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Phenotypic diversity in the threespine stickleback *Gasterosteus aculeatus* LINNAEUS, 1758 (Teleostei: Gasterosteidae) in western Austria – the four-spined form

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Abstract

Threespine sticklebacks in the Rhine valley and some brooks entering Lake Constance in western Austria are characterised by an unusual high number of specimens (about 6%) with an additional fourth dorsal spine. These populations of western Austria, where *Gasterosteus aculeatus* was introduced in the second half of the 19^{th} century, are phenotypically characterised by three lateral plate morphs, completely, partially and low plated. Specimens with four dorsal spines occur in all three morphs, although mainly in the complete morph. The fourth spine is always positioned between the second and third dorsal spine, but not always on the same pterygiophore. The occurrence on different pterygiophores indicates that these additional spines are not based on the same genetic information. The possible role of such a fourth spine in reinforcing the defensive complex in *Gasterosteus aculeatus* is discussed.

Zusammenfassung

Dreistachlige Stichlinge in Westösterreich, aus dem Rheintal und aus Nebengewässern des Bodensees, sind durch eine ungewöhnlich hohe Anzahl (etwa 6%) von Individuen mit einem vierten Dorsalstachel charakterisiert. Die Populationen von *Gasterosteus aculeatus* in Westösterreich sind phänotypisch durch drei Lateralplattenformen charakterisiert: vollständig, teilweise und wenig beschildert. Dreistachlige Stichlinge mit vier Dorsalstacheln finden sich in allen drei Lateralplattenformen, sie kommen aber am häufigsten in der vollständig beschilderten Form vor. Dieser zusätzliche Stachel ist immer zwischen dem ersten und dem zweiten Dorsalstachel positioniert, jedoch nicht immer auf dem selben Pterygophor. Das Auftreten eines vierten Stachels auf unterschiedlichen Pterygophoren deutet darauf hin, dass diese Stacheln nicht auf die selbe genetische Information zurückgehen. Die Möglichkeit, dass solch ein zusätzlicher Dorsalstachel den Defensivkomplex bei *Gasterosteus aculeatus* verstärkt, wird diskutiert.

Introduction

Gasterosteus aculeatus, widely distributed in coastal marine and freshwaters of all three northern continents, is greatly differentiated ecologically and morphologically (summarised in WOOTTON 1984, BELL & FOSTER 1994). It is believed that the threespine stickleback has originated in marine habitats, but many populations have been isolated in freshwater. These freshwater populations represent the vast majority of a wide array of phenotypes of this most variable fish known. Much interest has been given to the bony armour of *G. aculeatus* morphologically (e.g. MÜNZING 1963, REIMCHEN 1983, BANBURA 1994, BELL & ORTI 1994) and genetically (e.g. MÜNZING 1959, HAGEN &

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GILBERTSON 1973, ZIUGANOV 1983, COLOSIMO & al. 2004, SHAPIRO & al. 2004). Nevertheless, populations of threespine sticklebacks with four-spined specimens are rarely investigated in detail.

In Europe, threespine sticklebacks with four dorsal spines usually comprise 1%-2% of the specimens in a population (HEINCKE 1889, GROSS 1978, PAEPKE 1981) although this percentage is distinctly higher in some populations (PENCZAK 1965, GROSS 1978). Introduced *G. aculeatus* populations in western Austria often contain more specimens with four dorsal spines than native populations in northern central Europe (AHNELT & al. 1994) or introduced populations in eastern Austria (AHNELT unpublished), possibly because of genetic drift or founder effect. PENCZAK (1963, 1965), GROSS (1978), BELL (1984) and KLEPAKER (1993) discussed the heredity of a fourth spine and also its possible causes by environmental factors. No consideration has been given to whether such an additional dorsal spine may reinforce the defensive complex of threespine sticklebacks. Positioned next to the two long dorsal spines, a well-developed fourth spine may function as part of this complex.

The first two dorsal and the pelvic spines, their basal plates, the pelvic girdle, and the associated lateral plates together form a complex of external bony structures (HOOGLAND & al. 1957, REIMCHEN 1983). Each structure may show morphological differentiation related to differences in habitat, life history and predatory pressure, thus changing its importance within the complex. Together they form a functional unit which aids against predation (REIMCHEN 1983, 1992, 2000), thus the term "defensive complex" (BAUMGARTNER 1992) seems more appropriate than the term "defensive apparatus" (GROSS 1978).

