the most part. The study therefore supports the segregation of *Gerris costae* into three subsepcies in the area considered. Linear discriminant functions are given to allocate additional material to the subspecies.

INTRODUCTION

Intraspecific variation is of major interest for the study of evolutionary processes such as speciation (ENDLER, 1977), and, in conjunction with biogeographic evidence, to infer the distributional history of taxa (JANSSON, 1980; THORPE, 1984). Moreover,

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geographic variation is of taxonomic importance in the context of the subspecies concept (BLACKWELDER, 1967; BARROWCLOUGH, 1982; MAYR, 1982; SMITH and PATTON, 1988). Morphometric methods can be used to analyze various aspects of variation (PIMENTEL, 1979; THORPE, 1983), and have therefore been widely utilized in recent investigations. Examples of morphometric studies on geographic variation in insects include ZIMMERMAN and LUDWIG (1974), BRYANT (1977), BRYANT and TURNER (1978), FOOTTIT and MACKAUER (1980), and RUTTNER (1988).

To be of evolutionary or taxonomic importance, geographic variation has to be genetically determined, at least to a large extent (ATCHLEY, 1983). Variation in the quality of habitats can generate environmental variation in morphometric traits (e.g., SMITH and PATTON, 1988) which may be confounded with genetic variation. Laboratory rearings under standardized conditions can be used to control for environmental effects in studies of the genetic determination of morphometric differentiation (BRYANT, 1977; BRYANT and TURNER, 1978; SORENSEN and SAWYER, 1989).

The waterstrider *Gerris costae* (Herrich-Schaeffer) has a wide and rather disjunct distribution in the southern palearctic region, and exhibits considerable geographic variation in morphological traits. Several subspecies have been proposed in the European part of its range (Fig. 1). *G. c. costae* (Herrich-Schaeffer), *G. c. fieberi* Stichel, and *G. c. poissoni* Wagner and Zimmermann have been described in detail by WAGNER and ZIMMERMANN (1955) using genital morphology and coloration as main characters, and were included by NIESER (1978) in his compilation of European semiaquatic and aquatic bugs. NIESER (1978) did not, however, recognize *G. c. arvernensis* from the French Massif Central (distinguished by male genital morphology, POISSON, 1957) or *G. c. avernensis* from the Pyrenees (a name proposed without any further details or description by RICHARD, 1967), as subspecies. Another subspecies, *G. c. sahlbergi* Distant, occurs in Central Asia (KANYUKOVA, 1982).

The nominate subspecies *G. c. costae* was reported from the Alps (WAGNER and ZIMMERMANN, 1955; NIESER, 1978), and from various parts of Italy (SERVADEI, 1967; NIESER, 1978; TAMANINI, 1979). It is confined to high altitudes (in Switzerland mainly above 1200 m; in Tyrol mainly above 800 m, lowest record 550 m, HEISS, 1969), where it is found mostly on small pools and ponds in meadows and bogs. *G. c. fieberi* is distributed in Italy (WAGNER and ZIMMERMANN, 1955; SERVADEI, 1967; TAMANINI, 1979) and in south-eastern Europe (Balkans, Carpathian Mountains, eastwards to the north-western coast of the Black Sea; WAGNER and ZIMMERMANN, 1955; NIESER, 1978), and its range extends throughout Asia Minor to Lebanon, Israel, Syria and Iraq (HOBERLANDT, 1948; WAGNER and ZIMMERMANN, 1955; NIESER and MOUBAYED, 1985). It occurs in very diverse habitats on stagnant as well as flowing water (HOBERLANDT, 1948; ZIMMERMANN, 1982; pers. obs.), and is not restricted to high elevations, but can also be very abundant in river basins at low altitudes (pers. obs.). *G. c. poissoni* is found on small bodies of stagnant water in mountainous areas of France and the Iberian peninsula, and in Great Britain and Ireland (WAGNER and ZIMMERMANN, 1955; POISSON, 1957; NIESER, 1978), where it also occurs at low altitudes under harsh climatic conditions. The subspecific status of *Gerris costae* in large parts of the western Soviet Union is unclear (e.g., records of the nominate subspecies reported by KANYUKOVA, 1982).

Genital morphology and coloration, the main characters in previous taxonomic studies, exhibit considerable variability within samples from single localities. Individuals differ seasonally in certain characters, such as the bright patch on the pronotum that often is visible to its full extent only after overwintering. BÄCHLER (1985) found no correspondence between enzyme electrophoretic similarities and described subspecies, mainly

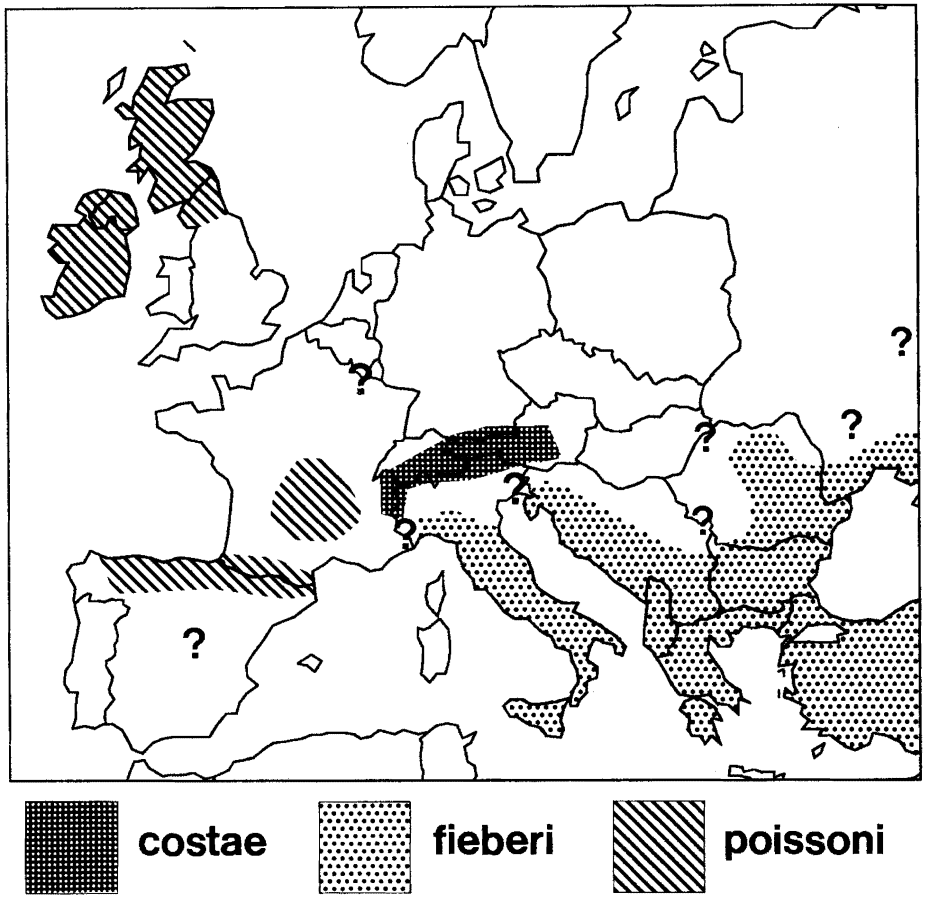

FIG. 1.

