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Morphometric differentiation in populations of the Central European sculpin *Cottus gobio* L., a fish with deeply divergent genetic lineages

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Abstract: The morphological differentiation between four population collectives of the Central European sculpin *Cottus gobio* from southwestern Germany and adjacent France (the contact region of the Rhine, Danube, and Rhône drainage basins) was evaluated using multivariate analysis of 34 morphometric and 3 meristic characters. Body shape separated Neckarian and Danubian sculpins, Rhenish stocks taking a somewhat intermediate position. These morphological differences between populations from various drainage basins were slighter than the deeper allozyme differentiation observed in a previous study. However, both allozymes and morphometry indicated the same population groupings. It is concluded that *C. gobio* displays an accelerated rate of allozyme evolution, or that stabilizing selection conserves its phenotype despite ongoing evolution at the protein level. The morphological data do not support the distinction of species in the area of the Rhine/Danube watershed.

Résumé : Une analyse multidimensionnelle de 24 caractères morphométriques et de trois caractères méristiques a permis d'établir la différenciation morphologique entre quatre groupes de populations du chabot *Cottus gobio* du centre de l'Europe, dans le sud-ouest de l'Allemagne et la zone française adjacente (soit la région de contact entre les bassins hydrographiques du Rhin, du Danube et du Rhône). La forme du corps distingue les stocks de chabots de la Neckar et du Danube, les chabots de Rhénanie occupant une position plutôt intermédiaire. Ces différences morphologiques entre les populations des divers bassins sont moins importantes que ne le laissaient entendre les résultats de la différenciation des allozymes obtenus au cours d'études antérieures. Cependant, les deux types d'étude ont donné lieu aux mêmes regroupements de populations. Il semble donc que *C. gobio* ait subi un rythme accéléré d'évolution des allozymes ou que la sélection stabilisante ait fait en sorte que le phénotype soit maintenu en dépit de l'évolution enregistrée au niveau des protéines. Les données morphologiques ne justifient pas la distinction d'espèces différentes dans la zone de jonction des bassins hydrographiques du Rhin et du Danube.
[Traduit par la Rédaction]

Introduction

Sculpins of the genus *Cottus* (Cottidae, Scorpaeniformes) are small bottom-dwelling freshwater fish of Holarctic distribution. In North America, *Cottus* has undergone a significant radiation, producing some 30 species (Hocutt and Wiley 1986). However, a considerable degree of mensurable and meristic variation within most species has complicated taxonomic decisions (Daniels and Moyle 1984). Delimitation of North American sculpin species entirely on the basis of their external morphology is difficult, if not impossible (Maughan 1978). Europe is the home of only two species, the European sculpin, *Cottus gobio* L., and the Siberian sculpin, *Cottus poecilopus* Heckel, 1893. The taxonomic validity of a third species, *Cottus petiti* Bacescu and Bacescu-Mester, 1964, which is restricted to the upper part of the Lez River in southern France, has been debated (Koli 1969). Siberian sculpins are widely distributed throughout Eurasia westwards to the mountains of southern Poland (Witkowski 1979). *Cottus poecilopus* had become extinct in Germany by 1978 (Bless et al. 1994).

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This leaves *C. gobio* as the most widely distributed European representative of its genus. Its range extends continuously from northern Spain to Siberia (Witkowski 1995). McAllister and Lindsey (1961) suggested that *Cottus ricei* in North America may be most closely related to *C. gobio*. A number of taxonomic studies focusing on morphological variability (Heckel and Kner 1858; Gratzianov 1907; Bacescu and Bacescu-Mester 1964; Marinov and Dikov 1986) led to the acceptance of four subspecies: *C. g. gobio* from Central and Western Europe, the prickly sculpin, *C. g. koshewnikowi* Gratzianow, 1907 from northern and eastern Europe, *C. g. ferrugineus* Heckel and Kner, 1858 from northern Italy, Slovenia, and Croatia, and *C. g. haemusi* Marinov and Dikov, 1986 from the Vit River in Bulgaria, a Danubian tributary. The taxonomic validity of the first two subspecies is based on the study of sufficient specimens (Koli 1969; Witkowski 1979; 1995), but no thorough studies have been conducted to validate the latter two subspecies.

Morphometric studies on *C. g. gobio* have demonstrated conspicuous phenotypic variability, even among the stocks from a single catchment basin (Oliva 1960; Oliva and Hensel 1962; Škorepa 1967; Bacescu and Bacescu-Mester 1964; Koli 1969; Witkowski 1972, 1979, 1995). Our genetic inventory of 261 sculpins from 27 different populations in southwestern Germany and adjacent France, based on 29 allozyme loci, produced similar results (Riffel and Schreiber 1995). Sculpin stocks from the Neckar basin (a tributary of the Rhine, Atlantic drainage) and the Danube (Black Sea drain-

age), and from the rivers Rhine and Doubs (an affluent of the Rhône, Mediterranean drainage), differed at up to six loci fixed for different alleles, resulting in standard genetic distances (D) of up to 0.2267. Genetic distances and the numbers of loci fixed for different alleles between sculpins of the Neckar, the Rhine/Danube, and the Doubs fell within the range of valid, morphologically distinct sculpin species from North America and Japan (Zimmerman and Wooten 1981; Strauss 1986; Matthews 1980; Andoh and Goto 1988). Moreover, sharp genetic discontinuity has been disclosed within the catchment of the Rhine, separating sculpin populations of the Oberrhein (Fig. 1) from those of one of its affluents, the Neckar. Four loci fixed for different alleles between sculpins of the Oberrhein and Neckar indicate restricted gene flow between the stocks in these rivers, despite the absence of known present-day geographical, ecological, or anthropogenic barriers. Rhenish sculpins were more similar to Danubian stocks in their allozymes than were Neckarian sculpins to those living in other parts of the Rhine catchment. The previous allozyme study, therefore, suggested the existence of hitherto overlooked taxa within *C. gobio* in south-central Europe (Riffel and Schreiber 1995).