Within the defensive complex (DC) the lateral plates, in combination with the basal plates of the dorsal spines and the ascending process of the pelvic girdle, help to protect the fish against lethal injury upon the first attack of a predator. Such attacks are mostly directed against the head and the anterior part of the body (REIMCHEN 1992). This area is densely armoured in all three lateral plate morphs (completely, partially and low plated) of the threespine stickleback in western Austria (AHNELT & al. 1998).

Gasterosteus aculeatus shows a high variability in morphology but in some features also canalised phenotypes. With six dorsal pterygiophores preceding the soft dorsal fin and with three dorsal spines the number of these bony structures is very constant in threespine sticklebacks (summarised in WOOTTON 1984, BOWNE 1994, PAEPKE 2002). This is also the case for the dorsal pterygiophores even the spine number is increased. In these specimens the fifth pterygiophore, spineless in the three-spined form, supports the fourth spine (GROSS 1978, PAEPKE 1981, AHNELT & al. 1994). Nevertheless, besides the variation in spine number also the number of the dorsal pterygiophores is variable (HEINCKE 1889, TAGLIANI 1926).

The aim of the present study is to compare the number and position of pterygiophores and dorsal spines in the four-spined form of the threespine stickleback with those of the three-spined form in the same populations of tributaries of the Alpine Rhine and Lake Constance (western Austria). We also compare the position of the additional dorsal spine and the position of the additional pterygiophore of the four-spined form. Besides the arrangement and the number of these bony structures of the first dorsal fin also their possible function within the DC of the four-spined specimens is investigated. Therefore we compare the DC of *G. aculeatus* with three versus four dorsal spines and investigate whether the development of an additional spine and an additional pterygiophore possibly reinforces the DC.

Materials and Methods

Values given below for preserved specimens of *Gasterosteus aculeatus* are: sampling site, number of specimens with three or four dorsal spines respectively, standard length in mm and date of sampling.

Western Austria, Vorarlberg, five tributaries of the River Rhine (Alpine Rhine) and Lake Constanze. A total of 305 specimens. **Harder Dorfbach**, 200 m before it enters Lake Constanze, 46 spec., three dorsal spines, 30.1 - 60.9 mm SL; 4 spec., four dorsal spines, 30.4 – 50.6 mm SL; 23 September 1993 (NMW 92819, ZMB 32418, uncatalogued). **Birkengraben near Hard**, 2 spec, three dorsal spines, 25.8 – 47.9 mm SL (uncatalogued), 23 August 1993. **Gillbach near Altach**, 126 spec., three dorsal spines, 13.2 – 72.7 mm SL; 10 spec., four dorsal spines, 9.9 – 46.2 mm SL; 01 September 1993 and 10 July 1995 (NMW 94974, uncatalogued). **Rheintal Binnenkanal near Hohenems**, 76 spec., three dorsal spines, 17.2 – 52.9 mm SL; 4 spec., four dorsal spines, 31.3 – 46.2 mm SL; 22 March and 01 September 1993 (NMW 94975, uncatalogued). **Lustenauer Kanal near Lustenau**; 37 spec., three dorsal spines, 15.7 – 35.5 mm SL; 11 and 23 August 1993 (NMW 92250). All specimens were collected by E. & M. Amann, A. Lunardon and E. & O. Bösch.

Institutions: NMW = Naturhistorisches Museum Wien. ZMB = Museum für Naturkunde der Humboldt-Universität, Zoologisches Museum. Uncatalogued = collection H. Ahnelt.

The specimens were collected by dip net, fixed in 4% formaldehyde and stored in 70% ethanol. For better identification of the external bony structures, 177 specimens were cleared and stained (DINGERKUS & UHLER, 1977).

Abbreviations used: ADS, additional dorsal spine; APT, additional dorsal pterygiophore; DC, defensive complex; LP, lateral plate; PT, pterygiophore.

Nomenclature of the lateral plate morphs: The characterisation of the three plate morphs (low, partial and complete) and the two ecological forms (anadromous and resident freshwater) follows WOOTTON (1984). In the nomenclature of the plate morphs we follow BAKKER & SEVENSTER (1988). These authors proposed following descriptions for the designations leiurus, semiarmatus and trachurus: low plated for leiurus, partially plated for semiarmatus and completely plated for trachurus.