Approximate distribution ranges of the subspecies of *Gerris costae*, mainly based on NIESER (1978) and WAGNER and ZIMMERMANN (1955).

because of the low level of genetic differentiation in the loci considered. Although WAGNER and ZIMMERMANN (1955) mentioned that body size varies between subspecies, morphometric methods have not yet been used to examine the geographic variation of *G. costae*.

The purpose of this paper is to assess the geographic variation of *Gerris costae* in Europe by means of multivariate morphometrics, to show the genetic base of variability by comparing samples collected in the field and reared in the laboratory, and to examine if the pattern of geographic variation is consistent with the segregation into subspecific taxa.

MATERIAL AND METHODS

MATERIAL AND MEASUREMENTS

Material from various parts of Europe (Table 1) was used in this study. Samples from the Alps, the French Massif Central, the Pyrenees, and from Greece, including living

TABLE 1

Samples sizes by subspecies, geographic region, and sex. For laboratory cultures, geographic regions refer to parent populations.

Presumed subspecies	Geographic region	Sample size Males	Females
G. c. costae	Central Alps	118	153
	Eastern Alps	53	33
	Southern Alps	14	20
G. c. fieberi	Northern Greece	65	40
	Northern Italy	1	2
	Calabria	1	–
G. c. poissoni	Scotland	9	9
	Northern Ireland	4	1
	French Massif Central	36	29
	Pyrenees	39	36
	Laboratory cultures		
G. c. costae	Central Alps	34	36
G. c. fieberi	Northern Greece	33	34
G. c. poissoni	French Massif Central	25	28
G. c. poissoni	Pyrenees	30	33

specimens for rearing experiments, were collected during field trips in 1986 and 1987. Additional material was obtained from collections at the Zoological Institute of the University of Bern. Laboratory mass rearings were carried out under standardized conditions (temperature 20°C, stationary long-day photoperiod 18L:6D) as specified by GROSSEN and HAUSER (1982), using frozen cockroaches (*Nauphoeta cinerea*) as food. Since food was provided in large quantities, and the numbers of larvae in different rearings were similar, effects of different rearing densities (crowding) can be ruled out. After the imaginal molt, the laboratory-reared offspring were kept until their cuticles were completely hardened, and then killed by deep-freezing.

All specimens were stored in 70% ethanol for at least two months before measuring. By this treatment the bright markings (e.g., the patch on the pronotum) became clearly visible and distinctly delimited, even in specimens that had not yet overwintered and appeared uniformly dark brown before storage. Only individuals with firmly hardened cuticle were included. Specimens for which one or more characters could not be measured were excluded from the study.

Measurements were taken in millimetres using a Wild M5 dissecting microscope fitted with a Wild MMS 235 digital length measuring equipment. In a preliminary step 56 variables were measured in males and 57 in females (Table 2, Figs 2, 3). Of these, 12

TABLE 2

List of measured variables. Morphological terms according to Andersen (1982). Variables marked with an asterisk were selected for the reduced set of characters.

1. Total length.
2. Pronotum length.
3. Distance from anterior margin of pronotum to mesopleural tubercles.
4. Length of bright median line on pronotum.
5. Length of bright pronotum patch.
6*. Eye length.
7. Distance from first to second pair of cephalic trichobothria.
8. Distance from first to third pair of cephalic trichobothria.
9. Distance from third to fourth pair of cephalic trichobothria.
10. Length of radial cell.
11. Length of cubital cell.
12. Distance between apical ends of radial and cubital cells.
13. Pronotum width.
14*. Width of bright pronotum patch.
15. Distance between anterior corners of pronotum.
16*. Distance between mesopleural tubercles.
17. Distance between outermost points of mesoacetabula.
18. Distance between outermost points of metaacetabula.
19*. Head width.
20*. Smallest distance between eyes.
21. Distance between outermost points of antennal sockets.
22. Distance between first cephalic trichobothria.
23*. Distance between second cephalic trichobothria.
24. Distance between third cephalic trichobothria.
25. Distance between fourth cephalic trichobothria.
26*. Abdominal width at posterior margin of second abdominal segment.
27. Abdominal width at posterior margin of sixth abdominal segment.
28. Distance between end points of connexival spines.
29. Width of fore wing.
30. Distance between end points of longitudinal ridges of mesosternum.
31. Prosternum length.
32. Mesosternum length.
33. Metasternum length.
34. Distance between anterior margin of prosternum and posterior end points of coxal clefts of mesoacetabula.
35*. Distance between anterior margin of prosternum and outermost visible points of borderline between metaacetabula and hind coxae.
36. Distance between metathoracic scent orifice and posterior margin of metasternum.
37. Length of second abdominal sternite.
38. Length of third abdominal sternite.
39. Length of fourth abdominal sternite.
40. Length of fifth abdominal sternite.
41. Length of sixth abdominal sternite.
42. Length of seventh abdominal sternite.
43*. Length of first antennal segment.
44. Length of second antennal segment.
45. Length of third antennal segment.
46. Length of fourth antennal segment.
47. Length of fore femur.
48. Length of fore tibia.

49*. Length of middle femur.
50*. Length of middle tibia.
51. Length of first segment of middle tarsus.
52. Length of second segment of middle tarsus.
53*. Length of hind femur.
54. Length of hind tibia.
55. Length of first segment of hind tarsus.
56. Length of second segment of hind tarsus.
57. (Measured for females only). Distance between midpoint of posterior margin of seventh abdominal sternite and endpoints of connexival spines.

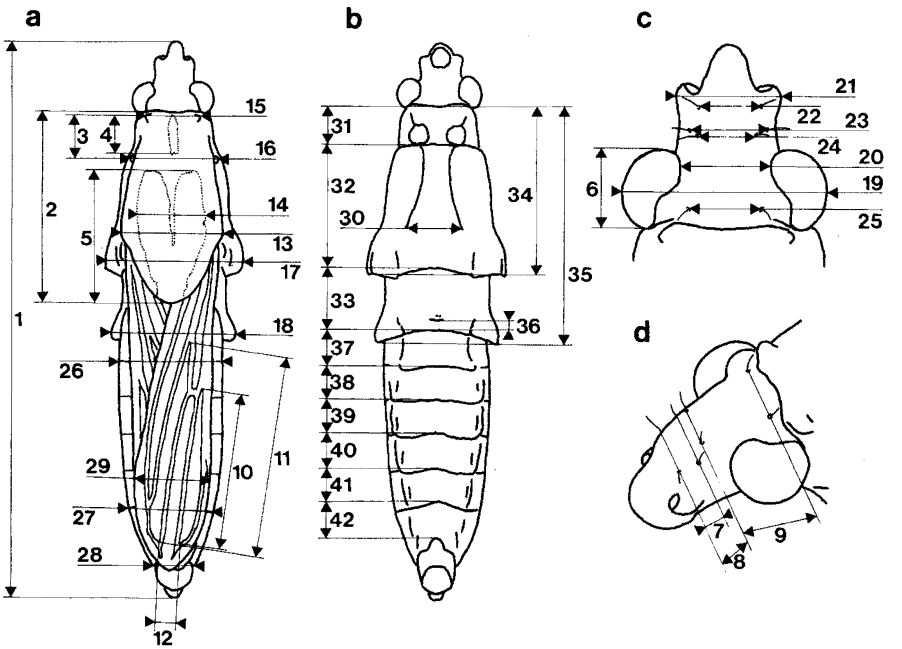

FIG. 2.