The study area is of considerable biogeographical interest for several reasons. It represents the contact zone between three major European rivers, the Rhine (Atlantic), the Danube (Black Sea), and the Rhône (Mediterranean Sea), with numerous connections and stream captures throughout the Pleistocene (Hantke 1993). Most of our study area was ice-free during the Pleistocene, allowing cold-adapted fish species to survive north of the Alpine ice sheet. Like many other cold-adapted fish species such as grayling (*Thymallus thymallus*), char (*Salvelinus alpinus*), and pike (*Esox lucius*), sculpins (*C. gobio*) met with suitable conditions during the Pleistocene glaciations in the ice-free belt in what is now central and southern Germany, intercalated between the northern and Alpine glaciers (Thienemann 1950). W. Torke (personal communication, 1996) found fossil remains of *C. gobio* in the upper Danube region of the German state of Baden-Württemberg, dated approximately 40 000 B.P. until the Middle Ages, indicating that sculpins persisted in southern Germany throughout the last (i.e., Würmian) glaciation. In the German state of Thuringia, otoliths of *C. gobio* have been found in pre-Würmian deposits from the Eemian interglacial phase (Hebig 1978).

The Pleistocene glaciations caused major changes in the ichthyofauna in temperate-zone rivers (Thienemann 1950). Many limnic organisms were forced to retreat to ice-free refugia at the height of these glaciations, extending their ranges again during the subsequent interglacial phases (Berg 1932). On a longer time scale, the drainage history of the two large rivers in southwestern Germany, the Rhine and the Danube, is characterized by the ongoing backward erosion of the Rhine itself and its main tributaries, the Main and the Neckar, into the Danubian drainage system. A number of previous Danubian affluents were captured by the Rhine, Main, and Neckar during the late Tertiary and Pleistocene (Hantke 1993), resulting in complex limnofaunal exchange (Thienemann 1950), which is easily recognizable in the allozyme differentiation patterns of *C. gobio* (Riffel and Schreiber 1995). The complex drainage history of our study region offers a feasible explanation of the population genetic pattern of *C. gobio*. The

deeply divergent genetic lineages identified by protein electrophoresis call for a more detailed investigation of the phenotypic differences between the population collectives of *C. gobio* than data supplied by older treatises on the regional fish fauna (Günther 1853; Heckel and Kner 1858; Klunzinger 1881; Siebold 1863). The purpose of this paper is to examine the extent of morphological divergence of *C. gobio* in the contact zone of the Rhine, Danube, and Rhône river basins in order to assess the congruence between allozymic and morphological variation.

Material and methods

Sampling

A total of 262 sculpins from 25 sampling sites were collected between 1992 and 1995 by electroshocking or the use of small hand nets. The collection sites (Fig. 1), river systems, and sample sizes are listed in Table 1. The specimens were pooled into four geographical collectives, Rhine (n = 62), Neckar (n = 83), Danube (n = 84), and Doubs (n = 33), according to their population genetic affiliations derived from our allozyme survey (Riffel and Schreiber 1995).

Thirty-four morphometric measurements and 3 meristic characters were recorded for each specimen, including 5 conventional morphometric variables: standard length (SL), total length (TL), orbit–operculum length (OO), orbit diameter (OD), and interorbital width (IO). Apart from these measurements, the truss system (Strauss and Bookstein 1982) served for the superior characterization of the body shape. Each fish was positioned in a straight posture on waterproof paper, 29 points were pinned, and the distances between the points were recorded to the nearest 0.1 mm (Fig. 2). D1-2 denotes the distance between landmarks 1 and 2.

Three meristic variables were counted, i.e., the ray numbers in the first and second dorsal fins and the anal fin. Fin-ray counts refer to the fin base and include each detectable ray, including the last two elements, even when these are close together at the fin base.

Since the size distribution of the four groups varied significantly, the size component had to be removed to avoid spurious differences between groups. The morphometric measurements were corrected for outliers and standardized to a grand mean standard length of 87.41 mm, using the formula

$$M_s = M_o(L_s/L_o)^b$$

where M_s denotes the standardized measurement, M_o the observed measurement, L_s the grand mean standard length for all specimens examined (87.41 mm), and L_o the standard length of the specimen. The regression coefficient, b, was estimated as the slope of the regression of log M_o on log L_o. This allometric regression is a useful method of removing the variation in measurements among individuals of different body sizes (Reist 1985). As the slopes of the log–log regressions of the morphometric characters against standard length did not differ significantly between groups (F test, Bonferroni correction, $p < 0.05$), a common coefficient, b, was used to standardize every character (Elliott et al. 1995). The log–log regressions of character D3-6 against body size deviated significantly from parallelism between groups. It was therefore excluded from further analysis.

All analyses were conducted on $\log_{10}$-transformed variables to improve the approximation to multivariate normality. The statistical analysis was performed without distinguishing the sexes. Morphometric and the meristic variables were analyzed separately (Edge et al. 1991). After the normality of the standardized morphometric characters had been verified, the differences between groups were examined by univariate methods (ANOVA), including an a posteriori Scheffé test. Since the precondition of normality was not fulfilled for meristic variables, these were examined by means of nonparametric tests.