The dorsal pterygiophores: In the most common phenotype of *G. aculeatus*, six dorsal pterygiophores precede the soft dorsal fin, but their number may vary (PENCZAK 1965, BELL & BAUMGARTNER 1984, PAEPKE 2002, REIMCHEN & NOSIL 2002). The three dorsal spines are positioned on PTIII (third pterygiophore), PTIV and PTVI. The fifth pterygiophore (PTV) lacks a spine in specimens with the typical three dorsal spines (Fig. 1). In the four-spined phenotype the additional spine (ADS) is positioned on PTV or on an additional (seventh) pterygiophore (APT) (Figs. 2B, 4). We determined the position of an additional dorsal spine corresponding with a distinct pterygiophore and with lateral plates in specimens >30 mm SL. The dorsal pterygiophores are indicated by Roman numerals. The pterygiophores carrying the dorsal spines are also termed basal plates (BP) (Fig. 1).

The defensive complex: The nomenclature of the bony elements of the DC follows REIMCHEN (1983). The DC of *G. aculeatus* typically consists of 20 bony elements combined into three units: (1) first and second dorsal spines along with their basal plates (pterygiophores), (2) the lateral plates LP4 – LP8 and (3) the pelvic girdle with its anterior, its ascending and its ventral processes and the two pelvic spines. The pelvic girdle is a bilateral structure of two medially sutured pelvic plates which bear the pelvic spines (BELL & ORTI 1994, BOWNE 1994). These three parts of the two pelvic plates may be variously reduced or have divergent forms or sizes (compare REIMCHEN 1994). We designate the three parts of the two pelvic plates, the anterior, the ascending and the ventral processes, as three separate elements: the two ascending processes and the anterior and the ventral process (which are fused and treated each as one element).

A completely developed DC is separated into a central and peripheral sections. The central section is formed by the first and second dorsal spines and their basal plates, the lateral plates (LP) LP5 – LP7, the dorsal process of the pelvic girdle, and the two pelvic spines (Fig. 1). Only those LP belong to the central section of the DC which are dorsally overlapped by the basal plates of the first and the second dorsal spines and ventrally by the ascending process of the pelvic girdle, usually LP5, LP6, LP7. The lateral plates anterior and posterior to them, LP4 and LP8, merely buttress the basal plates of the first dorsal spine (LP4) and the second dorsal spine (LP8), but not the ascending process of the pelvic girdle. LP4 and LP8 are therefore elements of the peripheral sections of the DC (Fig. 1).

Results

The overall percentage of *G. aculeatus* with an additional fourth spine in western Austria is 5.9% of 305 specimens. They occur in three of five localities, i.e. 18 (6.8%) of 265 specimens. The ratio between sticklebacks with three versus four dorsal spines is somewhat lower in the Rheintal Binnenkanal population (4.7%), whereas it reaches 7.7% in the Gillbach and 8.0% in the Harder Dorfbach.

The elements of the DC are well developed in all investigated specimens, whether with three or with four dorsal spines. Deviations within the DC are minor. LP6 is the only lateral plate which often, but not always buttresses the basal plates of the two first dorsal spines (Figs. 1, 2). The ventral process of the pelvic girdle is long and slender. The pelvic spines are long, when fully depressed their tips extend up to a vertical through the anterior quarter of the basal plate of the third dorsal spine.

The basic development of the DC in the sticklebacks with four dorsal spines is identical to that with three dorsal spines (Figs. 1, 2A, 2C) in 16 of 18 investigated specimens with a fourth dorsal spine. The remaining two, however, differed as more than the usual five lateral plates were included in this functional unit because LP9 buttresses the basal plate of the fourth spine (Fig. 2B). In these specimens six lateral plates occur in the DC. No reductions or complete loss of DC elements were observed.