Drawings illustrating the measurements nos 1-42. (a) Dorsal view. (b) Ventral view. (c) Head in dorsal view. (d) Head in oblique view. Legs, antennae, and rostrum are omitted. Descriptions of the characters are given in Table 2.

variables were selected for detailed study using linear discriminant analysis (see below). Character 57 was included for females due to the taxonomic value attributed to the connexival spines in the paper of WAGNER and ZIMMERMANN (1955), but could not be measured for males.

STATISTICAL ANALYSIS

This study only considers morphometric variation in adult specimens of *Gerris costae*. Because the adult is a clearly defined stage in insects, and since environmental

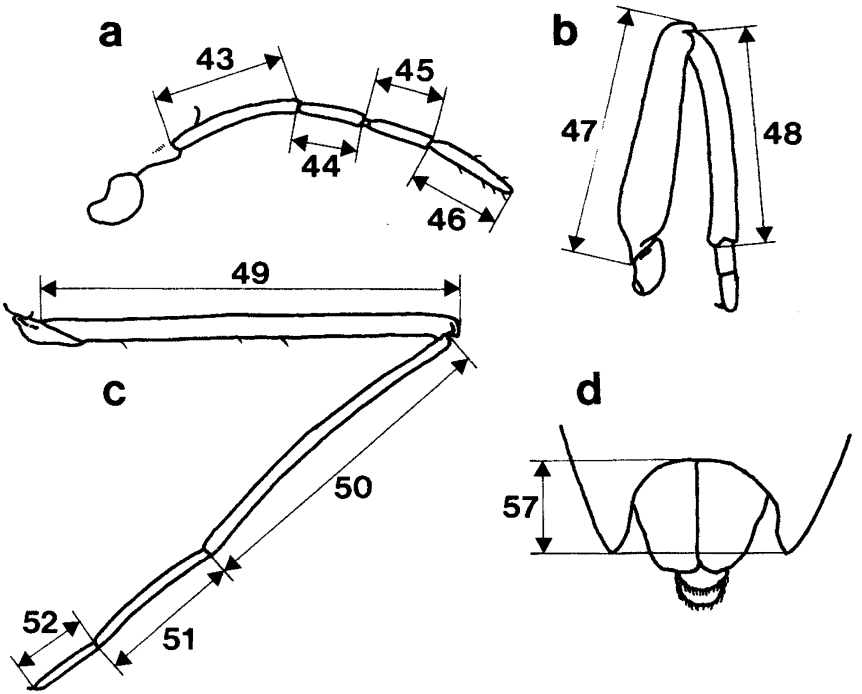

FIG. 3.

Drawings illustrating the measurements nos 43-57. (a) Antenna. (b) Fore leg. (c) Middle leg. Characters nos 53-56 are measured analogously on the hind leg. (d) Abdominal end of a female in ventral view. Descriptions of the characters are given in Table 2.

effects are controlled by the rearing experiments, there was no need to exclude variation in size from the analysis (ATCHLEY, 1983; THORPE, 1983). All analyses were carried out separately for males and females on untransformed variables using the SAS statistical software package (SAS INSTITUTE INC., 1985). Formal statistical testing and calculation of standard errors was not appropriate due to the inhomogeneity of the material and disparate sample sizes. Therefore, congruence of results from separate analyses for both sexes provided a measure of reliability of the observed patterns.

Specimens from the field were allocated to the three subspecies *G. c. costae*, *G. c. fieberi*, and *G. c. poissoni* according to their sampling locations, using the subspecies division and the distribution ranges given by WAGNER and ZIMMERMANN (1955) and NIESER (1978), as shown in Fig. 1. The only ambiguity about subspecies status concerned the samples from Northern Italy (see Introduction). They were provisionally allocated to *G. c. fieberi* becvause of three reasons: the general appearance of the specimens (they were very large and bright brown in color), the low altitude of the locations (200 m and 500 m), and their habitats (A. Scholl, pers. comm.) that seemed more similar to Greek than to Alpine populations.

In a preliminary step all the 56 or 57 variables were included. This dataset comprised 216 males and 201 females altogether, that were collected in all the geographic regions listed in Table 1 (except Calabria), and from the rearings of specimens from the Central Alps, the Franch Massif Central, and the Pyrenees.

Linear discriminant functions between each pair of subspecies were calculated separately for the field samples of both sexes by means of linear regression (FLURY and RIEDWYL, 1985, 1988). For every pairwise comparison of subspecies, a pseudo-variable was coded either 0 or 100 according to the subspecies status of each specimen. This pseudovariable was then used as dependent variable in a linear regression analysis. The number of variables was reduced by backward elimination, using multivariate standard distance (FLURY and RIEDWYL, 1986, 1988) as a criterion for the separation of groups. In all analyses, standard distance fell slowly during the first elimination steps, but more rapidly when fewer than 10-12 variables were retained. As the sets of variables retained in the different analyses were similar, it was possible to select 12 variables as a 'compromise' between subspecies comparisons, weighting difficult distinctions (*G. c. costae* – *G. c. poissoni*) somewhat more than others.

Congruence between the full and reduced sets of variables was expressed as the product-moment correlation coefficients of corresponding component scores of PCAs (pooled samples from the laboratory and the field) using the two character sets.

After measuring additional specimens for the 12 selected characters (see Table 1 for sample sizes), an ordination by means of principal component analysis (PCA) was used to display the multivariate structure of the data from field samples in fewer dimensions (PIMENTEL, 1979; FLURY and RIEDWYL, 1988). The correlation matrix of the untransformed variables of the pooled samples was used for PCA.

Canonical variate analysis (CVA; ALBRECHT, 1980; CAMPBELL and ATCHLEY, 1981; REYMENT *et al.*, 1984) was used to check if the separation into the three subspecies was reflected by the data for the field samples. Two different CVAs were carried out for each sex, the geographic regions listed in Table 1 and the subspecies status (as specified above) being used as criteria for the definition of *a priori* groups.

Comparisons of specimens from laboratory rearings with field-collected samples of their parental populations were carried out by CVA. If offspring from such cultures show a pattern of variation between rearings similar to that between corresponding field populations, this is taken as evidence for the genetic determination of morphometric variation. However, if differing environmental conditions determine to a large extent the variation between geographic regions, less variation between laboratory cultures is expected than between field populations, and patterns of variation between rearings will differ from the patterns between their parent populations.

The linear discriminant functions were recalculated with extended sample sizes. Multivariate standard distance was used as a measure of separation between groups. The discriminant functions were tested for reliability in two ways: by establishing percentages of individuals misclassified by the discriminant functions, and additionally by means of a cross-validation procedure (EFRON and GONG, 1983). For the latter, discriminant functions were recalculated omitting one sample location (test sample) from the analysis, and then the mean discriminant score of the test sample was evaluated. This step was repeated for all subspecies comparisons and all sampling locations in both sexes, yielding 106 separate analyses.

RESULTS

COMPARISON OF CHARACTER SETS

A reduced set of 12 characters was selected from the full set using stepwise discriminant analysis. This reduced set represents most body parts, although some imbalance exists. One length (variable 6) and three width measurements (19, 20, 23) of the head, two variables of thoracic (14, 16) and one of abdominal (26) width, one thoracic length (35), but no abdominal length measurements are included. Appendages are represented by lengths of one antennal (43) and three leg segments (49, 50, 53).