Fig. 1. Collection sites of sculpin populations and drainage affiliations within our study area. Populations are identified in Table 1.
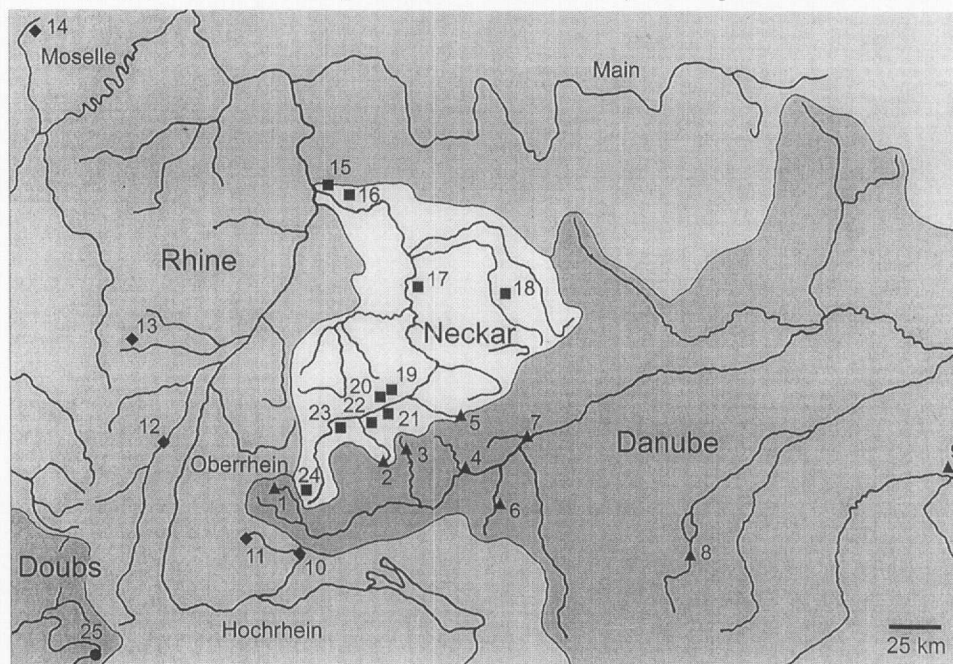
Fig. 2. Morphometric landmarks of the truss system following Strauss and Bookstein (1982). For example, truss variable D1-2 designates the distance between the landmarks 1 and 2.
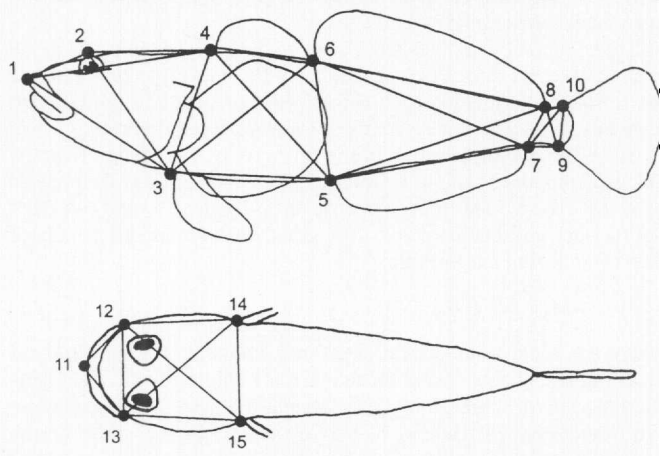
A principal component analysis (PCA), based on the covariance matrix of mensural characters, was performed to determine if the groups identified by means of protein electrophoresis (Riffel and Schreiber 1995) also differed in their morphology. Because size-adjusted measurements were entered into the PCA, the principal components (PC) extracted reflected shape differences only. This is confirmed by the absence of significant correlations between between PC scores and standard length. PC scores were compared among groups by univariate ANOVA (Pimentel 1979). Throughout our analysis the level of significance chosen was $p < 0.05$. Sequential Bonferroni tests were applied to adjust the level of significance in multiple tests (Rice 1989).

Results

Univariate analysis

The mean and standard deviation of each single morphological character for the four groups of *C. g. gobio* are shown in

Table 2. Standardized character measurements did not correlate significantly with standard body length. Hence, the size effect had been removed successfully by the allometric transformation procedure described above (cf. Methods). When mensurable characters were compared among groups, significant differences were found in 24 of the 32 measurements (Table 2).

Principal component analysis

A PCA on the covariance matrix of standardized morphometric data was performed to clarify if the groups identified by means of protein electrophoresis (Riffel and Schreiber 1995) could be recognized from morphometric variables. With the exception of the Doubs population, each of these groups consisted of several local populations. The first five principal components extracted reflected 64.8% of the total variance. As the mensural data were standardized for a common body size, the first principal component (PC1) reflected shape differences only and was not related to body length.

PC1 accounted for 20.86% of the total variation (Table 3). A simultaneous ANOVA performed on the PC1 scores distinguished all groups compared, except the Neckarian and Rhenish stocks, with high statistical significance.

Neckarian sculpins differed significantly from the Doubs and Danube groups, as indicated by an a posteriori Scheffé test. Significance was consistently higher when the Danubian sculpins were included in comparisons. Sculpins from the Rhine and those from the Neckar did not differ significantly. PC1 weighted characters of head dimensions (length and width) most heavily (D11-12, D11-13, D12-15, D13-14, D12-13) (Fig. 2, Table 4). All factor loadings of these variables were positive (Table 4). The factor values of Neckarian and Rhenish sculpins ranged predominantly in the negative portion of the multivariate space, while those of the Danubian and Doubs stocks were positive (Fig. 3). When related to the same standard length, Neckarian and Rhenish sculpins were

Table 1. Sample locations and numbers of *Cottus gobio* used in this analysis.