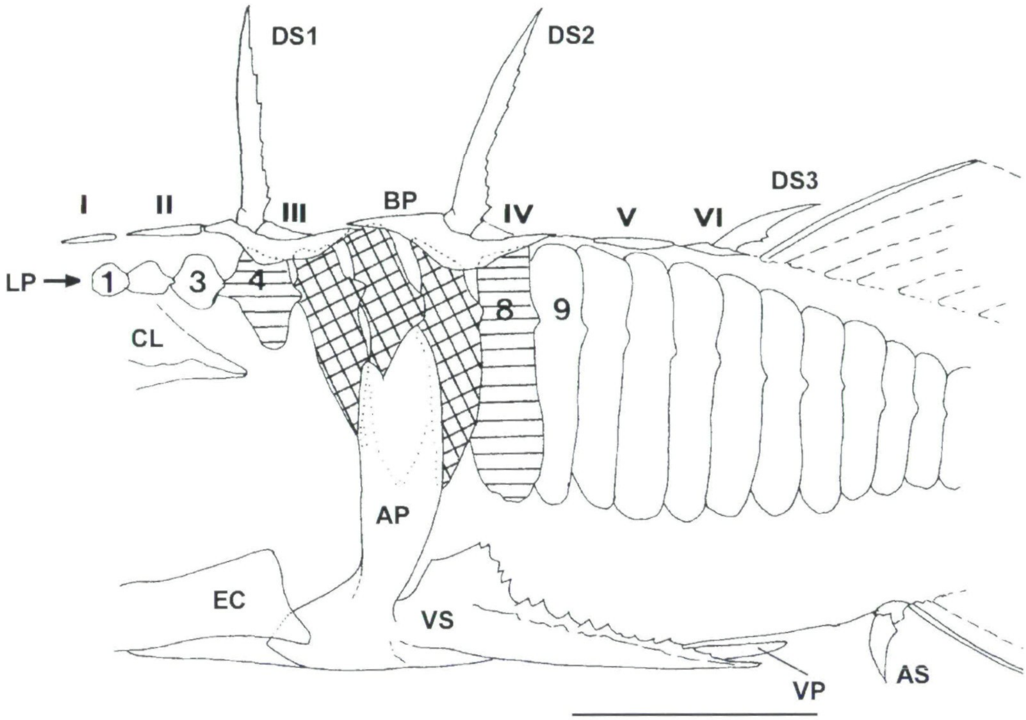


Fig. 1: *Gasterosteus aculeatus*, western Austria, Rhine valley, Gillbach, completely plated, 45. 9 mm SL. Structural associations between spines and lateral plates. Hatched = lateral plates of the peripheral part of the defensive complex; cross hatched = lateral plates of the central part of the defensive complex (for detailed explanation see text). AP, ascending process of the pelvic girdle; AS, anal spine; BP, basal plate of dorsal spine; CL, cleithrum; DS1, DS2, DS3, first, second and third dorsal spines; EC, ectocoracoid; LP, lateral plates; VP, ventral process of the pelvic girdle; VS, ventral spine. Pterygiophores with Roman numbers, lateral plates with Arabic numbers. Scale bar – 5 mm.

A fourth spine, when present, is positioned between the second and the third spines, never anteriorly to the first spine or between the first and second spines. This additional dorsal spine is developed in ten adult specimens (76.9%) of the completely plated, in one (7.7%) of the partially plated, and in two (15.4%) of the low plated forms. The development and number of the lateral plates was not completed in five juvenile specimens with four dorsal spines (9.9 – 16.8 mm SL).

The additional dorsal spine is well developed, shorter than the first two spines, but longer than the third spine (Fig. 3). It is vestigial in one specimen and forked in another (Fig. 2A). The basal plate of the fourth spine is positioned dorsal to LP9 – LP10. In most specimens these plates are distinctly distant from the ventral edge of the basal plate, in some specimens they come close to it. In 10 of 13 adult specimens this basal plate does not touch a lateral plate on either the left or the right side. In one specimen the basal plate of the additional dorsal spine is buttressed by LP9 of both sides, in an other specimen by LP9 on the right side only. Thus, in these two specimens the fourth spine, its basal plate and the LP9 are included into the DC, although in one case only on the right side. The five juveniles whose lateral-plate development was incomplete were not taken into consideration.

In 38% (n = 5) of sticklebacks with a fourth dorsal spine (n = 13) there were seven pterygiophores anterior to the soft dorsal fin, and the typical six in 62% (n = 8). In specimens with six pterygiophores and the fourth spine on the fifth pterygiophore, PTV is always positioned immediately anterior to PTVI, distinctly distant from PTIV, dorsal to LP10 – LP11 and never dorsal to LP9 (Fig. 2A, 4B). The additional pterygiophore is always positioned immediately posterior of PTIV (Fig. 4C, 4D) and, dorsal to LP9 (Fig. 2B). This additional pterygiophore is in fifth position within the pterygiophore row. In specimens with four dorsal spines and seven pterygiophores, the additional dorsal spine is generally positioned on the additional pterygiophore (Fig. 2A, 4D). Only in such a case a fourth spine may improve the DC.