The congruence between character sets, measured as the product-moment correlation between first principal component (PC) scores from analyses using the reduced character set and all variables, is 0.98 for males and 0.97 for females. The first PC in males takes up 51% of the total variance in the analysis of the full character set, and 60% in the analysis of the reduced set. Corresponding figures for females are 45% and 53%. Correlations between second PC scores are 0.86 for males, where the second PC explains 8% of total variance in the full character set and 14% in the reduced set. For females, where second PCs explain 9% and 16% of total variance of the full and reduced character sets respectively, the correlation coefficient between these components is 0.88. Correlations of the third and following PCs are lower, and in females third and fourth components are interchanged with respect to those of the males. Because these components have considerably smaller eigenvalues, however, differences in components between the character sets are less important. Thus, the reduced character set represents the main patterns of variation in the data fairly well.

TABLE 3

Principal component analysis. Tabled values are correlations of the original variables with the first, second, and third PCs for males and females, and corresponding eigenvalues and percentages of total variance.

Character number	Males PC 1	Males PC 2	Males PC 3	Females PC 1	Females PC 2	Females PC 3
6	0.775	–0.043	0.267	0.727	–0.039	–0.482
14	0.714	0.062	–0.521	0.783	0.105	–0.218
16	0.845	0.141	–0.172	0.825	0.130	–0.011
19	0.880	0.225	0.045	0.862	0.287	–0.222
20	0.739	0.471	–0.054	0.739	0.549	0.102
23	0.483	0.681	0.386	0.524	0.604	0.423
26	0.767	0.138	–0.346	0.662	0.226	–0.206
35	0.924	–0.134	–0.046	0.927	–0.132	–0.003
43	0.851	–0.304	0.117	0.779	–0.439	–0.022
49	0.899	–0.274	0.102	0.879	–0.323	0.242
50	0.875	–0.249	0.138	0.858	–0.233	0.243
53	0.897	–0.295	0.134	0.869	–0.382	0.185
Eigenvalue	7.92	1.11	0.71	7.56	1.34	0.71
Percentage	66.0	9.3	6.0	63.0	11.2	6.0

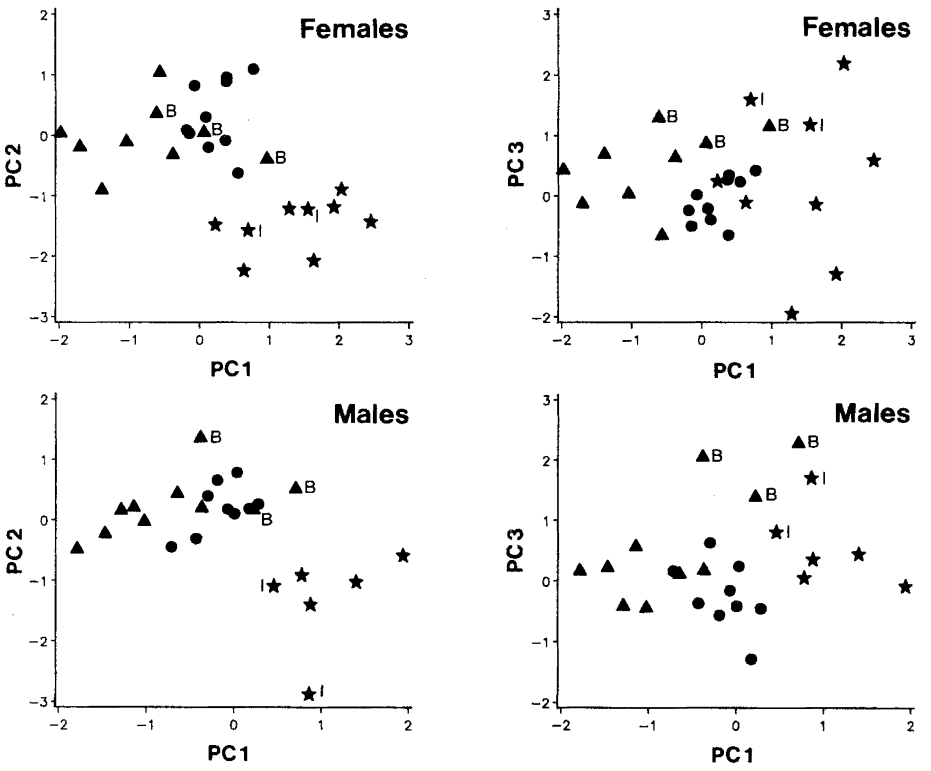

FIG. 4.

Principal component analysis. Plots of second and third versus first principal component scores for specimens from free-living populations. Points represent sample centroids. Symbols for subspecies: dots, *G. c. costae*; stars, *G. c. fieberi*: triangles, *G. c. poissoni*. Letters for geographic regions mentioned in the text: B, British Isles; I, Italy (Upper Italy and Calabria).

PRINCIPAL COMPONENT ANALYSIS

The PCAs, using the reduced character set and all specimens from free-living populations, revealed that about 80% of total variance is accounted for by the first three principal components (PCs; Table 3). The first PCs alone take up about two thirds of total variance. Scores of the first PCs have positive and relatively high correlations with all 12 original variables in both sexes. The character 23 (distance between second cephalic trichobothria) has distinctly smaller component correlations compared to the other characters. Some other differences in component correlations, although smaller, are also consistent in both sexes. The second PCs mainly contrast antennal and leg segments (characters 43, 49, 50 and 53; large negative component correlations) with measurements of head width (19, 20, 23; large positive component correlations). Correlations of the third PCs with the original variables are all low to moderate (positive for variables 49, 50, 53, and 23; negative for 14 and 26) for males and females, and show considerable differences between the sexes (especially in variables 6 and 19). As the proportion of total variance for which the third PC accounts is rather small, sampling error may be important in this component.

The patterns revealed by the first three PCs are similar for both sexes (Fig. 4). The plots of second versus first PC scores show rather clear discontinuities in the scatter of sample centroids (points representing sample means) between the presumed subspecies *G. c. fieberi* and the other groups. *G. c. costae* and *G. c. poissoni* are not clearly separable in these plots, although *G. c. poissoni* samples tend to have lower first PC scores than *G. c. costae* samples. Within *G. c. poissoni*, samples from the British Isles tend to have both higher first and second PC scores than samples from continental Europe. The one male *G. c. fieberi* from Upper Italy scores extremely low on the second PC, whereas the one from Calabria and the two females from Upper Italy are within the clusters of samples from Greece. The third PCs mainly contain variation within subspecies, but there is a tendency towards separation between *G. c. costae* and *G. c. poissoni* sample centroids, *G. c. costae* having lower third PC scores than most *G. c. poissoni* samples with similar first PC scores. Again, there is some differentiation between *G. c. poissoni* from the British Isles and continental Europe. Further, there is considerable variation among samples from Greece. In all three PCs considered here *G. c. costae* forms rather tight clusters, and thus there seems to be little geographic variation among the different regions of the Alps.

CANONICAL VARIATE ANALYSIS

The results of CVA, using the three presumed subspecies as an *a priori* classification criterion, are also very similar for the two sexes (Fig. 5). *G. c. fieberi* is again well separated from the other groups, mainly by the first CVs, which account for 73% of the between-subspecies variance in males and for 65% in females. The second CVs, which explain the remaining between-subspecies variance, separate *G. c. costae* and *G. c. poissoni* sample centroids into two distinct groups, although there is some overlap if one considers individual specimens. Two samples of male *G. c. poissoni* from the Pyrenees have considerably lower first and higher second CV scores than the other samples of this subspecies.