Collective and sample	No.	Drainage system	Sample size
Danube			
Kirnach	1	Danube	2
Steinlach*	2	Neckar	2
Lauchert	3	Danube	2
Schmiech	4	Danube	25
Fischbach*	5	Erms—Neckar	17
Umlach	6	Riss—Danube	21
Blau	7	Danube	3
Hardtbach	8	Isar—Danube	7
Tuerkenbach	9	Inn—Danube	5
			84
Rhine			
Josbach	10	Hochrhein	25
Wutach	11	Hochrhein	3
Ill	12	Oberrhein	2
Fischbaechle	13	Oberrhein	26
Muehlbach	14	Mosel—Mittelrhein	6
			62
Neckar			
Gruppenbach	15	Kocher—Neckar	20
Eiterbach	16	Steinach—Neckar	28
Katzenbach	17	Neckar	14
Kurzach	18	Murr—Neckar	2
Ammer	19	Neckar	3
Aischbach	20	Neckar	3
Eschach	21	Neckar	3
Stunzach	22	Eyach—Neckar	5
Goldersbach	23	Neckar	3
Landgraben	24	Neckar	2
			83
Doubs			
Doubs	25	Saône—Rhône	33

Note: The samples are pooled into geographic collectives according to their population genetic affiliations (Riffel and Schreiber 1995). The collection sites are shown in Fig. 1.

*Although these stocks inhabit tributaries of the Neckar River, they clearly belong to the "Danubian" group according to the population genetic data and thus are considered to have been relocated after stream capture (Riffel and Schreiber 1995).

characterized by shorter and broader heads, while the heads of the Danubian and Doubs stocks tended to be elongated and smaller. Rhenish sculpins occupy an intermediate position between the collectives from the Neckar and Danube, with a strong affiliation to the Neckarian stocks.

PC2 accounts for 18.10% of the total variance, summarizing various length dimensions of the tail (D7-9, D8-10, D8-9) (Tables 3, 4). Significant differences in the factor scores could not be observed between the groups (Table 3). This variance is not explained by divergence between the groups, but could be caused by intracollective variance (e.g., individual differences in breeding state, sex, or age). PC3 was determined by characters connected with head length (D12-14, D13-15); it accounted for 12.37% of the total variance (Table 4). The factor loadings of these variables were negative. Neckarian sculpins differed significantly from all other groups with generally negative factor scores, reflecting their shorter head. Significant differences were also observed between Rhenish sculpins and those from the Doubs. The remaining principal

components, PC4 and PC5, explained 8.15 and 5.32% of the total variance, respectively (Table 3), D7-8 and D3-5 being the variables with high loadings. PC4 separated the Rhenish stock from all other groups and PC5 distinguished the Rhenish sculpins from the Neckar and Doubs collectives.

Accordingly, PCA of morphometric characters recognizes the groups defined by means of protein electrophoresis, at least as far as the Neckarian and Danubian sculpins are concerned. This conclusion is clearly illustrated by a plot of PC scores, i.e., PC1 against PC3 (Fig. 3). Despite some overlap, the majority of Neckarian and Danubian sculpins aggregate at opposite positions in the multivariate space, while groups from the Rhine and the Doubs resided within the range of phenotypic variation of the Neckarian and Danubian stocks. Rhenish sculpins strongly resemble the Neckarian population.

Meristic variables

Significant deviations from the normal distribution within geographical collectives were observed for all three meristic

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Table 2. Means and standard deviations of the size-adjusted mensural characters of population samples of *Cottus gobio gobio* (grand mean standard length 87.41 mm) and *F* values from the analysis of variance ($p < 0.05$).

Variable	Neckar		Rhine		Danube		Doubs		$F_{[3,262]}$
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
TL	102.33	1.38	101.98	1.22	102.31	1.90	102.18	1.90	0.893
OO	13.48	1.02	13.58	0.92	14.69	1.12	14.85	1.03	29.75
OD	4.34	0.45	3.99	0.44	4.24	0.44	4.10	0.54	7.37
IO	7.39	0.96	7.42	0.90	8.34	0.89	8.25	0.68	21.51
D1-2	9.95	0.80	10.04	0.93	10.46	0.84	11.00	1.04	13.22
D1-3	22.34	1.55	22.34	2.25	23.98	1.88	23.10	1.84	13.55
D3-4	16.67	1.92	17.24	2.07	17.95	1.41	17.60	1.34	7.61
D2-4	19.65	1.27	20.24	1.40	20.80	1.29	19.95	1.87	9.94
D1-4	28.23	1.72	29.10	1.40	30.19	1.55	29.28	1.59	22.30
D2-3	19.41	1.26	19.39	1.99	20.65	1.50	20.23	1.67	10.99
D3-5	27.13	1.73	29.27	2.76	27.27	1.83	26.83	1.87	17.85
D4-6	16.71	1.34	17.55	1.63	17.22	1.42	18.82	1.57	17.78
D5-6	15.66	1.20	16.13	1.19	16.38	1.24	15.20	1.66	9.35
D4-5	26.78	1.72	27.59	1.59	27.14	1.40	26.81	1.41	3.94
D5-7	25.15	1.97	23.43	2.12	23.12	1.60	25.45	1.54	26.23
D6-8	36.58	1.63	35.34	2.06	34.07	1.78	34.80	1.82	27.40
D7-8	10.27	1.11	10.46	1.22	10.49	1.12	10.05	1.05	1.56
D5-8	33.14	1.65	31.76	1.76	31.17	1.90	32.92	2.35	18.86
D6-7	32.41	1.72	30.95	2.38	30.26	1.74	30.56	1.37	20.70
D7-9	13.00	1.66	14.10	1.78	13.81	1.70	14.44	1.30	7.87
D8-10	6.30	1.24	6.44	1.33	6.77	1.44	6.98	1.38	2.44
D9-10	7.74	0.67	7.88	0.77	7.97	0.67	7.92	0.80	1.35
D7-10	15.29	1.57	15.65	1.75	15.88	1.79	15.59	1.23	1.93
D8-9	9.3	1.00	10.02	1.09	10.38	1.10	10.50	1.27	4.46
D11-12	9.30	1.48	8.85	1.33	9.80	1.23	8.87	0.85	7.66
D12-14	12.55	1.42	12.73	1.45	14.23	1.64	14.13	1.43	24.87
D11-13	9.55	1.66	9.30	1.95	10.37	1.46	9.11	0.64	7.43
D13-15	12.20	1.67	12.71	1.79	13.95	1.63	13.93	1.29	20.96
D14-15	20.26	1.74	21.53	1.57	21.56	1.24	22.52	1.04	23.45
D12-15	21.25	1.44	21.80	1.85	23.20	1.44	22.81	1.18	26.04
D13-14	21.30	1.59	21.61	1.72	22.96	1.30	22.49	1.20	19.96
D12-13	14.54	1.93	14.29	1.85	15.23	1.81	14.06	1.14	4.61