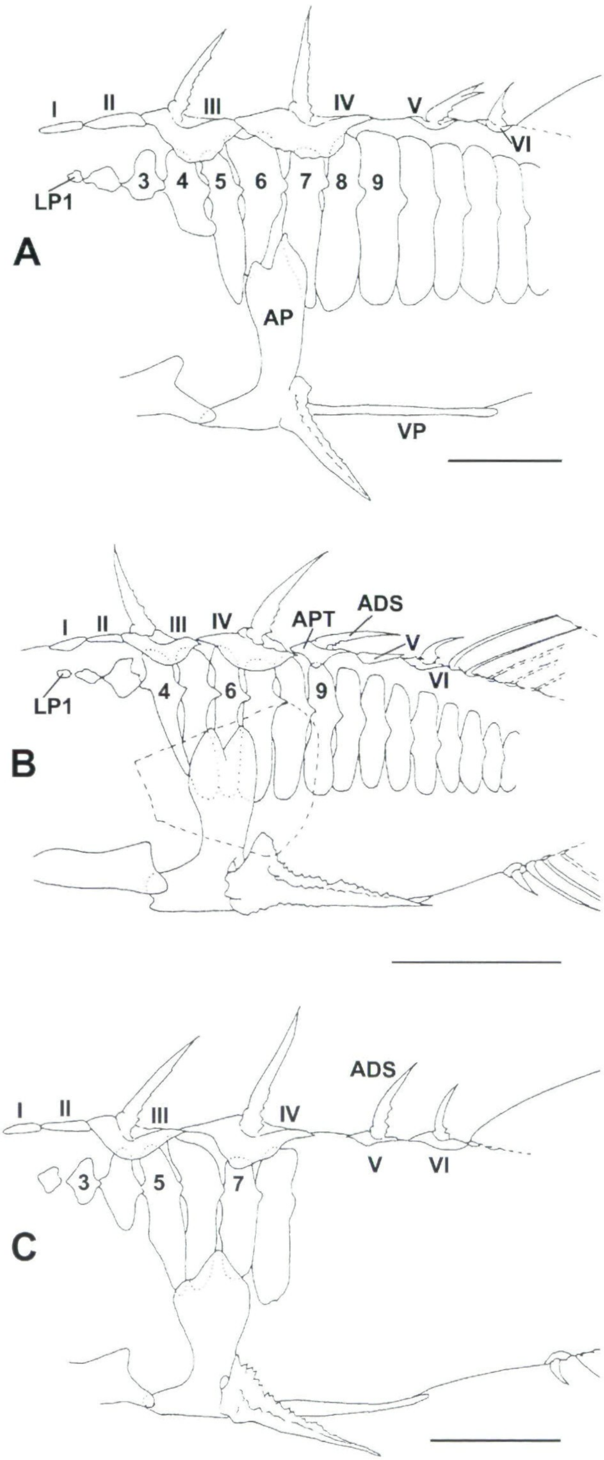
In our material an additional pterygiophore occurs distinctly more often in specimens with four dorsal spines. In sticklebacks with three dorsal spines only 2.6% have seven pterygiophores. One specimen was found with only five pterygiophores.

Discussion

In Austria *G. aculeatus* is an introduced fish species (AHNELT 1986, AHNELT & al. 1994, MIKSCHI 2002). In western Austria it was probably introduced in the Alpenrhein, Lake Constance and some of their tributaries by aquarists (HELLER 1870), around 1860. Introductions occurred several times, possibly from both polymorphic and monomorphic populations (AHNELT & al. 1995), but their origin is unknown. The most likely source is northern Central Europe. Most native resident freshwater populations of *G. aculeatus* in Europe, with the exception East Baltic Sea and Poland, consist nearly 100% of low plated forms (GROSS 1978, BANBURA 1994, PAEPKE 1996, 2002). This is the same for the native populations of the Rhine (except its lower part) upstream as far as Basel (Switzerland) (LEUTHNER 1877). In contrast, the populations from western Austria are all polymorphic and dominated by the completely plated form (AHNELT & al. 1994, 1998). This is strong evidence that they did not originate from an upstream migration of the native low plated sticklebacks from the Upper Rhine.

Gasterosteus aculeatus was able to establish large populations around Lake Constance and in the lake itself (BERG 1993). In three of the five investigated populations, up to 8% of the specimens have four dorsal spines. This is distinctly higher than known in most native populations from northern Central Europe (HEINCKE 1889, PENCZAK 1965, PAEPKE 1981) or for Canada (NELSON & HARRIS 1987). PENCZAK (1965) and GROSS

Fig. 2: *Gasterosteus aculeatus* from western Austria, Rhine valley, with four dorsal spines. Associations between spines and pterygiophores. A: completely plated, Harder Dorfbach, 50.4 mm SL; additional spine associated with fifth pterygiophore. B: completely plated, Rheintal Binnenkanal, 31.3 mm SL; additional spine associated with additional pterygiophore; hatched = outline of pectoral fin. C: low plated, Harder Dorfbach, 44.3 mm SL; additional spine associated with fifth pterygiophore. ADS, additional dorsal spine; APT, additional pterygiophore; LP1, first lateral plate; other abbreviations as in Fig. 1. For further information see text. Scale bar – 5 mm.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(1978) conclude that even if the overall percentage of specimens with an additional dorsal spine is usually low in large areas, about 5% of a population may have a non-typical number of dorsal spines (two or four spines).