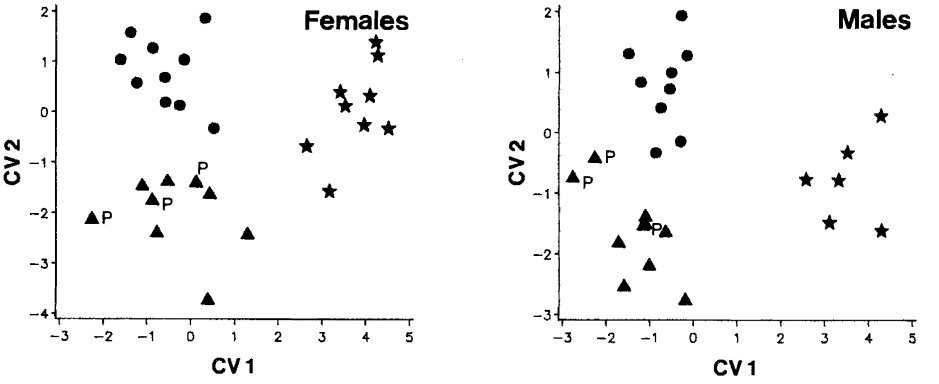

FIG. 5.

Canonical variate analysis, using subspecies status as *a priori* grouping criterion. Plots of second versus first canonical variates for specimens from free-living populations. Points represent sample centroids. Symbols for subspecies: dots, *G. c. costae*; stars, *G. c. fieberi*; triangles, *G. c. poissoni*. The samples from the pyrenees are labeled with the letter P.

If the 10 geographic regions listed in Table 1 are used as a grouping criterion for CVA instead of the presumed subspecies status, the plots of second versus first CV scores (Fig. 6) are strikingly similar to the results of the previous analysis. The first CVs explain 61% and 55% of between-region variance for males and females, the second CVs 23% and 28% respectively, and the third CVs 8% for both sexes. There is, mainly in the third CVs, substantial variation within subspecies, especially for *G. c. poissoni*, where samples from the French Massif Central have the lowest, and samples from the British Isles the highest third CV scores. Within *G. c. fieberi*, there is no clear distinction between Italian and Greek specimens.

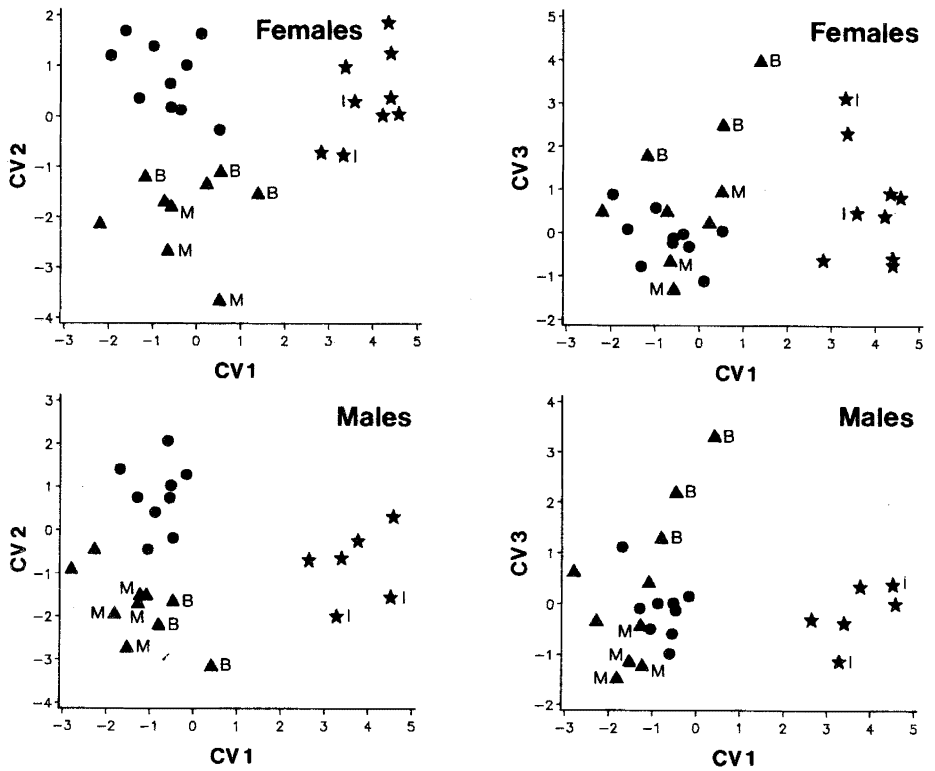

FIG. 6.

Canonical variate analysis, using geographic region as *a priori* grouping criterion. Plots of second and third versus first canonical variates for specimens from free-living populations. Points represent sample centroids. Symbols for subspecies: dots, *G. c. costae*; stars, *G. c. fieberi*; triangles, *G. c. poissoni*. Letters for geographic regions mentioned in the text: B, British Isles; I, Italy (Upper Italy and Calabria); M, French Massif Central.

LINEAR DISCRIMINANT ANALYSIS

As evidenced by the multivariate standard distances, linear discriminant analysis revealed a clear separation of *G. c. fieberi* from the other two subspecies, and a less important differentiation between *G. c. costae* and *G. c. poissoni* (Table 4). The coefficients of the discriminant functions are not easily interpretable, because the variances of the variables differ widely, as variables were not standardized prior to analysis, and because of intercorrelations of the variables. Yet it can be seen that variables 14, 16, and 23 are more important in differentiating between *G. c. costae* and *G. c. poissoni* than in other comparisons. Besides some similarities between sexes, there are also marked differences (e.g., in character 43).

TABLE 4.

Linear discriminant analysis. Subspecies considered for each pairwise comparison, coefficients of discriminant functions, and corresponding classification limits and standard distances (D_{12}). % mis (ssp. 1) and % mis (ssp. 2) denote percentages of misclassified specimens from subspecies 1 and 2, respectively.

Subspecies 1 Subspecies 2	*G. c. costae* *G. c. poissoni*		*G. c. costae* *G. c. fieberi*		*G. c. fieberi* *G. c. poissoni*	
Character	Males	Females	Males	Females	Males	Females
6	447.07	615.86	711.42	638.45	–116.09	–529.15
14	–139.24	– 94.33	5.45	– 0.40	– 23.67	0.29
16	184.46	162.05	– 49.31	– 15.17	86.75	28.95
19	–470.51	–501.79	–422.50	–381.20	189.59	314.19
20	–395.57	–354.37	–144.58	–366.43	–155.95	235.35
23	618.03	485.70	– 78.00	64.60	371.15	47.54
26	– 65.20	– 96.48	– 14.85	– 17.70	– 11.91	10.90
35	20.31	– 1.33	104.59	69.35	– 62.76	– 61.61
43	–198.00	– 62.59	142.40	97.07	–242.70	–110.50
49	–108.20	– 35.64	– 62.03	– 43.01	– 21.46	29.71
50	– 17.58	– 40.63	– 5.21	9.86	11.45	– 1.51
53	119.36	62.14	59.12	53.06	11.26	– 44.15
Intercept	648.05	675.96	–174.14	– 57.56	290.53	185.79
Limit	43.31	40.13	45.33	41.18	51.04	52.42
D12	2.74	2.63	4.52	4.40	4.72	4.54
% mis (ssp. 1)	7.6	10.2	0	0	1.5	0
% mis (ssp. 2)	6.8	9.3	1.5	2.4	0	1.3

Note. – For each comparison, subspecies 1 and 2 were coded 0 and 100, respectively. Thus, new samples should be allocated to subspecies 1 if their discriminant score is lower than the classification limit, and to subsepcies 2 if the discriminant score exceeds the limit.