Note: For an explanation of variables see the text. Significant *F* values are indicated by shading.

variables, i.e., the ray counts in the first and second dorsal fins and the anal fin (Kolmogoroff–Smirnoff test, $p < 0,05$). Various comparisons of geographical groups by means of nonparametric tests yielded significant differences between groups (Kruskal–Wallis ANOVA, $p < 0,05$) (Table 5).

Neckarian sculpins
Most Neckarian sculpins (82%) possessed 17 or more rays in the second dorsal fin, but Danubian sculpins possessed 16 rays or fewer. Numbers also differed, though less significantly, in the anal fin: more than 77% of the Neckarian sculpins had 13 anal fin rays or more, while 73% of the Danubian stock had 12 rays or fewer. Sculpins from the Neckar had predominantly seven rays in the first dorsal fin (or fewer), but most Doubs sculpins had eight such rays or more. Fin-ray numbers did not separate the Neckarian and Rhenish sculpins.

Rhenish sculpins
Three-quarters of the Rhenish stock did not exceed seven rays in the first dorsal fin, while more than 80% of the Doubs sculpins had eight or more rays. This numerical difference proved to be highly significant ($p < 0.01$). Approximately two-thirds of the Rhenish sculpins had 17 rays (or more) in the second dorsal fin, but the ray count in 87% of the Danubian sculpins was 16 or fewer.

The meristic characters of the Neckarian and Rhenish sculpins differed only slightly; no statistical significance was recorded. The Rhenish sculpins tended to have fewer than 13 rays in the anal fin, but the Neckarian sculpins exceeded this number.

Danubian sculpins
The Danubian sculpins were characterized by 16 (or fewer) rays in the second dorsal fin and 12 (or fewer) in the anal fin, compared with 17 rays in Neckarian and Rhenish sculpins. Another significant difference between the Neckarian and Danubian sculpins was the number of rays in the anal fin, with the Danubian stocks predominantly having 12 rays or

Table 3. Relative contribution of five principal components (PC1-PC5) to the total morphological variance, and probabilities of univariate post hoc Scheffé tests of principal component scores between the four sample collectives of *Cottus gobio gobio*.

	Rhine	Danube	Doubs
PC1 (20.86%)			
Neckar	ns	<0.0001	<0.001
Rhine		<0.0001	<0.05
Danube			<0.05
PC2 (18.1%)			
Neckar	ns	ns	ns
Rhine		ns	ns
Danube			ns
PC3 (12.37%)			
Neckar	<0.05	<0.001	<0.0001
Rhine		ns	<0.05
Danube			ns
PC4 (8.15%)			
Neckar	<0.001	ns	ns
Rhine		<0.05	<0.05
Danube			ns
PC5 (5.32%)			
Neckar	<0.001	ns	ns
Rhine		ns	<0.01
Danube			ns

Table 4. Factor loadings of the first five principal components (PC1-PC5).

	PC1	PC2	PC3	PC4	PC5
GL	0.124	−0.140	0.058	0.054	−0.003
OO	0.578	0.010	−0.292	0.087	0.118
OD	0.156	−0.281	0.082	0.196	0.083
IO	0.592	−0.113	−0.321	0.039	0.111
D1-2	0.388	−0.061	−0.134	0.146	0.177
D1-3	0.549	−0.242	−0.075	0.211	0.350
D3-4	0.509	0.004	−0.175	−0.144	−0.491
D2-4	0.381	−0.116	−0.280	−0.107	−0.100
D1-4	0.533	−0.143	−0.367	−0.064	0.028
D2-3	0.619	−0.239	−0.103	0.144	0.105
D3-5	−0.186	0.164	−0.079	−0.365	−0.720
D4-6	0.014	0.108	−0.262	−0.133	−0.385
D5-6	0.305	−0.137	−0.001	0.035	−0.191
D3-6	0.219	0.151	−0.313	−0.333	−0.707
D4-5	0.013	−0.006	−0.102	−0.082	−0.534
D5-7	−0.297	−0.382	0.059	0.561	0.265
D6-8	−0.469	−0.445	0.270	−0.049	0.340
D7-8	0.234	−0.048	0.078	−0.788	0.354
D5-8	−0.223	−0.449	0.115	−0.007	0.490
D6-7	−0.371	−0.404	0.215	0.475	0.154
D7-9	0.214	0.612	0.114	−0.585	0.158
D8-10	0.135	0.928	0.106	0.290	0.009
D9-10	0.286	−0.029	−0.018	0.116	0.139
D7-10	0.204	0.544	0.123	−0.525	0.330
D8-9	0.291	0.698	0.073	0.293	0.015
D11-12	0.663	−0.192	0.574	0.055	−0.074
D12-14	0.485	0.042	−0.650	0.110	0.077
D11-13	0.664	−0.166	0.570	−0.004	−0.070
D13-15	0.478	−0.019	−0.715	0.025	0.098
D14-15	0.499	−0.029	−0.181	−0.099	−0.155
D12-15	0.864	−0.144	−0.241	0.021	−0.045
D13-14	0.858	−0.126	−0.192	0.083	−0.024
D12-13	0.709	−0.273	0.492	0.062	−0.062