A rapid increase of four-spined specimens has been reported from a former marine, completely plated population of *G. aculeatus* in Norway isolated in freshwater for about 40 years (KLEPAKER 1993). Over a 9-year period the frequency of sticklebacks with an additional spine increased from 5% to 12%. GROSS (1978), who found low frequencies of such offspring in one-third of its crosses, and in one a high frequency (35%), assumed that alleles for varying numbers of dorsal spines may exist in *G. aculeatus* and that, under natural selection, homozygous specimens (with four spines) may occur in such populations. This is possibly supported by observations on the increase of dorsal spines (KLEPAKER 1993) and pectoral fin rays (KRISTJANSSON & al. 2004) in populations isolated in freshwater.

In polymorphic populations in western Austria, completely, partially and low plated forms occur, although not in the same ratio (AHNELT & al., 1994, 1998). However, the occurrence of a fourth spine is not linked to a specific plate morph: the additional spine may occur primarily on low plated sticklebacks (GROSS 1978) or on completely plated specimens (BELL & BAUMGARTNER 1984, KLEPAKER 1993, present data). Nevertheless, such a fourth dorsal spine is seemingly not uncommon in European populations of *G. aculeatus*. GROSS (1978) found specimens with four dorsal spines in 27 (50.9%) of 53 localities across Europe. But obviously the number of individuals with such an additional spine is usually low. From 2813 specimens only 69 (2.5%) were corresponding to the four-spined form and, in only five samples (9.4%) more than three specimens with a fourth dorsal spine occurred (GROSS 1978, Tab.1).

Divergent spine number in *G. aculeatus* is well documented. Less well known are divergent position of these spines and divergent pterygiophore numbers. Additionally these extra spines may be positioned on different pterygiophores (Figs. 2, 4) (PENCZAK 1965, PAEPKE 1981, BELL & BAUMGARTNER 1984). In teleost fishes the dorsal pterygiophores support the spines and the rays of the dorsal fins. A high number of spineless pterygiophores in the fin skeleton is uncommon and often linked with the reduction of external fin elements (spines, rays), but with the internal elements (pterygiophores) still present (BIRDSONG & al. 1988, AHNELT 2003). The occurrence of additional spines on different pterygiophores in the threespine stickleback indicates that these extra spines and extra pterygiophores are based on different genetic information.

PAEPKE (1996) concludes that divergent spine number may indicate that threespine sticklebacks descended from an ancestor with more than three dorsal spines. If this hypothesis is correct, the additional dorsal spines of *G. aculeatus* could be the re-expression of ancestral morphologies – an atavism. This would be the same for the additional dorsal pterygiophore which often occurs together with a dorsal spine, but not necessarily (Fig. 4C, 4D). Such a character state is not a phylogenetic character reversal or taxic atavism, but possibly a spontaneous atavism *sensu* STIASSNY (1992). Nevertheless, this is a feature possibly favoured under to date unknown environmental conditions.

The few well-documented reports of more abundant four-spined specimens underline that this phenomenon is mostly restricted to isolated and/or introduced freshwater populations (GROSS 1978, BELL & BAUMGARTNER 1984, KLEPAKER 1993). Freshwater habi-

Fig. 3: *Gasterosteus aculeatus* from western Austria, Rhine valley, Gillbach, 33.2 mm SL, with four dorsal spines. Cleared and stained specimen. Additional spine on fifth pterygiophore, near second dorsal spine.

tats are marginal environments for the *G. aculeatus* complex *sensu* NELSON (1994), which is dominated by marine and anadromous populations. This might explain why threespine sticklebacks with four dorsal spines are rarely found in anadromous populations (GROSS 1978, KLEPAKER 1993). It supports the view of BELL & BAUMGARTNER (1984) that genetic drift plays an important role, but also indicates that unusual environmental conditions may be responsible for such an unusual phenotype. Genetic drift and founder effect combined with new physiological conditions may also have caused an increased number of pectoral fin rays in isolated freshwater populations in Iceland (KRISTJANSSON & al. 2004).