The percentages of misclassified specimens (Table 4) should be interpreted with caution, because the discriminant functions were both computed and tested on the same set of data, and therefore the probabilities of misclassification tend to be underestimated (but see the results of cross-validation, below). Yet it can be seen that far more false classifications occur in discriminating between *G. c. costae* and *G. c. poissoni* than in the

other comparisons. The cross-validation procedure was designed to test the discriminant functions for errors in classifying sample means (centroids). In the 106 separate discriminant analyses so obtained, the test sample was always allocated to the correct subspecies, although the mean discriminant score of some samples differed from the classification limit by as little as 1.7. However, the discriminant functions can be said to be reliable tools for identifying subspecies, at least when sample means are considered.

Comparison of laboratory cultures and free-living parental populations

The results of CVAs show that laboratory cultures exhibit a pattern of variation clearly corresponding to the pattern among the respective parental populations (Fig. 7). It also closely resembles the results of CVAs for all samples from the field (Figs 5, 6). However, there is a consistent shift in first, second, and third CV scores from wild populations to their laboratory offspring.

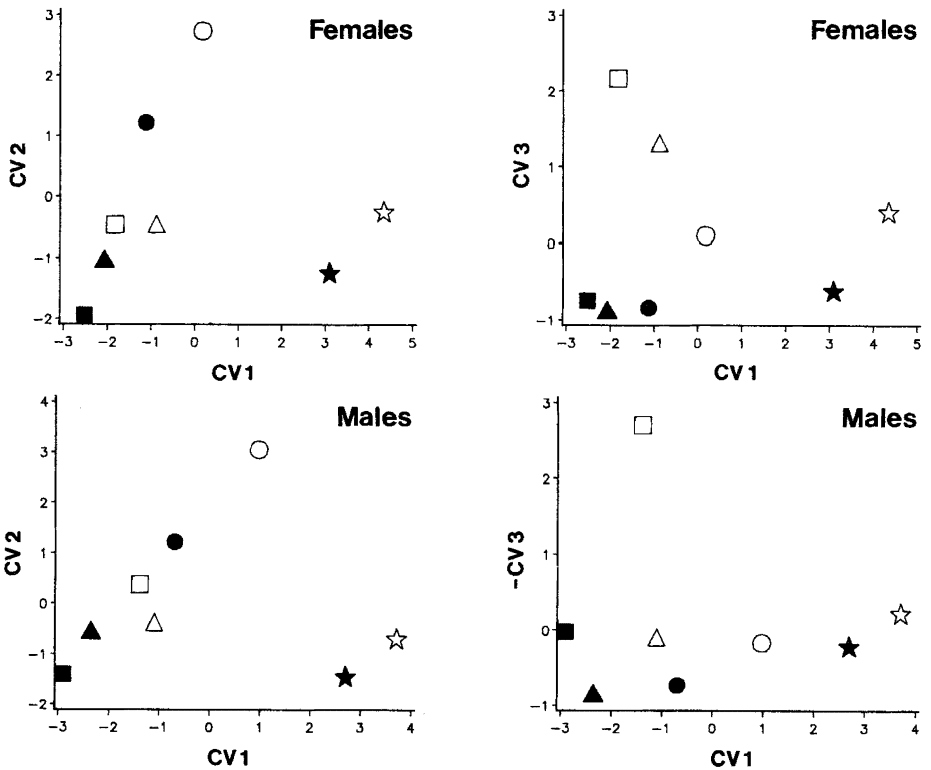

Fig. 7.

Comparison of specimens from laboratory cultures and corresponding free-living populations. Plots of second and third versus first canonical variate scores. Centroids of population samples and laboratory rearings are represented by solid and open symbols respectively: dots, central Alps; stars, northern Greece; squares, French Massif Central; triangles, Pyrenees.

A general pattern of variation in first and second CVs holds for both sexes in the laboratory cultures and in the field. The populations from the Pyrenees and the French Massif Central have both low first and second CV scores. The animals from the Alps have low to intermediate first but high second CV scores. The Greek specimens score high on the first and low on the second CV. For males 59% and 24% of between-group variance are explained by the first and second CV, and 59% and 22% for females, respectively. Only comparatively minor differences in the relative positions of the centroids of wild and laboratory samples occur, especially between the populations from the Pyrenees and the Massif Central. The upward shift in second CV scores between wild and laboratory-reared specimens seems to be less marked for the Pyrenees population than for the others. This is most clearly visible for males.

These differences become more apparent in the third CV, which accounts for 10% and 11% of between-group variance for males and females respectively. In females, this CV separates the parent populations from their laboratory offspring, most markedly for the specimens from the Pyrenees and the Massif Central. The sign of the third CV for males had to be reversed, but this does not influence the interpretation of patterns of variation. There is a large difference in third CV scores between wild and laboratory samples for the Massif Central population, but only small differences for the others. A considerable difference between sexes occurs in the relative position of the laboratory-reared specimens of the Pyrenees population.

PCAs carried out with specimens from laboratory cultures and corresponding parent populations revealed results similar to those for all field samples. Laboratory cultures always had higher scores than corresponding parent populations on the first PC which contained 'size' variation. Some smaller differences were also found in 'shape' components.

DISCUSSION

Three multivariate statistical procedures were applied to study different aspects of morphometric variation in *Gerris costae*. Principal component analysis (PCA) of all samples together was used as an ordination procedure to display the overall pattern of variability in fewer dimensions (PIMENTEL, 1979; REYMENT *et al.*, 1984). PCA does not require any *a priori* grouping of the specimens included in the analysis. However, it should be kept in mind that such an analysis reveals an overall pattern but does not distinguish variation within groups from differentiation among groups (THORPE, 1983; GIBSON *et al.*, 1984; AIROLDI and FLURY, 1988). Canonical variate analysis (CVA) is especially suitable to assess the pattern of variation between groups defined *a priori*, e.g., different free-living populations, and to compare laboratory-reared and field samples (ALBRECHT, 1980; CAMPBELL and ATCHLEY, 1981; REYMENT *et al.*, 1984). Finally, linear discriminant analysis was used to evaluate phenetic distance, and to classify additional samples into subspecies (PIMENTEL, 1979; REYMENT *et al.*, 1984).

The main patterns of variation in the full set of variables are well represented by a much smaller number of characters. Congruence between the full and reduced character sets, as measured by product-moment correlations of principal component scores, is comparable in magnitude to the corresponding asymptotic values in THORPE's (1985) computer simulations of character selection.