Note: For an explanation of variables see the text.

fewer and the Neckarian sculpins 13 or more. Danubian sculpins differ from the Doubs stock in having, on average, 1 ray fewer in the first dorsal fin (i.e., 7 rays) and 1 ray more in the anal fin (i.e., 13).

Doubs sculpins

The majority (>80%) of the Doubs sculpins had eight or more rays in the first dorsal fin. This is a major difference from all other groups and yielded high statistical significance in each comparison (Kruskal—Wallis ANOVA, $p < 0.05$). Approximately 70% of the Doubs sculpins had 13 rays (or more), and more than 90% of the Danubian stock had 12 rays (or fewer). Doubs sculpins did not differ in any other meristic character from the other stocks.

In conclusion, the Neckarian sculpins differed from the Danubian group chiefly by having different body proportions, i.e., a relatively shorter head and longer posterior parts of the body, concomitant with a greater number of rays in the second dorsal fin. Rhenish sculpins emerged as the collective least differentiated from all the others, occupying an intermediate position between Neckarian and Danubian sculpins in both mensurable and meristic measurements.

Discussion

Cottus gobio has been the subject of a number of morphometric analyses (Oliva 1960; Oliva and Hensel 1962; Škorepa 1967; Koli 1969; Witkowski 1972; 1979; Marinov and Dikov 1986). Most authors have emphasized the tendency of this species to diversify into geographical stocks that can be distinguished by means of univariate statistical analysis. In these studies, data were commonly standardized by using each

character as a percentage of standard body length. However, this is an inadequate method for standardizing morphometric data efficiently (Reist 1985). Investigations applying multivariate statistics are lacking. In the most extensive morphometric analysis of *C. gobio*, Koli (1969) concluded that the number of fin rays did not vary in Scandinavian sculpins. Scandinavian populations allocated to the subspecies *C. g. koshewnikowi* reportedly have a tendency towards reduced dorsal fin-ray counts compared with *C. g. gobio* (Koli 1969). However, an examination of 1393 sculpins from 54 populations in Poland did not confirm this difference, although both subspecies were included (Witkowski 1995). *Cottus gobio* gobio and *C. g. koshewnikowi* differ by the latter having the lateral line partly reduced and complete prickling of the body. Both subspecies intergrade in a zone that extends from eastern Poland to northern Scandinavia (Koli 1969; Witkowski 1979, 1995). Northern Italy, Slovenia, and Croatia are the home of *C. g. ferrugineus* Heckel and Kner, 1858, which is characterized by a smaller number of rays in the unpaired fins. However, the description of this taxon is not

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Fig. 3. Plot of principal component scores (PC1 against PC3). ■, Neckarian sculpins; ◆, Rhenish sculpins; ▲, Danubian sculpins; ●, Doubs sculpins.
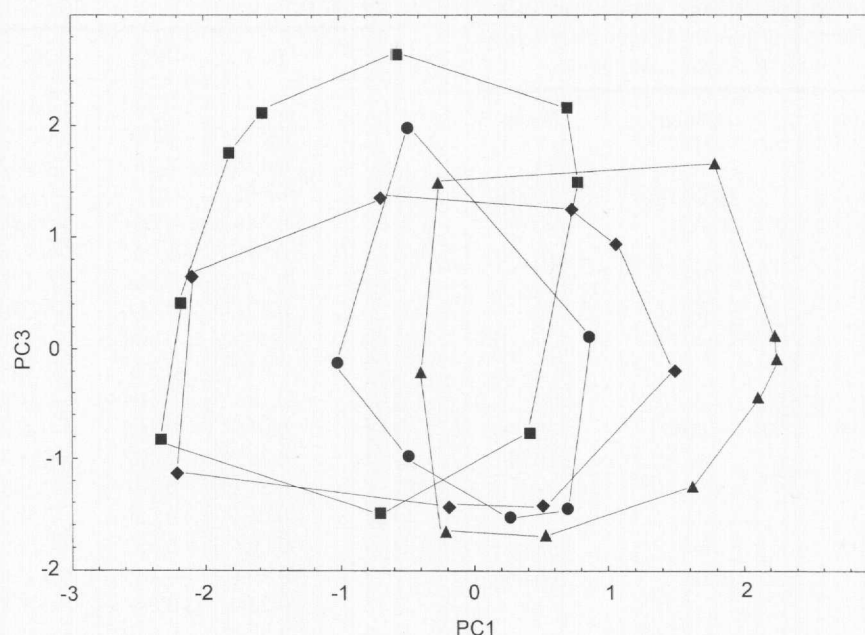
Table 5. Ray counts (means ± standard deviation) in the first and second dorsal fins and the anal fin of four groups of *Cottus gobio* from different drainage basins.