Heredity of a fourth spine was not revealed by crosses by PENZCAK (1963) but results of LINDSEY (1962) and GROSS (1978) indicate that dorsal spine number has a genetic basis. BELL (1984) notes that a genetic influence on dorsal spine number may interact with environmental conditions. This author points to the possibility that high temperature may induce four-spined offspring. But the results of GROSS (1978) do not corroborate a temperature dependent number of dorsal spines. GROSS (1978) showed that the majority of populations with four-spined specimens occur north to 50° latitude (63.2% of the northern populations). Contrary, only 13.3% of the populations south of 50° latitude are characterised by specimens with four dorsal spines. KLEPAKER's (1993) data seemingly also indicate a genetic basis of the dorsal spine number. This author reports that the number of specimens with an additional spine increased distinctly in a Norwegian population within less than a decade (from less than 1% to about 12%). Additionally the frequency of threespine sticklebacks with four dorsal spines increased steadily, which points to a process of selection (KLEPAKER 1993).

It might be assumed that once such a feature, a fourth dorsal spine, occurs in an isolated population and is not disadvantageous, then selection will not eliminate it, resulting in a population with a high ratio of specimens with the four dorsal spine phenotype. However, we follow GROSS (1978) in assuming that alleles for varying numbers of dorsal spine may exist, which, under natural selection, produce threespine sticklebacks with an additional dorsal spine. This extra spine in specimens from western Austria is positioned on different pterygiophores, an indication that these additional spines are not homologous.

The lateral plates which buttress the basal plates of the dorsal spines distribute the forces occurring from spine deflection during manipulation by gape-limited predators (e.g. HOOGLAND & al. 1957, REIMCHEN 1980, 1983). The longer the spines, the larger their leverage and the larger the potential injuries caused by deflections of their basal plates. Thus, the buttressing of these basal plates by lateral plates is a prerequisite for the development of the typical strong and long spines of *G. aculeatus*.

Structural associations between spines and lateral plates of the threespine stickleback in western Austria are similar to the DC-types A and B of REIMCHEN (1983). No reductions or complete loss of DC elements were observed as is described for populations e.g. from eastern Austria (AHNELT & al. 1998), western North America (REIMCHEN 1983, 1994, BELL & al. 1993) or Scotland (CAMPBELL 1985).

A fourth spine, when present, is generally positioned between the second and the third spines, which corresponds to the most common types designated by HEINCKE (1889). This author described a few specimens in which the fourth spine occurs in front of the first or between the first and the second dorsal spines. In such positions, a fourth spine (if it is not vestigial) becomes part of the defensive complex in the sense of REIMCHEN (1983). These positions are extremely rare in native populations (PAEPKE 1996), allowing the conclusion that there is no selective advantage.

Authors who investigated threespine sticklebacks with four dorsal spines (PENCZAK 1965, GROSS 1978, BELL & BAUMGARTNER 1984, KLEPAKER 1993) did not indicate whether such a spine was incorporated into the defensive complex, as is the case in the two western Austrian specimens (Fig. 2B). Even in populations like those of the present study, which show an unusual high percentage of four-spined forms, it is unlikely that the DC will be distinctly improved compared with threespine specimens. Nevertheless, selection may favour even a small advantage. It is interesting to note that the fourth spine is included into the defensive complex only in those sticklebacks which have seven instead of the typical six pterygiophores anterior to the soft dorsal fin. The basal plate of the additional spine is in contact with the lateral plate only when this spine is positioned on the additional pterygiophore. In none of the investigated sticklebacks with four dorsal spines is this spine and its basal plate included into the defensive complex when the spine is positioned on the pterygiophore close to the third dorsal spine (usually PTV).

KLEPAKER (1993), who reports a steadily increasing frequency of four dorsal spines in an Norwegian population, concludes that this points to a selection process. Such changes in isolated populations, also concerning the shift from monomorphic completely plated forms to polymorphic populations within a few generations, is understood as a drastic reorganisation of the genetic composition of the entire population (KLEPAKER 1993, KRISTJANSSON & al 2004). REIMCHEN'S (1980) demonstration of variable spine numbers related to predation by vertebrates or by insects is interpreted by BELL (1984) as a possible explanation for spine number variation in sticklebacks in general.