The results of PCA show that the overall pattern of variation concerns both 'size' and 'shape'. Especially *G. c. fieberi* differs considerably from the other groups in the first (mainly 'size') component, but is also clearly distinguished by the second PC. The other two subspecies are more similar, although *G. c. costae* scored somewhat higher on the

'size' component than *G. c. poissoni*, as is consistent with univariate measurements (WAGNER and ZIMMERMANN, 1955). In a similar study of the housefly and the face fly in the United States, BRYANT and TURNER (1978) also found congruence between sexes in PCAs, although it is noteworthy that they excluded one strongly dimorphic character from the analysis in males. The 'size' component (first PC) accounted for more than 83% and 90% of the total variance in the housefly and the face fly, respectively, whereas these figures are considerably lower in *Gerris costae* (66% and 63% in males and females). This may be due to the fact that in *G. costae* there is more geographic diversification in habitat and niche utilization, and thus increased morphometric differentiation, than in the two fly species, where even the more variable species was considered to have uniform ecological requirements across the United States (BRYANT, 1977).

The CVA using the 10 geographic regions of Table 1 as *a priori* group criterion revealed a pattern of variation of the first two canonical variates (Fig. 6) strikingly similar to that of the CVA using the three presumed subspecies as groups (Fig. 5). As the same pattern was revealed by CVAs with two different *a priori* grouping criteria, this pattern is not an artifact of the analysis using subspecies groups. In both sexes more than 80% of the between-region variance is accounted for by the first two CVs which clearly reflect the division into the three subspecies, whereas other variation patterns only appear from the third CVs onward, and explain comparatively minor portions of between-group variance. Thus the three subspecies recognized by WAGNER and ZIMMERMANN (1955) and NIESER (1978) in fact reflect the main pattern of geographic variation between samples from different regions of Europe. The variability within subspecies is greatest in *G. c. poissoni*, particularly reflecting some differentiation between populations from the British Isles and from continental Europe. This intrataxon variation makes discrimination between *G. c. costae* and *G. c. poissoni* somewhat difficult.

Linear discriminant analysis yielded results consistent with PCA and CVA. The standard distances indicate that *G. c. costae* and *G. c. poissoni* are more similar to each other (D_{12}-values about 2.6) than to *G. c. fieberi* (D_{12}-values about 4.5). Standard distance, which equals the square root of Mahlanobis' generalized distance D^2 (FLURY and RIEDWYL, 1986), can easily be compared among different studies. In their study of an aphid, FOOTITT and MACKAUER (1980) found three main phenetic groups which they later recognized as subspecies (FOOTITT and MACKAUER, 1983), with averaged standard distances between groups of about 4 to 5.3 (18 mensural characters). In a study of an aquatic beetle (ZIMMERMAN and LUDWIG, 1974), standard distances between samples ranged from about 1 to 6 (14 mensural characters), and partitioned the samples into two major groups. In the present study, error rates of discriminant functions in allocating individuals are comparable in magnitude to those of FOOTITT and MACKAUER (1980). The cross-validation test showed that the discriminant functions given here can be considered as reliable tools for the identification of samples or populations.

The main pattern of variation among free-living populations was also found in offspring reared under standardized laboratory conditions, suggesting a major role of genetic determination in explaining the observed geographic variation. This interpretation is consistent with the analyses of BRYANT (1977) and BRYANT and TURNER (1978), showing a considerable genetic component of geographic variation in two species of flies. In *G. costae* the effects of differences in environmental conditions between the habitats of the parental populations seem to be rather small compared to the effects imposed by the transfer to laboratory conditions. Thereby a factor of prime importance for explaining the increased size of laboratory-reared specimens probably is increased availability of food in captivity. Field enclosure experiments demonstrated that supplying *Gerris buenoi* larvae

with additional food increased the weight they achieved as adults (SPENCE, 1986), and also linear size (J. R. Spence, pers. comm.). Increased size in laboratory-reared specimens was also found in waterstriders of the genus *Limnoporus* (J. R. Spence, pers. comm.). In other studies of environmental factors, larval crowding (which relates to food availability; MURDIE, 1969; BLACK and KRAFSUR, 1986), food quality (BERNAYS, 1986), and temperature (MURDIE, 1969) have been reported to influence insect morphology. The relative importance and interactions of genetic and environmental determination of morphometric variation certainly merit further study. Crossbreeding experiments with specimens of different geographic origin revealed no evidence of premating barriers of F_1 hybrid inviability between presumed subspecies (pers. obs.; R. Hauser, C. Largiadèr, pers. comm.).

Although the objective of this paper is not a formal taxonomic revision of the European subspecies of *Gerris costae*, the results can be interpreted as supporting the segregation into the three subspecies recognized by WAGNER and ZIMMERMANN (1955) and NIESER (1978). They form coherent units characterized by genetically determined morphometric differences. Even in *G. c. poissoni*, which is the most heterogeneous group, a clear morphometric separation of the populations from the Brisith Isles from those of the European continent is not possible. The few specimens from Italy that were available for this study were similar to the animals from Greece, suggesting that Italy should be considered as part of the range of *G. c. fieberi* (this conclusion is supported by additional material, C. Largiadèr, pers. comm.). The subspecific status of *Gerris costae* in the USSR and western Asia (NIESER, 1978; KANYUKOVA, 1982) remains to be resolved.

Some interesting questions arise in relation to the distribution ranges of the subspecies. It is tempting to speculate about Pleistocene refuges of the present subspecies (see also JANSSON, 1980; THORPE, 1984). As a possible scenario it can be imagined that *G. c. fieberi* had its refuge in southeastern Europe or Asia Minor, the refuge of *G. c. poissoni* extended along the Atlantic coast from Iberia to the present British Isles (which was then contiguous because of the eustatic sea level drop), and *G. c. costae* remained between the Alpine and Scandinavian ice shields. This historical scenario suggests an explanation for the present distribution ranges and the ecological differentiation of the subspecies. In northern Italy and in northern Yugoslavia or southern Austria there are two areas where *G. c. costae* and *G. c. fieberi* possibly come into close contact (see Fig. 1). The ecological differences between these two subspecies, *G. c. costae* being restricted to high altitudes whereas *G. c. fieberi* also occurs at low elevations and in a wider range of habitat types, make such possible secondary contact zones particularly interesting. Further work on this topic is in progress (C. Largiadèr, pers. comm.).

SUMMARY

Geographic variation of *Gerris costae* (Herrich-Schaeffer) in Europe was studied by three multivariate morphometric methods. Twelve characters were selected from a larger set (56 or 57 variables for males and females, respectively) by means of stepwise discriminant analysis (backward elimination). Good congruence between full and reduced character sets was found. Various aspects of the patterns of geographic variation were assessed using principal component analysis and canonical variate analysis, and revealed three main groups consistent with the previously described subspecies *G. c. costae* (Herrich-Schaeffer), *G. c. fieberi* Stichel, and *G. c. poissoni* Wagner and Zimmermann. Comparisons of patterns of morphometric variation between populations of different geographic origin and between laboratory-reared offspring showed that geographic variation is genetically determined for the most part. Linear discriminant functions are provided to allocate new samples to the subspecies groups.

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REFERENCES

AIROLDI, J.-P. and B. K. FLURY, 1988. An application of common principal component analysis to cranial morphometry of *Microtus californicus* and *M. ochrogaster* (Mammalia, Rodentia). *J. Zool.* 216: 21-36.

ALBRECHT, G. H. 1980. Multivariate analysis and the study of form, with special reference to canonical variate analysis. *Am . Zool.* 20: 679-693.