	First dorsal fin	Second dorsal fin	Anal fin
Neckar (1)	7.0±0.56	17.2±0.81	13.0±0.70
Rhine (2)	7.0±0.73	16.8±0.72	12.5±0.77
Danube (3)	6.5±0.70	16.0±0.60	12.1±0.67
Doubs (4)	7.9±0.60	16.4±0.87	13.0±0.39
Comparison with significant differences*	1 vs. 4, 2 vs. 4, 3 vs. 4	1 vs. 3, 2 vs. 3	1 vs. 3, 3 vs. 4

*Mann—Whitney test, $p < 0.05$ (Bonferroni-corrected).

based on a thorough study. Marinov and Dikov (1986) described *C. g. haemusi* from the Vit River system in Bulgaria, which is a tributary of the Danube. According to their findings, *C. g. haemusi* is characterized by a very short head relative to standard length, but morphometric data from only 30 fish from one population were compared with those on much larger collectives of sculpins from the literature. Therefore, the validity of *C. g. haemusi* has been doubted (Witkowski 1995).

Our results demonstrate a significantly longer head for sculpins of the upper Danube basin than for stocks from the Neckar, Rhine, and Doubs. The fin-ray counts in *C. g. haemusi* did not coincide with those in the Danubian sculpins analyzed by us. A thorough taxonomic review of *C. gobio* throughout its range is lacking. None of the specimens analyzed by us demonstrated such qualitative markers as lateral prickles or an incomplete lateral line. All sculpins analyzed by us matched the phenotype of the nominate subspecies *C. g. gobio*. Univariate and multivariate statistics nevertheless demonstrated morphometric and meristic variation between four regional groups from the contact area of the catchment basins of the Rhine, Rhône, and Danube. No quali-

tative character has been revealed that permits the unequivocal allocation of specimens to a geographical grouping. This lack represents a striking difference from the divergence between the same groups revealed by allozyme electrophoresis (Riffel and Schreiber 1995). Allozymes and morphometrics clearly separate the Neckarian and Danubian stocks, but the morphometric difference is recognized through multivariate statistical analysis only. This might be one of the reasons why these populations of *C. gobio* have not been split, despite the deep separation of genetic lineages, into several taxa by previous investigators (Günther 1853; Heckel and Kner 1858; Siebold 1863; Klunzinger 1881). Body shape chiefly differentiated the Neckarian and Danubian sculpins (which had already emerged as distinct groups in the previous population genetic analysis). The Rhenish stock took a somewhat intermediate position in terms of biometric and meristic variables, as it did in the allozyme survey. Interestingly, although their restricted sample size limits conclusions, Doubs sculpins more closely resembled the Danubian group than the other groups in the multivariate space, indicating a similar body shape (although the Doubs sculpins were much smaller, on average). The morphological similarity of the Danubian and

Doubs sculpins affects a population pair separated by a electrophoretic standard distance (D) of 0.2174 (Riffel and Schreiber 1996). Comparable genetic distances have been obtained from morphologically distinct North American sculpin species (Zimmerman and Wooten 1981; Strauss 1986; Matthews 1980). The Neckarian sculpins differed from the Danubian group chiefly by having a comparatively shorter head, a longer posterior part of the body, and a higher number of rays in the second dorsal fin. An elongated head, concomitant with shorter dimensions of the posterior body and fewer rays in the second dorsal fin, characterizes the Danubian sculpins. Differences in the latter variable might have occurred purely by chance, because the ray counts in the second dorsal fin of both stocks fit well into the range (15.28–17.83) observed by Witkowski (1995) in sculpins from Poland and by Koli (1969) in Scandinavian populations (range 15.9–17.5). Anal fin ray counts were highest in the Neckar and Doubs sculpins (13.0) and lowest in the Danubian sculpins (12.0). Again, the Rhenish stock was found to intercalate between Neckarian and Danubian sculpins. These values agree with the ranges found by Koli (1969) and Witkowski (1995). Doubs sculpins were distinguished from all other stocks, where the majority of specimens had seven or six rays, by having predominantly eight rays in the first dorsal fin. In 102 Scandinavian sculpin populations, Koli (1969) observed a mean of 7.1 rays (range 6.6–7.6). In 54 popuations from Poland, the numbers of rays in the first dorsal fin ranged between 6.3 und 7.9, the majority of specimens ($n = 1202$) having 7 (Witkowski 1979). In *C. g. haemusi* from Bulgaria, the mean fin-ray count in the first dorsal fin was 7.97 (range 7–9) (Marinov and Dikov 1986). The highest fin-ray count in the second dorsal fin was found in Neckarian sculpins (mean 17.2) and the lowest in the Danubian stock (mean 16.0). Both Rhenish and Doubs stocks were intermediate. Again, these numbers fit well into the ranges of 15.3–17.8 (Witkowski 1995) and 15.9–17.5 (Koli 1969). The data from our study and those presented by Witkowski (1979) and Koli (1969) suggest that there might be a northward clinal increase in number of rays in the second dorsal fin. D.E. McAllister (personal communication in a letter, 1997) found a strong meristic northward cline in *Cottus ricei*, a close relative of the Palearctic *C. gobio*.