It is not known if the high number of threespine sticklebacks with four dorsal spines in western Austria is caused by genetic divergence. A selective advantage is not obvious. It is also not clear to which extant genetic drift accounts for the relative abundance of

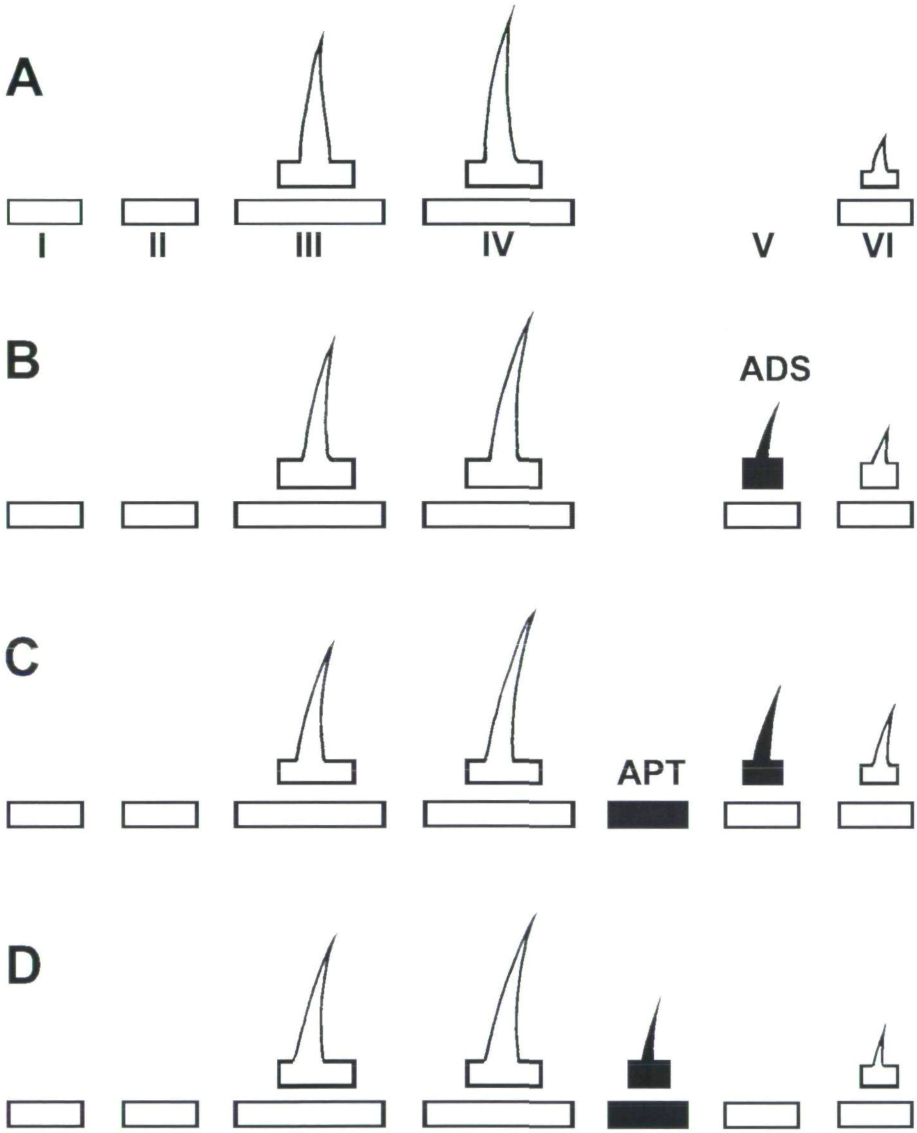

Fig. 4: Number and position of the dorsal spines and the dorsal pterygiophores of *Gasterosteus aculeatus* in western Austria, stylised. A: typical number and position of spines and pterygiophores (outlined). B – D: positions of the additional spines and the additional pterygiophore (black). B: additional spine associated with the fifth pterygiophore; C: additional pterygiophore between fourth and fifth pterygiophores, additional spine associated with fifth pterygiophore; D: additional spine associated with additional pterygiophore. I – VI: pterygiophores; ADS: additional dorsal spine; APT: additional dorsal pterygiophore.

four-spined specimens in these non-native populations. But taking in consideration that these populations have been introduced in the second half of the 19th century this feature should have disappeared if disadvantageous. In many specimens the fourth dorsal spine is fully functional but in most it is not incorporated into the defensive complex, thus its possible aid against predation seems to be minor if existing at all. Latter is seemingly supported by observations in the Norwegian population which is characterised by a steadily increasing frequency of four dorsal spines although these specimens are not under predatory pressure (KLEPAKER 1993).

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