ANDERSEN, N. M. 1982. The semiaquatic bugs (Hemiptera, Gerromorpha), phylogeny, adaptations, biogeography and classification. *Entomonograph 3. Scandinavian Science Press Ltd., Klampenborg.*

ATCHLEY, W. R. 1983. Some genetic aspects of morphometric variation. In: Numerical taxonomy: 346-363. J. Felsenstein (Ed.), *Springer Verlag, Berlin.*

BÄCHLER, M. 1985. Geographische Isolation und genetische Differenzierung des alpinen Wasserläufers *Gerris costae* (Hemiptera: Gerridae). *Lic. phil. nat. thesis, University of Bern.*

BARROWCLOUGH, G. F. 1982. Geographic variation, predictiveness, and subspecies. *Auk* 99: 601-603.

BERNAYS, E. A. 1986. Diet-induced head allometry among foliage-chewing insects and its importance for graminivores. *Science* 231: 495-497.

BLACK, W. C. and E. S. KRAFSUR, 1986. Geographic variation in house fly size: adaptation or larval crowding? *Evolution* 40: 204-206.

BLACKWELDER, R. E. 1967. Taxonomy: a text and reference book. *John Wiley & Sons, New York.*

BRYANT, E. H. 1977. Morphometric adaptation of the housefly, *Musca domestica* L., in the United States. *Evolution* 31: 580-596.

BRYANT, E. H. and C. R. TURNER, 1978. Comparative morphometric adaptation of the housefly and the face fly in the United States. *Evolution* 32: 759-770.

CAMPBELL, N. A. and W. R. ATCHLEY, 1981. The geometry of canonical variate analysis. *Syst. Zool.* 30: 268-280.

EFRON, B. and G. GONG, 1983. A leisurely look at the bootstrap, the jackknife, and cross-validation. *Am. Stat.* 37: 36-48.

ENDLER, J. A. 1977. Geographic variation, speciation, and clines. *Princeton University Press, Princeton, NJ.*

FLURY, B. and H. RIEDWYL. 1985. T^2 tests, the linear two-group discriminant function, and their computation by linear regression. *Am. Stat.* 39: 20-25.

— 1986. Standard distance in univariate and multivariate analysis. *Am. Stat.* 40: 249-251.

— 1988. Multivariate statistics: a practical approach. *Chapman and Hall, London.*

FOOTTIT, R. G. and M. MACKAUER, 1980. Morphometric variation between populations of the balsam woolly aphid, *Adelges piceae* (Ratzeburg) (Homoptera: Adelgidae), in North America. *Can. J. Zool.* 58: 1494-1503.

— 1983. Subspecies of the balsam woolly aphid *Adelges piceae* (Homoptera: Adelgidae), in North America. *Ann. Entomol. Soc. Am.* 76: 299-304.

GIBSON, A. R., A. J. BAKER, and A. MOEED, 1984. Morphometric variation in introduced populations of the common myna (*Acridotheres tristis*): an application of the jackknife to principal component analysis. *Syst. Zool.* 33: 408-421.

GROSSEN, B. and R. HAUSER, 1982. Über den Umgang mit Wasserläufern (Hemiptera, Gerridae). *Mitt. schweiz. entomol. Ges.* 55: 394-395.

HEISS, E. 1969. Zur Heteropteren-Fauna Nordtirols I: Wasserwanzen (Corixidae-Hydrometridae). *Alpin-Biol. Stud. Univ. Innsbruck* 3: 1-28.

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, plates 9-17.

JANSSON, A. 1980. Postglacial distributional history of the water boatman, *Arctocorisa carinata* (Heteroptera: Corixidae). *Entomol. Gen.* 6: 235-245.

KANYUKOVA, E. V. 1982. [Water-striders (Heteroptera, Gerridae) of the fauna of the USSR.] *Trudy Zool. Inst. Akad. Nauk SSSR* 105 (1981): 62-93. [In Russian.]

MAYR, E. 1982. Of what use are subspecies? *Auk* 99: 593-595.

MURDIE, G. 1969. Some causes of size variation in the pea aphid, *Acyrthosiphon pisum* Harris. *Trans. R. Entomol. Soc. Lond.* 121: 423-442.

NIESER, N. 1978. Heteroptera. In: Limnofauna Europaea: 280-285. J. Illies (Ed.), 2nd edn, *Gustav Fischer Verlag, Stuttgart.*

NIESER, N. and Z. MOUBAYED, 1985. Les Hétéroptères aquatiques du Liban I. Inventaire faunistique. *Annls. Limnol.* 21: 247-252.

PIMENTEL, R. A. 1979. Morphometrics. The multivariate analysis of biological data. *Kendall/Hunt Publishing Company, Dubuque, Iowa.*

POISSON, R. 1957. Hétéroptères aquatiques. Faune de France, Vol. 61. *Editions Paul Lechevalier, Paris.*

REYMENT, R. A., R. E. BLACKITH, and N. A. CAMPBELL, 1984. Multivariate morphometrics. 2nd edn, *Academic Press, London.*

RICHARD, G. 1967. Heteroptera. In: Limnofauna Europaea: 236-241. J. Illies (Ed.), 1st edn, *Gustav Fischer Verlag, Stuttgart.*

RUTTNER, F. 1988. Biogeography and taxonomy of honeybees. *Springer Verlag, Berlin.*

SAS INSTITUTE INC. 1985. SAS user's guide: statistics, version 5 edition. *SAS Institute Inc., Cary, North Carolina.*

SERVADEI, A. 1967. Rhynchota. Heteroptera-Homoptera-Auchenorrhyncha. Catalogo topografico e sinonimico. Fauna d'Italia. IX. *Edizioni Calderini, Bologna.*

SMITH, M. F. and J. L. PATTON, 1988. Subspecies of pocket gophers: causal bases for geographic differentiation in *Thomomys bottae*. *Syst. Zool.* 37: 163-178.

SORENSEN, J. T. and S. M. SAWYER, 1989. Assessing the multivariate evolutionary responses of phenological differentiation for sibling species: biosystematics in the *Macrosteles fascifrons* complex (Homoptera: Cicadellidae). *Ann. Entomol. Soc. Am.* 82: 250-261.

SPENCE, J. R. 1986. Relative impacts of mortality factors in field populations of the waterstrider *Gerris buenoi* Kirkaldy (Heteroptera: Gerridae). *Oecologia* 70: 68-76.

TAMANINI, L. 1979. Etterotteri acquatici (Heteroptera: Gerromorpha, Nepomorpha). Guide per il riconoscimento delle specie animali delle acque interne italiane. 6. *Consiglio nazionale delle ricerche, Verona.*

THORPE, R. S. 1983. A review of the numerical methods for recognising and analysing racial differentiation. In: Numerical taxonomy: 404-423. J. Felsenstein (Ed.), *Springer-Verlag, Berlin.*

— 1984. Primary and secondary transition zones in speciation and population differentiation: a phylogenetic analysis of range expansion. *Evolution* 38: 233-243.

— 1985. Character number and the multivariate analysis of simple patterns of geographic variation: categorical or "stepped clinal" variation. *Syst. Zool.* 34: 127-139.

WAGNER, E. and S. ZIMMERMANN, 1955. Beitrage zur Systematik der Gattung *Gerris* F. (Hemiptera-Heteroptera, Gerridae). *Zool. Anz.* 155: 177-190.

ZIMMERMAN, J. R. and J. A. LUDWIG, 1974. Multiple-discriminant analysis of geographical variation in the aquatic beetle, *Rhantus gutticollis* (Say) (Dytiscidae). *Syst. Zool.* 24: 63-71.

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