A thorough morphometric analysis of *C. gobio*, based on multivariate statistics, with which to compare our results is lacking. In a PCA of 30 morphometric characters, Strauss (1986) could distinguish *Cottus cognatus* from *Cottus bairdi* stocks from the Susquehanna River drainage in Pennsylvania. A comparison of three populations of *Cottus klamathensis*, allocated to three different subspecies from different drainage systems, produced the high rate of over 95% correct assignments in a discriminant function analysis (Daniels and Moyle 1984). However, the number of individuals per group, and thus the intrasample variation, were considerably lower than in our study. Lyons (1990), using comparable morphometric and statistical approaches to analyze the phenotypic divergence of nine populations of *C. cognatus* in the north-central United States, found only slight differences among populations. Lyons (1990) concluded that the study population had not originated from different glacial refugia. Unfortunately, an accompanying allozyme survey is absent.

There are no allozyme or other genetic data with which to compare the extensive results of Witkowski (1979, 1995) and Koli (1969), and both studies were also based on many more specimens collected from large areas than our study, two differences with obvious effects on in-group variance. Although our study area is centered on the German state of Baden-Württemberg, with an area of approximately 35 000 km² only, different meristic counts were observed that might indicate local population divergence. When Neckarian and Rhenish sculpin populations were grouped together, as they inhabit the same (i.e., Rhine) drainage system, the otherwise clear morphological differences between Neckarian and Danubian sculpins were obfuscated because of the intermediate position of the Rhenish population. Only the previous results of our population genetic analysis (Riffel and Schreiber 1995) enabled some degree of morphological divergence to be detected, because geographical populations were grouped according to their genetic affiliations. Basing the clustering of geographical groups on the drainage basins only is evidently dangerous in a region with pronounced fluvial dynamics, characterized by many captured and diverted brooks and rivers, owing to the backward erosion of the Atlantic Rhine drainage basin into the elevated Danube area, which continues to lose ground.

The Danube River basin has been the main Pleistocene refugium for Central European freshwater fishes (Berg 1932; Banarescu 1991). After the retreat of the Alpine ice sheet at the end of the various Pleistocene glaciation phases, numerous temporary pathways enabled the exchange of limnic organisms between the Danube and Rhine river basins (Thienemann 1950), as did the many captures of the Rhine, Main, and Neckar. The modern limnofauna of the Rhine is composed of several faunistic layers originating from the different immigration phases, e.g., from the Danube and to a lesser extent the Rhône.

Our comparison of morphological and allozyme differentiation in *C. gobio* indicates that European sculpins represent a morphologically conservative taxon (species) containing deeply divergent, but phenotypically rather cryptic, genetic lineages. These intergrade into one another at certain locations but meet at others, exhibiting relatively sharp genetic discontinuities. Similar zoogeographical patterns have been found for North American and Japanese *Cottus* species (Strauss 1986; Andoh and Goto 1988). In conclusion, either the rate of biochemical evolution is enhanced in sculpins, or natural selection decelerates the evolutionary transformation of the external phenotype. We are unable to explain this phenomenon at present, but a number of possibly relevant arguments may be mentioned. As discussed before, the degree of genetic drift may be fairly high in philopatric species with a very limited capacity for dispersal (Riffel and Schreiber 1995). The local genetically effective population sizes may therefore be rather small. Moreover, *C. gobio* has a polygynous mating system with female mate choice (Bisazza and Marconato 1988). Polygyny might accelerate the fixation of allozyme polymorphism between demes (Riffel and Schreiber 1995; Schreiber et al. 1998). Species exhibiting a similar mating system are prone to phenotypic divergence in the males, owing to the females' preference for male phenotypes (Schreiber et al. 1997, 1998). However, sexual dimorphism in the sculpins studied by us appeared to be rather slight, except for the larger body size of males. During the breeding season, male *C. gobio*

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can be recognized from their darker head and by a yellowish edge along the first dorsal fin (Smyly 1957). Stabilizing natural selection could also preserve the morphotype in the harsh environment of fast-running streams, where sculpins coexist with competing carnivores (e.g., salmonids). Danubian fish species with the life strategy of a sluggish benthic ambush hunter include, apart from the cottid *C. gobio* itself, the percid *Romanichthys valsanicola* and the gobiid *Proterhorinus marmoratus*. These fish seem to occupy comparable niches and are quite similar in appearance.

Sculpins persisted throughout the Pleistocene in the ice-free areas of Central Europe and North America (Cumbaa et al. 1981; Lindsey and McPhail 1986; W. Torke, personal communication, 1996), i.e., sufficient time may have passed to allow the evolution of deeply divergent population lineages in situ. According to D.E. McAllister (personal communication in a letter, 1997), *Cottus* species fall into two geographical groups: periglacial species such as the Palearctic *C. gobio* and the Nearctic *C. cognatus*, which may have geographically differentiated populations because of differences in the degree of isolation during glaciation and in subsequent migration patterns, and the more southerly species, which survived south of the glaciations and may possess more stable populations not influenced as greatly by glacial events.

Further, relative phenotypic stasis, concomitant with biochemical–genetic divergence among populations, is a common feature of ancient relict taxa; Schreiber et al. (1992) could distinguish the sibling species *Priapulus caudatus* and *P. tuberculatospinosus* (phylum Priapulida) from circum-Arctic or circum-Antarctic oceans by the molecular masses of blood serum peptides despite their almost identical external appearance, and Schreiber et al. (1996) observed that these phenotypically very similar but grossly disjunct priapulid sibling taxa did not share identical allozyme alleles at two polymorphic loci. Prolonged evolution in isolation in widely separated habitats, coupled with stabilizing selection on the phenotypes, has been invoked to explain this deep, but phenotypically largely cryptic, separation. Morphologically inconspicuous variation has also been revealed in plethodontid salamanders (Wake and Yanev 1986), tuataras (Daugherty et al. 1990), and onychophorans (Briscoe and Tate 1995) by means of population genetic approaches.

Acknowledgments

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